INVESTIGATIONS INTO USING VEGETATIVE INDICES IN SOYBEAN BREEDING

by

RANDI R. CLARK

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College of Agriculture

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Approved by:

Major Professor

William T. Schapaugh

Abstract

Yield in soybean (*Glycine max* (L.) Merr) needs to dramatically increase across the world to feed the growing population. Remote sensing and high-throughput phenotyping may provide a tool to better phenotype soybean genotypes. This research was conducted to: 1) examine the relationships between NDVI and CT with seed yield, maturity, lodging, and height, 2) determine if the time of day and growth stage have an effect on the spectral readings, 3) examine the relationships between spectral reflectance and traits associated with drought tolerance, and 4) evaluate how weather variables impact the ability of vegetative indices and canopy temperature to detect differences among genotypes**.** Ninety genotypes from the mapping population derived from the cross between KS4895 x Jackson were evaluated in Manhattan, KS, in 2013 and in McCune, Pittsburg, and Salina, KS in 2014. Genotypes were planted in a randomized complete bloc design in four-row, 3.4m long plots spaced 76 cm apart. Plant height, lodging, maturity and seed yield was collected on the center two rows of each plot. Spectral readings used to calculate a normalized differential vegetative index (NDVI) and canopy temperature (CT) were taken during reproductive growth. Nitrogen fixation trait and drought tolerance data was collected by the University of Arkansas. This population exhibited a substantial genetic variation for all traits evaluated. Correlations of NDVI and CT entry means with the agronomic traits were small and inconsistent. Time of day and growth stage were not important in differentiating genotypes. Differences in NDVI and CT did account for some genetic variation in drought tolerance traits, however, the strength of the associations were small. None of the weather variables were consistently associated with an increase or decrease in entry or error variance across the four environments. Stronger associations need to be established to use NDVI or CT to characterize differences in genotypes in a plant breeding program

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Dedication

This work is dedicated to my Mom and Dad who without their love support and encouraging words I wouldn't be where I am today.

Chapter 1 - Literature Review

Introduction

Soybean is an important oil seed crop in the world [*Glycine max* (L.) Merr.] (Manavalan et al., 2009; Pereyra-Irujo et al., 2012). Soybean is in high demand for food, feed and industrial applications (Deshmukh et al., 2014). With changing climates and the increasing population, soybean yields will need to increase 55% by 2050 (Deshmukh et al., 2014). Because of this increased demand there will need to be a faster and better way to develop high yielding, stress resistant varieties (Cobb et al., 2013).

Phenotyping and Remote Sensing

Remote sensing is the use of measuring radiation reflected from plants (Mulla, 2013). Remote sensing using satellites to collect data has been used since the 1980's (Govender et al., 2009). Advances in technology have brought about hand held sensors to use in field for multiple observations (Vicente-Serrano et al., 2006). There are two types of sensors, passive and active sensors. Passive sensors rely on solar radiation to collect data and can be influenced by things such as dust, pollen, and cloud cover (Fitzgerald, 2010). Active sensors do not need to rely on solar radiation and are day light independent (Rochon et al., 2003; Winterhalter et al., 2013) and should not be influenced by pollen, dust, and cloud cover (Elsayed et al., 2015). Two active sensors have been widely used. The first is the GreenSeeker (NTech Industries Inc., Ukiah, California) the second is the Crop Circle ACS-470 (Holland Scientific Inc., Lincoln, Nebraska) (Elsayed et al., 2015). Currently there is a down side to active sensors, they are limited to only a few wavebands whereas passive sensors can be hyperspectral and contain hundreds of wavebands (Elsayed et al., 2015). Having narrower wave bands such as used in the Crop Circle or Greenseeker have been shown to be highly correlated to nitrogen content, plant pigments, and carbon amount (Campbell et al., 2007).

The use of multiple wavebands means that different vegetative indices can be calculated such as the normalized differential vegetative index (NDVI) that can be related to the amount of absorption (Choudhury, 1987). An NDVI can be calculated by using the red and the near infrared (NIR) portions of the spectrum (RED-NIR)/(RED+NIR) (Gamon et al., 1995). Hoyos-Villegas and Fritschi (2013) used the red wavelengths, 550-675 nm, and the NIR, 700-1300 nm, to determine if vegetative indices can be used as tools to predict yield and biomass. Chlorophyll highly absorbs in the red and blue wavebands (400 – 500 nm) (Hatfield et al., 2008). Red wavebands are long and do not scatter as easily as the short wavebands of the blue light, therefore blue light is not widely used for remote sensing (personal communication with Kevin Price). Spectral indices can be designed to detect small changes in the vegetation (Lausch et al., 2013). These indices are useful not only for detecting those small changes, but the indices also minimize the background noise, such as soil reflectance (Hatfield and Prueger, 2010). In wheat, high NDVI values have been found to correlate to high photosynthetic rates and high yields (Guasconi et al., 2011). When a plant is stressed Sridhar and Parihar (2000) observed an increase in reflection of red and a decrease in reflectance of the NIR. In a review of remote sensing Govander et al. 2009 quoted several articles indicating NIR has been correlated to the relative water content in vegetation and soil. The red edge has been shown to be a good indicator of crop stress in cotton. The red edge is the area where there is a dramatic increase in reflectance between 690 nm and 730 nm and shifts between the larger wavebands and shorter wavebands when stress is present (Read et al., 2002).

High thermal infrared readings of plant leaves, or canopy temperature, is linked to water availability in durum wheat (Idso et al., 1977; Jackson et al., 1981). In wheat it has been found that irrigation leads to better stomatal conductance, however measuring this is expensive. Using thermal infrared technologies has shown to work just as well (Amani et al., 1996). Many studies have shown, high heat or disease disrupts the transpiration, causing plant temperatures to increase (Pinter et al., 2003). McKinney et al. (1989) concluded that canopy temperature readings of soybeans were erratic under drought stressed environments.

One of the biggest challenges facing phenotyping, for example for a trait like seed yield, is the amount of time it takes and the cost (Furbank and Tester, 2011). Also, phenotyping can be difficult because plants have different responses when placed in different environments (Cobb et al., 2013). With the changing environments it is hard to find a trait with high heritability (Deshmukh et al., 2014; Passioura, 2012). Most things being phenotyped are time sensitive, meaning data must be collected during a certain time of growth (like reproduction stages) (Passioura, 2012). The use of high-throughput phenotyping may help relieve some of this time and cost. If selection systems can be developed that better combine genotyping with improved phenotyping techniques, breeding advances may be accelerated (Cabrera-Bosquet et al., 2012).

Several challenges are associated with using remote sensing. Effects associated with weather, such as cloud cover, may impact the quality of the readings being taken. Sridhar and Parihar (2000) noted the impact of cloud cover on the scattering of the radiation wavelengths. Gardner, B.R. (1992) noted that different crops require different amounts of time after cloud cover to reach a steady state temperature. Other weather variables, such as relative humidity and wind speed may also impact the quality of the readings. Another challenge associated with using remote sensing is the potential impact of background effects include influences which could be attributed to reflectance from the soil (Hilker et al., 2011), or the variation of vegetation due to seasonal and climate-induced changes (Dudley et al., 2015). More research is needed on how weather can affect the data collected.

Drought

All organisms require adequate water to survive. Water is vital for plant photosynthesis, nutrient uptake, and respiration (Govender et al., 2009). Drought is an accumulation of multiple factors such as high temperatures, lack of water, and shading. For soybeans, it is considered one of the most limiting factors for yield production because it affects most growth stages (Abdel-Haleem et al., 2012; Devi and Sinclair, 2013; King et al., 2009; Pathan et al., 2014). When drought stress occurs it affects the cell walls, turgor presser, and the water content in the plant (Govender et al., 2009). Drought is the leading cause of yield reduction in the world causing about 50% reduction across all crops (Araus et al., 2008; Mutava et al., 2015; Pardo et al., 2015). It is possible to combat drought using farming practices such as irrigation, but this can be costly (Seversike et al., 2013), and with rising concerns about agricultural water use it is not considered sustainable in many parts of the world. Another option to combat drought, is to breed drought tolerant crops, however breeding for drought tolerance is complex both genetically and physiologically (Abdel-Haleem et al., 2012; Dhruv et al., 2015; Montes et al., 2011). In the past this has not been seen as a good option because it was believed that drought tolerance was considered to have a negative effect on yield (Blum, 2005; Mutava et al., 2015). Recently, there has been more research into drought tolerance showing that a yield penalty is not always the case when looking at different breeding options (Blum, 2005). There have been several mechanisms associated with drought tolerance such as dehydration avoidance (Blum, 2005), better ability to fix nitrogen (Sinclair et al., 2007), slow canopy wilting (King et al., 2009), and water use efficiency (Mutava et al., 2015). Drought tolerance traits also tend to have low heritability (Blum, 2005; Manavalan et al., 2009). Drought tolerance has been linked with different mechanisms of resistance such as, transpiration rate (Fletcher et al., 2007; Seversike et al., 2013), nitrogen fixation rate (King et al., 2009; King et al., 2014; Ries et al., 2012; Sinclair et al., 2007), and having greater stored moisture (Ries et al., 2012)

Slow-wilting

Phenotyping for the drought stress response in soybeans has focused on canopy wilting (King et al., 2009). Wilting is the first symptom of drought in soybeans and cultivars differ in how quickly they wilt during water stress (Charlson et. al., 2009, King et. al., 2009). In the early 1980's delayed wilting in soybeans was observed in several hundred-plant introductions (PI) for drought stress in North Carolina (Ries et. al., 2012). Two plant introductions, PI 471938 and PI 416937, were reported to possess a delayed expression of the phenotypic leaf wilting compared to other lines (Sloane et al., 1990; Hufstetler et al., 2007; King et al., 2009).

PI 471938 has been identified as a slow wilting trait that could be potentially used as a trait in soybeans. Devi and Sinclair (2013) found that the rate of nitrogen fixation in PI 471938 was more tolerant of soil drying than the other genotypes tested. They concluded that the delayed decrease in nitrogen fixation during soil drying may be the advantage in the slow-wilting phenotype.

PI 416937 is an introduction also identified as its slow canopy-wilting trait. It has shown more than one mechanism for drought resistance (Abdel-Haeleem et. al., 2012). A key mechanism seems to be a limitation of the transpiration rate to a maximum rate at high vapor pressure deficit, which delays the damages done to the plant tissue while available soil water is conserved (Seversike et al., 2013). Evaluating genotypes for slow-wilting can be accomplished in several ways. King et al. (2009), used a scale of 0 to 100, where; 0 was no leaf wilting and rolling in the top part of the canopy; 20 had slight wilting and leaf wilting at the top of the canopy; 40 had severe rolling of the leaves in the top of the canopy and moderate wilting throughout the rest of the canopy, as well as some loss of petiole turbidity; 60 is severe leaf wilting throughout the canopy and loss of turbidity in the petioles; 80 showed plants with severe petiole wilting and dead leaves through much of the canopy; and 100 was total plant death.

Nitrogen Fixation

Soybeans are inherently sensitive to soil drying and water stress because of the inability to fix nitrogen (Devi and Sinclair, 2013). Soybeans have a symbiotic relationship with a rhizobia group, primarily *Bradyrhizobium japonicum*, allowing them to fix nitrogen (Miransari et al., 2013). This relationship signals the plant to form root nodules (Hwang et al., 2014). Drought stress can disrupt the communication between the rhizobia and the plant and inhibit nitrogen fixation (Miransari et al., 2013). Nitrogen fixation is vulnerable to drought during soil drying because there is an accumulation of ureides in the shoot (King et al., 2014; Sinclair et al., 2007). During the fixation, N2 is converted into NH3 which is turned into ureides, allanation, and allantotate. When drought conditions start, large amounts of ureides accumulate in the shoot even when nitrogen has been limited. This is thought to serve as a signal to stop or decrease nitrogen fixation (Hwang et al., 2013).

Reducing nitrogen fixation increases the risk of nitrogen deficiency (Hwang et al., 2013). Hwang et al. (2013) developed recombinant inbred lines (RIL) from a cross between ‘KS4895’ and ‘Jackson’ to map quantitative trait loci (QTL) for shoot ureide and nitrogen concentration in soybeans. They developed this population based on previous research done by King et al. (2005 and 2006), and Purcell et al. (2000) that showed Jackson possessing low concentrations of nitrogen and shoot ureides during drought and KS4895, a high yielding variety, which had high concentrations of nitrogen and shoot ureides during drought. They found five QTLs associated with ureide concentrations and four QTLs associated with nitrogen fixation. Hwang et al. (2014) conducted a study looking at the nodule number, size, and weight using the same population derived from 'KS4895' and 'Jackson'. They found that nodule weight and total number were associated with increased nitrogen fixation.

Objectives

To keep up with the growing population there is a need to increase yields at a faster pace than current gains. Remote sensing and high-throughput phenotyping may provide a tool to better screen soybean genotypes to yield and drought response. This research will examine the effectiveness of using remote sensing and canopy temperature to evaluate soybean performance and traits related to drought stress. Specific objectives for Chapter 2 were to: 1) examine the relationships between normalized differential vegetative index and canopy temperature with seed yield, maturity, lodging and height, 2) determine if time of day and growth stage have an effect on the spectral readings, and 3) examine the relationships between spectral reflectance and traits associated drought tolerance. For chapter 3, the objective was to examine how weather variables impact the vegetative indices ability to detect differences among genotypes for relative seed yield.

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Chapter 2 - Using spectral reflectance indices to predict seed yield and traits linked to drought tolerance

Abstract

Yields for all crops need to dramatically increase across the world to feed the growing population. Remote sensing and high-throughput phenotyping may provide a tool to better screen soybean genotypes. This research was conducted to: 1) examine the relationships between NDVI and CT with seed yield, maturity, lodging, and height, 2) determine if the time of day and growth stage have an effect on the spectral readings, and 3) examine the relationships between spectral reflectance and traits associated with drought tolerance in soybean (*Glycine max* (L.) Merr). Ninety genotypes from the mapping population derived from the cross between KS4895 x Jackson were evaluated in Manhattan, KS, in 2013 and in McCune, Pittsburg, and Salina, KS, in 2014. Genotypes were planted in a randomized complete bloc design in four-row, 3.4m long plots spaced 76 cm apart. In Kansas, all genotypes had plant height, lodging, and maturity collected on the center two rows of each plot. Spectral readings were taken during reproductive growth using a Crop Circle AES to calculate a normalized differential vegetative index (NDVI). Canopy temperature (CT) was taken immediately following or at the same time as the spectral data was collected. Nitrogen fixation trait and drought tolerance data was collected by the University of Arkansas, in Arkansas trials. This population exhibited a substantial amount of genetic variation for all traits evaluated. Correlations of NDVI and CT entry means with the agronomic traits were small and inconsistent. Time of day and growth stage were not important in differentiating genotypes. Differences in NDVI and CT did account for some genetic variation in drought tolerance traits, however, the strength of the associations were small. Stronger

associations need to be established to use NDVI or CT to characterize differences in genotypes in a plant breeding program.

Introduction

Yields for all crops will have to dramatically increase across the world to feed the growing population. Between the 1950's – 1990's there was a dramatic increase in yield, however recently these increases have slowed (Araus et al., 2009). By 2050, soybean [*Glycine max* (L.) Merr.] yields will have to increase by 55% to sustain the population (Deshmukh et al., 2014). These increases will have to take place with less arable agricultural land (Araus et al., 2008; Cassman et al., 2003). Soybean projections for 2015/16 thought to be 465 million bushels produced in the United States, would be the highest production since 2006/07 (USDA Staff, 2015). There is still a high demand for food, feed, and industrial applications for soybean because it is considered the number one oil seed crop in the world (Deshmukh et al., 2014; Manavalan et al., 2009; Pereyra-Irujo et al., 2012).

Remote sensing is the gathering of data, such as radiation reflected from plants, at a distance from the plant (Mulla, 2013). It is a non-invasive technique that can be used to monitor various plant traits related to agronomic performance (Montes et al., 2007). Remote sensing techniques have been around since the 1980's utilizing satellites to gather data but recent advances have led to hand held sensors that can be used in-situ (Grovander et al., 2009; Vicente-Serrano et al., 2006). Two different types of hand held sensors have been developed, passive and active sensors. Passive sensors require solar radiation to collect data. Active sensors are equipped with their own light source (Elsayed et al., 2015; Rochon et al. 2003; Winterhalter et al., 2013). Active sensors have become popular in agriculture because they tend to be less effected by changing environmental conditions, such as cloud cover, compared with passive sensors (Elsayed et al., 2015; Fitzgerald, 2010). Two active sensors currently used in agriculture include the Green Seeker (NTech Industries Inc, Ukiah California), and the Crop Circle AES-470

(Holland Scientific Inc., Lincoln, Nebraska). The Crop Circle is equipped with three 10nm range waveband filters, but comes with a total of 6 filters (Holland Scientific Inc., Lincoln, Nebraska). It is also possible to order customized filters to look at specific waveband sections.

With multiple wavebands it is possible to calculate a normalized differential vegetation index (NDVI) (Choudhury, 1987). Vegetative indices are mathematical combinations of the reflectance in the visible and the nonvisible regions of the electromagnetic spectrum. Vegetative indices have been shown to vary with seasonal variation of the plants and within field variability (Vina et al., 2011). These indices are often species related and change based off of the leaf structure and canopy types (Vina et al., 2011). One index used often in agriculture is the red NDVI (referred to as NDVI in this chapter), which is calculated by using reflectance values in the red and near infrared (NIR) wavebands ((NIR-RED)/(NIR+RED)) (Hmimina et al., 2013). Hoyos-Villegas and F. B. Fritshi used 550-675nm for their red wavebands and 700-1300nm for their NIR wavebands in soybean to look at soybean growth and yield. When reflection in the red increases and reflection in the NIR decreases a lower NDVI is observed. Sridhar and Parihar (2000) related lower NDVI values to increased plant stress. When plants are stressed there is a decrease in the in leaf chlorophyll content, leading to a reduction in the absorption in red light (Grovander et al., 2009). The most sensitive waveband area to chlorophyll content falls between the 550 nm and 700 nm, however it is not agreed upon which of the wavebands is the best to assess the plant chlorophyll content (Grovander et al., 2009).

Thermal infrared technology another useful non-destructive way to characterize the physiological status of the plant (Amani et al., 1996). Thermal infrared readings or canopy temperature (CT) differs from the air temperature (Pinter et al., 2003). Studies have shown that canopy temperature is sensitive to water stress in plants (Jackson and Ezra, 1985; Moran et al., 1989). For example, CT has been linked to water availability in durum wheat (Idso et al., 2977; Jackson et al., 1981). Using CT to detect water stress is useful because when water is limiting reduced transpiration may cause leaf temperature to increase (Jackson, 1982). In soybean, high CT during the reproductive stages has been correlated to lower yields (McKinney et al., 1989). Significant genotypic differences have been found in soybean, millet, cotton, alfalfa, and wheat based on CT measurements (Amani et al., 1996).

There are different ways to collect CT. Two particular methods include using a thermal infrared camera or using an infrared thermometer (IRT) sensor. The thermal infrared camera captures images showing the thermal signature of the canopy or anything in the field of view (Jackson et al., 1981). The IRT collects the thermal data for the object the sensor is pointed toward, as long as the sensor is active. The IRT sensor is capable capturing an overall temperature for each plot when connected to a data logger.

Detecting stress in plants is an important part of breeding. Stress in plants can be caused by biotic stresses or abiotic stresses. One major abiotic stresses in agriculture is drought. Drought can be brought on by several different factors such as high temperatures, water deficits, and shading. For soybean, drought is the leading cause for yield reduction (Abdel-Haleem et al., 2012; Devi and Sinclair, 2013; King et al., 2009; Pathan et al., 2014).

Breeding for drought tolerance is complex both genetically and physiologically (Abdel-Haleem et al, 2012; Dhruv et al., 2015; Montes et al., 2011). Drought tolerance also tends to have low heritability (Blum, 2005; Mutava et al., 2015). In the past, breeding for drought tolerance was believed to have negative effects on yield (Blum, 2005; Mutava et al., 2015). Recently, several mechanisms have been found to be associated with drought tolerance (i.e.: dehydration avoidance (Blum, 2005), better ability to fix nitrogen (Sinclair et al., 2007), slow canopy wilting (King et al., 2009), and water use efficiency (Mutava et al., 2005)) that are not necessarily associated with a yield penalty.

Nitrogen fixation is of particular interest to soybean breeders. The symbiotic relationship between soybean and particular rhizobia groups such as, *Bradyrhizobium japonicum,* allow soybean to fix nitrogen (Miransari et al., 2013). Soybeans ability to fix nitrogen is an important process for the plant and is inherently sensitive to soil drying and water stress (Devi and Sinclair, 2013). Nitrogen fixation is vulnerable to drought during soil drying because ureides accumulate in the shoot of the plant (King et al., 2014; Sinclair et al., 2007). Nitrogen fixation occurs when N_2 is converted to NH_3, which is converted into ureides, allanation, and allantotate (Hwang et al., 2013). It is thought that the increase of ureides in the shoot serves as a signal to stop or decrease nitrogen fixation when drought occurs (Hwang et al., 2013). When this happens the risk of nitrogen deficiency increases (Hwang et al., 2013). Hwang et al. (2013) mapped quantitative trait loci for ureide and nitrogen concentration in soybeans. They developed recombinant inbred lines from a cross between ‘KS4895’ and ‘Jackson’. These parents were used because previous research done by King et al. (2005, 2006) and Purcell et al., (2000) showed that Jackson had low concentrations of nitrogen and shoot ureides under stress and KS4895 possessed high concentrations of both under drought stress. Hwang et al., 2013 found five QTLs associated with ureide concentration and four QTLs associated with nitrogen fixation in the mapping population.

To keep up with the growing population there is a need to increase genetic gain in yield. Increased efforts to genotype germplasm for various agronomic traits to improve genetic gain has created a bottleneck to phenotype progeny produced in breeding programs (Furbank and Tester, 2011). Phenotyping continues to be labor and time intensive (Furbank and Tester, 2011). Remote sensing and high-throughput phenotyping may provide a tool to better screen soybean genotypes to yield and drought response and complement the genotyping effort. This research will examine the effectiveness of using NDVI and CT to evaluate soybean performance and traits related to drought stress. Specific objectives include: 1) examining the relationships between NDVI and CT with seed yield, maturity, lodging, and height, 2) determining if the time of day and growth stage have an effect on the spectral readings, and 3) examining the relationships between spectral reflectance and traits associated with drought tolerance.

Materials and Methods

Field evaluations were done on non-irrigated land in Manhattan, KS (39°12'53.01"N 96°35'34.08"W), the plots were planted into Kahola silt loam soil on May 22 in 2013. In McCune, KS (37°23'36.88"N, 95° 3'7.40"W) plots were planted into Parsons Silt Loam soil on June 25. In Pittsburg, KS (37°20'28.30"N, 94°35'41.28"W) plots were planted into Cherokee silt loam soil on June 20. In Salina (38°40'43.03"N, 97°36'35.66"W), plots were planted into New Cambria silty clay soil on May 20. The 90 genotypes evaluated come from the soybean mapping population AR93705. This population is derived from a cross between KS4895 x Jackson. This population was chosen as a part of a cooperative effort with the University of Arkansas to phenotype nitrogen fixation traits linked to drought tolerance. The genotypes in this population have been shown to differ under drought stress conditions in yield and several nitrogen fixation traits (Hwang et al., 2015). Genotypes were planted in a randomized complete block design with three replications. Plots were planted with an Almaco planter 4 m long and 76 cm apart.

Spectral readings were taken using a Crop Circle AES (Holland Scientific Inc., Lincoln, Nebraska). The filters used were in the 680 nm, 715 nm, and 800 nm waveband regions. These waveband regions were used to calculate two normalized differential vegetative indices. The correlation between the normalized differential index (NDVI) 1((715nm - 800nm) / (715nm + 800nm)) and NDVI 2 ((680nm - 800nm)/(680nm + 800nm)) was extremely high ($r^2 = 0.98$) so results only NDVI 1 will be reported here and referred to only as NDVI. Data was taken in both the morning and afternoon between 0900 and 1500 hours. In 2013 it took roughly 60 minutes to complete all 270 plots due to the height and lodging of the plants. In 2014 it took roughly 20-30 minutes to complete readings per location. There was a total of 40 readings taken between all locations. Manhattan had readings taken between July 18 and September 6 with a total of 11 readings were taken. Salina had readings taken between July 18 and September 7 with a total of 11 readings taken. McCune had readings taken between August 20 and September 28 with a total of 9 readings taken. Pittsburg had readings taken August 20 and September 28 with a total of 9 readings taken. The readings were given a specific code that included the year, time of day, and location. The data was collected during both the vegetative and reproductive stages. Data was collected by walking over each plot. The Crop Circle data logger indicated the plot number being read. The sensor was held over one of the two middle rows of each plot. Each plot was monitored for about 3-5 seconds each. This produced about 25-50 data points for each plot. The Crop Circle was held about 76 cm inches above the canopy in 2014. Because of the tall plants in 2013, the sensor was held about 25 to 35 cm above the canopy.

Canopy temperature (CT) was taken immediately following or at the same time as the spectral data was collected to ensure a small temperature range. In 2013, CT was taken using a FLIR thermal infrared imaging camera. Images were collected by pointing the camera at the foliage of the second or third rows avoiding soil. The temperature were read in degrees Celsius. In 2014, CT was taken using an Ocean Optics Infrared Radiation Temperature (IRT) sensor. The temperature was taken by walking over each individual plot. The sensor was equipped with a button activation and light to indicate when a reading being taken. When the light was on the sensor was moved over the second row of the plot until the light turned off. It took the sensor 3-5 seconds to complete a reading and calculate an average temperature for the plot.

The nitrogen fixation trait data was collected on the mapping population AR93075 (Hwang et al., 2015) by Hwang et al. (2013) and Hwang et al. (2014). Hwang et al. (2013) focused on analyzing the nitrogen and shoot ureide concentration in the mapping population progeny. They planted the population in Kreiser, AR in 2009, Fayetteville, AR in 2005, 2007, and 2011. They collected biomass from the shoots of three to five plants during the R4 to R5 stages for the irrigated populations and the R2 stage for the drought population. They dried and weighed the samples after being ground. The ureide were extracted from the ground material. Hwang et al. (2014) collected data on the nodule size, weight, and number of the soybean plants. These evaluations were conducted in 2000, 2007 and 2011 in Fayetteville, AR under irrigated conditions. They collected the data by using intact roots form three plants between the V7 and V9 stages of growth. The roots and nodules were stored at 5 degrees C until they were washed. The nodules were taken off of the plants by hand dried and weighed. The individual nodule weight was derived from using the total number of nodules and the total weight of all of the nodules.

In Kansas, all genotypes had plant height, lodging, and maturity collected on the center two rows of each plot. Plant height was a measure of the average height in centimeters from the soil surface to the top of the main stem. Lodging scores were visually rated on a 1 to 5 scale: 1 represented all plants erect and 5 represented all plants prostrate. Maturity was taken as the date when 95% of the pods reached mature color. The center two rows were harvested with a plot combine. Seed yield was recorded as kg ha^{-1}, adjusted to 13% moisture.

Data was analyzed using SAS 9.4. The plot means for NDVI were calculated using PROC GLM. Sub-plot readings under .70 were assumed to be outliers, probably caused by influences of reflectance values from the soil, and were removed from the data set. NDVI entry means were obtained using PROC MIXED. Analyses of variance for NDVI and the agronomic traits were obtained using the MIXED procedure. Bloc was considered random, all other factors fixed. Each reading was coded for location, genotype, replication, plot, day, and time the reading was taken. PROC CORR was used to calculate correlations between the traits evaluated. Entry means were based on the overall mean averaged across all locations, or based on the entry means at individual locations. Entry means obtained from the University of Arkansas data were correlated to the NDVI data collected in the KS environments.

Results

Growing conditions varied between growing seasons and environments (Table 2.1). The season long rainfall at each of the four locations was lower than the 30-yr averages. Manhattan and Salina had lower total precipitation compared with McCune and Pittsburg. Rainfall received at Manhattan and Salina was well below the 30-yr average. The driest periods throughout the study were experienced in July at both Manhattan and Salina, when the total monthly rainfall was less than 20 mm. Air temperatures in 2013 and 2014 at the four locations were similar to the 30-yr averages. July and August tended to be the warmest months. Dew points observed at the locations also were similar to the 30-yr averages. Salina tended to have the lowest dew points compared to the other locations.

Sources of variation for environment, genotype, and genotype by environment were all significant for yield (Table 2.2). Among the four locations, McCune had the highest average yield and Salina the lowest (Table 2.3). The widest range in yield among the entries was observed at Manhattan, with a 1.5, 2.4, 2,9 and 3 fold difference observed between the lowest and highest yielding genotypes at McCune, Pittsburg, Salina and Manhattan, respectively. There was a significant genotype by environment interaction, however, the source of variation was relatively small compared to the genotypic source of variation. When looking at the yield ranks, the relative performance of many genotypes was fairly consistent across locations. For example, genotype 85 ranked in the top 8 across all locations and ranked first at both McCune and Pittsburg. Genotype 86 constantly ranked in the top 10. Genotype 55 ranked in the bottom 10 at each location. Genotype 90 ranked in the bottom 3 across all locations. Genotype 51 ranked in bottom 10% at 3 of 4 locations.

The sources of variation for environment, genotype, and genotype by environment were all significant for maturity (Table 2.2). The difference between entries for maturity ranged from just over one week, to almost three weeks across the four locations (Table 2.3). The largest difference seen between entry maturity dates was observed at Salina with the earliest entry matured Oct. 12 and the latest entry matured on Nov. 6. As with yield, there was a genotype by environment interaction but the relative maturity of the 90 genotypes was fairly consistent across environments.

As with yield and maturity, the sources of variation for environment, genotype, and genotype by environment were all significant for height (Table 2.2). The largest differences in entry means for height were observed at Manhattan and Salina (Table 2.3).This population was segregating for growth habit. Among the 90 entries, 57 were determinates and 48 were indeterminate genotypes and 5 were segregating for growth habit. The indeterminates were consistently taller than the determinant entries, which contributed to the entries remaining fairly similar in rankings for height across the four locations.

All environments saw some lodging among the entries, however lodging was most severe at Manhattan and Salina (Tables 2.2 and 2.3). Most of the entries in Manhattan had a lodging score of 2 or 3. At McCune and Pittsburg the lodging scores of a majority of the entries were assigned a value of 1. Salina had an average lodging score of 2. Entry 90 had the highest lodging score at each location, receiving a lodging score of 4 in Manhattan and Salina, a 3 in Pittsburg and a 2 in McCune.

This population of lines from the cross between KS4895 X Jackson exhibited a substantial amount of genetic variation for all of the agronomic traits evaluated, and while the genotype X environment interactions were significant, they did not overshadow the genetic effects. This represented a good genetic platform to test the informative value of capturing NDVI and CT in these environments.

All 40 plot readings for NDVI and all 22 readings for CT entry means were subjected to an ANOVA (Table 2.4). Sources of variation which were significant for NDVI and CT included environment, reading nested within environment, genotype, and the genotype by environment interaction. The relative performance of the genotypes across the four environments was not consistent for NDVI and CT. The reading by genotype sources of variation was not significant, indicating that the relative performance of the entries did not change as time of day and growth stage changes throughout the evaluation process.

The overall grand means for all locations for NDVI and CT were correlated to the overall grand means of the agronomic data for each location (Table 2.5). Earlier maturing and shorter plants tended to be higher yielding, and later maturing plants tended to be taller, but neither NDVI nor CT were correlated to the agronomic traits based on the overall means.

Because of the significant genotype by environment interactions for NDVI and CT measurements, an evaluation of the trends observed at each location needed to be performed. The overall entry means for all readings for NDVI and CT were correlated to the agronomic data collected in Manhattan (Table 2.6). As NDVI increased, there was a slight tendency for yield to increase ($r = 0.25^{**}$) and lodging to decrease ($r = -0.25^{**}$). CT was not correlated to any agronomic trait. Later maturing genotypes tended to be lower yield ($r = -0.29^{**}$) and shorter plants tended to be higher yielding ($r = -0.44^{**}$) as was observed in the grand means across environments.

The overall entry means for all readings for NDVI and CT collected in McCune were correlated to agronomic data collected in McCune (Table 2.7). NDVI and CT were not correlated to any agronomic traits. As NDVI increased for a genotype, there was a small tendency for CT to decrease (r = -0.23*). Genotypes with more lodging tended to be lower yielding (r = -0.22*) and more lodging tended to be observed in the taller, indeterminate, plants (r = 0.27**).

The overall entry means for all readings for NDVI and CT collected in Pittsburg were correlated to agronomic data collected in Pittsburg (Table 2.8). Once again, NDVI and CT were not correlated to any agronomic trait. Yield was correlated to lodging (r = - 0.24*), maturity was correlated to height (r = 0.26**), and height was correlated to lodging (r = 0.27**).

The overall entry means for all readings for NDVI and CT collected in Salina were correlated to the agronomic data collected in Salina (Table 2.9). Entries with higher NDVI values tended to be later in maturity (r = 0.25**). Higher NDVI values were also associated with lower CT values (r = -0.25**), as observed in McCune. The positive correlations between maturity and height (r = 0.34**), and lodging and height (r = 0.36**) were consistent with trends observed at McCune and Pittsburg.

An additional set of correlations were calculated across locations, using the entry means from each location (Table 2.10). When examining the data in this manner, as NDVI increased, CT (r = 0.59**), yield (r = 0.84**), maturity (r = 0.21**), height (r = 0.58**), and lodging (r = 0.20**) all increased. Higher CTs were associated with higher yields (r = 0.26**), taller plants (r = 0.77**), and more lodging (r = 0.67**). Yield was positively correlated to maturity (r = 0.23**), and height (r = 27**), and negatively correlated with lodging (r = -0.12*). Maturity was correlated to lodging (r = -0.11**). As seen in several of the individual locations, taller plants tended to be more lodging prone (r = 0.62**).

Yield (dryland and irrigated), nodule number (NN), nodule size (NS), shoot nitrogen (SN), shoot ureide (SU), and canopy wilt (CW) obtained by Hwang et al. (2013) had significant environment, genotype, and genotype by environment interactions (Table 2.11). NN, NS, SN, SU, and CW based on data collected in Arkansas were correlated with the overall grand means for NDVI, CT, and yield obtained in Kansas (Table 2.12). At the p = 0.10 level of probability, higher NDVI values were associated with higher yield (r = 0.20), shoot nitrogen (r = 0.20), and nodule number (r = 0.20). As CT values increased SN decreased (r = -0.25**). Higher yields in Kansas were associated with higher SN (r = 0.45**), higher SU (r = 0.37**), and lower NS (r = -0.19) based on measurement in Arkansas.

Discussion

Based on individual location results, higher NDVI values were associated with higher yields at Manhattan, lower lodging at Manhattan, and later maturity at Salina. These associations were consistent with what might be expected when using NDVI to characterize genotypic differences. Higher NDVI values have been associated with biomass and seed yield. It would be feasible that later maturing entries might have higher biomass than earlier maturing entries since they tend to have longer vegetative periods. Later maturing entries would also be expected to retain more active leaf area later in the season. Both of these situations might result in higher NDVI values among the later maturing entries compared with the earlier maturing genotypes. Lodging disrupts leaf orientation in the plant canopy. This disruption could negatively influence the plants ability to capture solar radiation, thus reducing yield and dry matter accumulation which could reduce NDVI values. If lodging was serve enough, it might reduce canopy thickness and leaf orientation enough to increase the likelihood that soil radiation could impact the spectral reflectance captured by a sensor. This also could result in lower NDVI values.

Within locations, entry means for CT were never correlated to entry means for yield, maturity, lodging or plant height. This was not expected. Tanner (1963), showed that CT can be valuable in determining water stress in plants. McKinney et al. (1989) found a strong association between seed yield and CT as well, which indicated that it has the potential to serve as a useful tool for selection.

Unfortunately, the correlations (r) of NDVI and CT entry means with the agronomic and traits were small (0.25 to -0.25). In two of the four environments (McCune and Pittsburg) none of the correlations between NDVI and the agronomic traits were significant. So, between the lack of consistency and the magnitude of the correlations, NDVI and CT values did not provide informative information on the relative performance of genotypes in this study for yield, maturity, lodging, and height.

This could be due to a number of factors. It is possible that the wavebands used to calculate NDVI were not capable of detecting differences among this group of genotypes. Gitleson (2013) suggested that using wavebands in the green or on the red-edge were the most sensitive to crops with moderate to high amounts of biomass such as soybean. Shiratsuchi et al. (2011) found using the NDVI containing wavebands in the red had lower correlations with the final grain yield in corn than other NDVI's they evaluated. We selected the visible wavelengths of 680 nm and 715 nm in the red region to calculate NDVI based on previous research, but perhaps an NDVI based on lower wavelengths in the green region, or higher in the red or red-edge would be more useful to differentiate soybean performance. However, it is interesting to note that NDVI had fairly strong correlations with the agronomic data when using entry location means (Table 2.10). For example, NDVI explained about 60% of the variation in yield among all the genotypes and locations based on the correlation of r = 0.84** between NDVI and yield. So while the NDVI was not informative at a genotypic level, the index did help characterize the performance observed across all four environments. So, a different index might have provided improved results, however, the inability to characterize genotypic differences also may have been related to the methods and frequency that the NDVI readings were collected that was unable to provide an adequate level of precision to consistently differentiate genotypes.

No genotype by reading interaction was observed for NDVI in this study. This was not expected. Amani et al. (1996) saw a significant effect on the genotype and a genotype by time interaction in wheat when collecting NDVI data. Hatfield (1983) also found that growth stage and time of day impacted the overall readings taken with spectral reading are taken in grain sorghum. This difference could be because of different crops examined of the use of different sensors in the experiments.

Correlations of NDVI and CT with NN, NS, SN, SU, and CW were either small or non-significant. This may have been because the data was collected from totally different environments, but it may have been due to the technology and methods used to capture the NDVI and CT data in Kansas that may have not resulted in the level of precision needed to characterize genotypic differences.

Conclusions

This research focused on the effectiveness of using NDVI and CT to evaluate soybean performance and traits related to drought stress. This population of lines from the cross between KS4895 X Jackson exhibited a substantial amount of genetic variation for all of the agronomic traits evaluated. It represented a good genetic platform to test the informative value of capturing NDVI and CT in these environments. Correlations of NDVI and CT entry means with the agronomic traits were small and inconsistent. The lack of consistency and the magnitude of the correlations indicated that NDVI and CT were not effective criteria to differentiate soybean performance. While 40 different NDVI and CT reading were captured for the genotypes across the four environments, no genotype by reading interaction was observed for NDVI or CT in this study. So either the time of day and growth stage were not important in differentiating genotypes, or the level of experimental precision was not adequate to evaluate this source of variation. Differences in NDVI and CT did account for some genetic variation in drought tolerance traits, however, the strength of the associations were small. Stronger associations need to be established to use NDVI or CT to characterize differences in genotypes in a plant breeding program.

Tables

Table 2.1 Summary of weather variables for all locations for 2013 and 2014 and 30-yr averages

Location	Year	Month	Average Air Temperature Average	Dew Point Average	Precipitation Average
			Degrees C	Degrees C	mm
Manhattan	2013	May	17.6	11.6	55
		June	23.7	16.8	245
		July	24.9	17.9	18
		Aug.	24.9	19.6	82
		Sept.	22.7	14.7	52
		Oct.	12.9	6.9	69
		Total	**126.7**	**87.5**	**521**
	30-yr average	May	18.4	11.7	119
		June	23.7	17	146
		July	26.7	19.1	107
		Aug.	25.7	18.4	106
		Sept.	20.6	13.3	89
		Oct.	14.1	6.4	68
		Total	**129.2**	**85.9**	**634**
McCune	2014	May	19.1	12.2	54
		June	23.1	19.2	142
		July	23.4	17.7	35
		Aug.	26.1	18.6	73
		Sept.	20.8	15.5	158
		Oct.	15.6	10.2	213
		Total	**128.1**	**93.4**	**675**
	30-yr average	May	18.7	18.9	162
		June	23.7	18	141
		July	26.3	19.4	102
		Aug.	25.8	18.6	86
		Sept.	20.7	14.2	119
		Oct.	14.2	13.6	86
		Total	**129.4**	**102.7**	**696**

Table 2.1 (continued) Summary of weather variables for all locations for 2013 and 2014 and 30-yr averages

Pittsburg	2014	May	19.3	.	46
		June	23.7	.	253
		July	24.3	.	39
		Aug.	26.7	.	43
		Sept.	21.2	.	132
		Oct.	16.3	.	183
		Total	**131.5**	**.**	**696**
	30-yr average	May	19.1	.	155
		June	24.1	.	159
		July	26.5	.	103
		Aug.	26.1	.	87
		Sept.	21	.	137
		Oct.	14.4	.	93
		Total	**131.2**	**.**	**734**
Salina	2014	May	19.9	10.2	100
		June	24.7	18	208
		July	26.2	16.1	19
		Aug.	27.6	17.3	126
		Sept.	21.2	14.3	105
		Oct.	15.5	8	40
		Total	**135.1**	**83.9**	**598**
	30-yr average	May	20.8	11.6	118
		June	24.6	16.2	101
		July	27.4	17.9	97
		Aug.	26.4	17.5	96
		Sept.	21.2	12.6	69
		Oct.	14.2	6.2	182
		Total	**134.6**	**82**	**661**

Table 2.2. Analyses of variance for agronomic traits.

Source	d.f.	Yield	Maturity	Height	Lodging
Environment (Env)	3	3006.77**	134.05**	1388.40**	548.85**
Genotype (Gen)	89	7.15**	17.18**	14.94**	6.41**
Gen x Env	267	1.79**	3.11**	1.27**	1.36**

* Significant at .05 probability level

** Significant at .01 probability level

†NDVI, Normalized Differential Vegetative Index; CT, Canopy Temperature

Table 2.3. Means, ranges and LSD values for agronomic traits for four environments.

Env†	Mean	LSD $_{(0.05)}$	Range	
	Seed yield (kg ha-1)			
Manhattan	2604	455.95	1476	4549
Salina	1344	287.51	673	1942
McCune	2826	230.87	2200	3362
Pittsburg	1571	224.05	962	2319
	Maturity (date)			
Manhattan	58	3.99	50	65
Salina	58	5.26	42	66
McCune	61	2.03	56	67
Pittsburg	58	2.27	46	66
	Height (cm)			
Manhattan	49	5.06	35	68
Salina	39	4.45	24	55
McCune	36	3.73	56	67
Pittsburg	32	3.04	22	45
	Lodging			
Manhattan	2.4	0.83	1	5
Salina	2.1	0.88	1	4
McCune	1.1	0.45	1	3
Pittsburg	1.2	0.64	1	4

†Environment

Table 2.4 Analyses of variance for NDVI and CT.

Source	d.f. 1	NDVI†	d.f. 2	CT
Environment (Env)	3	1129.22**	3	1798.54**
Reading(ENV)	36	414.06**	18	1966.79**
Genotype (Gen)	89	3.63**	89	1.49**
Gen x Env	179	3.88**	182	1.53**
Reading*Gen(Env)	3204	0.82NS	1603	.92NS

** Significant at .01 probability level

NS, non-significant

†NDVI, Normalized Differential Vegetative Index; CT, Canopy Temperature

Table 2.5. Pearson's correlation coefficients (r) between agronomic and spectral traits based on overall grand means (n=90).

Variable	NDVI†	CT	Yield	Maturity	Height
CT	-0.07				
Yield	0.17	-0.11			
Maturity	0.04	0.06	-0.30**		
Height	-0.17	0.13	-0.37**	0.25**	
Lodging	-0.002	0.09	-0.14	0.06	.33**

**Significant at .01 probability level

Table 2.6. Pearson's correlation coefficients (r) between agronomic and spectral traits for Manhattan (n=90).

Variable	NDVI[†]	CT	Yield	Maturity	Height
CT	0.01				
Yield	0.25**	-0.02			
Maturity	-0.03	-0.13	-0.29**		
Height	-0.14	-0.13	-0.44**	0.14	
Lodging	-0.25**	0.11	-0.08	0.02	-0.06

**Significant at .01 probability level
[†]NDVI, normalized differential vegetative index; CT, canopy temperature

Table 2.7. Pearson's correlation coefficients (r) between agronomic and spectral traits for McCune (n=90).

Variable	NDVI[†]	CT	Yield	Maturity	Height
CT	-0.23*				
Yield	0.04	-0.03			
Maturity	-0.01	-0.06	-0.10		
Height	-0.17	-0.01	0.02	0.15	
Lodging	0.08	-0.06	-0.22*	0.10	0.27**

* Significant at .05 probability level
**Significant at .01 probability level
[†]NDVI, normalized differential vegetative index; CT, canopy temperature

Table 2.8. Pearson's correlation coefficients (r) between agronomic and spectral traits for Pittsburg (n=90).

Variable	NDVI†	CT	Yield	Maturity	Height
CT	0.15				
Yield	-0.02	-0.06			
Maturity	0.04	0.03	0.08		
Height	0.03	0.03	-0.01	0.26**	
Lodging	0.02	-0.08	-0.24*	-0.05	0.27**

* Significant at .05 probability level

**Significant at .01 probability level

†NDVI, normalized differential vegetative index; CT, canopy temperature

Table 2.9 Pearson's correlation coefficients (r) between agronomic and spectral traits for Salina (n=90)

Variable	NDVI†	CT†	Yield	Maturity	Height
CT	-.25**				
Yield	0.07	0.07			
Maturity	0.25**	-0.06	-0.12		
Height	0.15	-0.02	-0.16	0.34**	
Lodging	0.07	0.03	-0.15	0.17	0.36**

* Significant at .05 probability level

**Significant at .01 probability level

†NDVI, normalized differential vegetative index; CT, canopy temperature

Table 2.10. Pearson's correlation coefficients (r) between agronomic and spectral traits across all locations (n=360 for CT and n=359 for NDVI).

Variable	NDVI†	CT	Yield	Maturity	Height
CT	-0.59**				
Yield	-0.84**	0.26**			
Maturity	-0.21**	-0.02	0.23**		
Height	-0.58**	0.76**	0.27**	0.06	
Lodging	-0.20**	0.67**	-0.12*	-0.11*	0.62**

* Significant at .05 probability level

**Significant at .01 probability level

†NDVI, normalized differential vegetative index; CT, canopy temperature

Table 2.11. F-values from analyses of variance for nitrogen fixation traits for soybean grown in Arkansas.

Source	d.f.	DLY	IRY	NN	NS	SN	SU	CW
Environment (Env)	2	687.19**	41.29**	157.31**	0.01 NS	19.16**	113.67**	7.53*
Genotype (Gen)	88	3.54**	2.53**	3.82**	0.5 NS	3.38**	4.44**	8.83**
Gen x Env	157	1.64**	1.65**	2.21**	0.29 NS	1.55**	2.49**	3.58**

* Significant at .05 probability level

** Significant at .01 probability level

§NS, Non-significant

† DLY, Dryland Yield; IRY, Irrigated Yield; NN, Nodule Number; NS, Nodule Size; SN, Shoot Nitrogen; SU, Shoot Ureide; CW, Canopy Wilt

Table 2.12 Pearson's correlation (r) between KS and Arkansas data for data collected in Arkansas and NDVI, CT, and yield collected in KS (n=89)

Variable	NDVI[†]	CT[†]	Yield
SN	0.20§	-0.25**	0.45**
SU	0.06	-0.18	0.37**
NN	0.20§	-0.1	0.15
NS	-0.18	0.06	-0.19*
CW	0.08	-0.05	0.09

§ Significant at .10 probability level

* Significant at .05 probability level

**Significant at .01 probability level

[†]NDVI, normalized differential vegetative index; CT, canopy temperature; SN, Shoot Nitrogen; SU, Shoot Ureide; NN, Nodule Number; NS, Nodule Size

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Chapter 3 - Effects of weather on the ability of spectral reflectance and canopy temperature to characterize genotypic differences in yield

Abstract

Studies characterizing soybean (*Glycine max* (L.) Merr) performance using spectral reflectance indices and canopy temperature have reported inconsistent results in the ability of remote sensing to detect differences in genotype performance. The objective of this research was to evaluate how weather variables impact the ability of vegetative indices and canopy temperature to detect differences among genotypes for relative seed yield**.** Ninety genotypes from the mapping population derived from the cross between KS4895 x Jackson were planted in Manhattan, KS, in 2013 and in McCune, Pittsburg, and Salina, KS, in 2014 in a randomized complete bloc design in four-row, 3.4m long plots spaced 76 cm apart. Seed yield was measured on the center two rows of each plot. Spectral readings (NDVI) and canopy temperature (CT) measurements were taken during reproductive growth. Weather data was collected from the nearest weather station of each environment. Variance components for entry and error for both NDVI and CT for each reading were correlated with weather data variables. Variation in weather was correlated with both the increase and decrease in entry and error components of variance. For example, as wind speeds increased the entry variance was reduced for CT at Manhattan and McCune, while increases in solar radiation were associated with reduced error variances for NDVI at Manhattan and McCune. However, none of the weather variables measured were consistently associated with an increase or decrease in entry or error variance across the four environments.

Introduction

The world demand for food and fuel has increased with global climate change and an increasing population (Deshmukh et al., 2014). Soybean [Glycine max (L.) Merr.] is the most important oil seed crop across the world (Manavalan et al., 2009; Pereyra-Irujo et al., 2012). To meet future demand, yields will have to increase by 55% by 2050 (Deshmukh et al., 2014). This means there needs to be a better and faster way of developing high yielding varieties (Cobb et al, 2013).

A bottleneck has been created in agriculture. Major advances have been made in genotyping, but little advances in phenotyping the genotypes have been made (Furbank and Tester, 2011). Remote sensing has been shown to be a useful tool (Grovender et al., 2009) as an early prediction of production (Vicente-Serrano et al., 2006). The main point of remote sensing is to determine the reflective signal from vegetation. Scientists originally utilized satellites to obtain vegetative indices, but it tended to have low spatial-temporal resolution causing the development of hand held devices with improved spatial temporal resolution (Grovender et al., 2009). As a tool, it is a nondestructive, instant, and quantitative assessment of a crops ability to photosynthesize light (Hatfield and Prueger, 2010; Hoyos-Villegas and Fritschi, 2013). Plants utilize chlorophyll to capture solar energy (Ferri et al., 2004). Different factors affect the reflective signal of the plants such as row spacing, soil, and the agronomic practices (Sridhar and Parihar, 2000). Plant properties that affect the reflection of vegetation include canopy architecture and leaf structure (Gitelson, 2013). Changes to these can be caused by different things such as abiotic stresses.

Canopy temperature has also shown to be a useful tool in predicting yield (Babar et al., 2006). Until recently thermocouple psychometry had been used to collect the canopy temperature (Jackson et al., 1981). However, the use of thermal imaging also has been used as a nondestructive and fast way of collecting canopy temperature (Amani et al., 1996). Pinter et al., (2003) summarized several studies saying that canopy temperature showed an interference with transpiration rates with canopy temperatures were high and a correlation between the plant water status and reductions to yield.

Overall, remote sensing seems to be a step in the right direction for indirect selection in soybean and to help improve the phenotyping process. However, reflectance indices and canopy temperature can be affected by exogenous factors such as cloud cover or other metrological phenomena (Pinter et al., 2003). The Soybean Breeding Project at Kansas State University, has noticed that some days returned better quality spectral and canopy temperature data than others. The quality of the data may be related to the impact of the environmental conditions on the readings. Weather conditions, such as cloud cover, can impact the scattering of the radiation wavelengths (Sridhar and Parihar, 2000). Possibly other weather variables such as relative humidity and wind speed could also affect remote sensing.

Results characterizing soybean performance using spectral reflectance indices and canopy temperature in Chapter 2 showed that the ability of an active sensor to detect differences in genotype performance was inconsistent within readings collected on the same day, or with readings collected across days throughout the growing season. In light of the erratic nature of the information, the objective of this research was to evaluate how weather variables impact the ability of vegetative indices and canopy temperature to detect differences among genotypes for relative seed yield.

Materials and Methods

In 2013, field evaluations were done in Manhattan, Ks (39°12'53.01"N 96°35'34.08"W) into Kahola silt loam soil on May 22. In 2014 field evaluations were done in McCune (37°23'36.88"N, 95° 3'7.40"W) into Parsons silt loam soil on June 25. In Pittsburg (37°20'28.30"N, 94°35'41.28"W) fields evaluations were planted into Cherokee silt loam soil on June 20. In Salina (38°40'43.03"N, 97°36'35.66"W) field evaluations were planted into New Cambria silty clay soil on May 20. All fields were non-irrigated. Ninety genotypes from the soybean mapping population AR93705, derived from a cross between ‘KS4895’ x ‘Jackson’, were planted in a randomized complete block design with three replications at each location. The plots were planted 4 m long and 76 cm apart with an Almaco planter.

Spectral readings were taken using a Crop Circle AES using three filters, two located in the red and one located in the near infrared (NIR). The wavebands used were 680 nm, 715 nm, and 800 nm. These bands were used to calculate two different normalized differential vegetative indices (NDVI). NDVI1 was calculated using the 715 nm range and the 800 nm range ((715nm-800nm)/(715nm+800nm)). NDVI2 was calculated using the 680 nm and the 800 nm range ((680nm-800nm)/(680nm+800nm)). The correlation between the two NDVI values calculated was high, so results of only one NDVI will be presented here. Data was taken in both the morning and the afternoon between 0900 and 1500 hours, across all locations, resulting in 40 total readings. Due to height and lodging of the plants, in 2013 it took roughly 60 minutes to complete the readings on the 270 plots at a location. In 2014, all readings took roughly 20-30 minutes to complete a test. The eleven readings were taken in Manhattan between July 18 and September 6. The nine readings taken in McCune were taken between August 20 and September 28. The nine readings taken in Pittsburg were taken between August 20 and September 28.

Eleven readings were taken in Salina between July 18 and September 7. Each reading was given a specific code to differentiate location, and time of day and plot.

Data collected by the Crop Circle was done by holding the sensor over either the second or third rows of the canopy and at least 30 inches above, with the exception of in 2013 when the plant heights exceeded 65 cm. The readings were taken by clicking a button at the start of each plot, walking with the sensor held over the plot for 2-3 seconds, and clicking the button again to signify the end of the plot. Each plot had roughly 25-50 data points which were arithmetically averaged to produce one reading per plot. Canopy temperature, taken in degrees Celsius, was taken immediately following or at the same time as the spectral readings. In 2013, CT was collected using a FLIR thermal imaging camera which was pointed directly at either the second or third row of each plot avoiding getting soil into the field of view. In 2014, CT was taken using an Ocean Optics Infrared Thermal Temperature (IRT) sensor. An average temperature was given after the sensor was held over the plot for 3-5 seconds.

All genotypes had plant height, lodging, and maturity collected on the center two rows of each plot. Plant height was a measure of the average height in centimeters from the soil surface to the top of the main stem. Lodging scores were visually rated on a 1 to 5 scale: 1 represented all plants erect and 5 represented all plants prostrate. Maturity was taken as the date when 95% of the pods reached mature color. The center two rows were harvested with a plot combine. Seed yield was recorded as kg ha^{-1}, adjusted to 13% moisture.

Weather data was collected from the nearest weather station and recorded by the Kansas Mesonet. The stations collected hourly data. Times were recorded for each reading. Then the spectral and canopy temperature data was matched with the weather data at the nearest time the reading were taken, from the nearest weather station. The weather data collected included: air temperature (AT), relative humidity (RH), dew point (DP), wind speed (WS), soil temperature (ST), solar radiation (SR), and the vapor pressure deficit (VPD). DP was calculated using the AT and RH. Lawrence, M. G. (2005), gave the equation for DP as AT – ((100-RH)/5)).VPD was calculated by using the AT, RH, saturated vapor pressure at dry bulb temperature (e^0), and the partial pressure of water vapor (e). Dry bulb temperature is found by looking up the air temperature (in degrees Celsius) in List, R. J. (1951) to obtain the dry bulb pressure. To obtain e the RH is divided by e^0. To obtain the VPD, e is then subtracted from e^0. The weather variables were chosen based on what was available, and what might have an influence on remotely sensed data.

Data was analyzed using SAS 9.4. Each reading was analyzed separately. The plot readings were averaged to produce an entry mean, for each entry at each location and reading. PROC VARCOMP was used to obtain the variance components for entry and error for both NDVI and CT. Proc Corr was used to calculate correlations between the weather data and the variance components for each of the 40 readings taken across the four environments.

Results and Discussion

There was a range in the weather and variances for entry and error experienced between locations and within locations (Table 3.1). A summary of the weather data in Table 3.2, shows that three of the locations (Manhattan, McCune and Pittsburg) experienced similar maximum temperatures around 34˚C, while Salina had the highest maximum temperature of 36.73˚C for a reading. Manhattan experienced the smallest range in temperatures with a 10.23˚C but had the highest mean average temperature of 30.02˚C. McCune had the overall lowest mean temperature at 25.4˚C as well as the overall lowest minimum temperature at 11.43˚C, but it also saw the biggest range in temperatures with a 23.08˚C difference. Pittsburg also saw a relatively large difference between the minimum temperature and the maximum temperature of 19.78˚C but the overall mean temperature was similar to Salina, ranging around 26˚C. Salina had the overall highest temperature across all locations at 36.73˚C, with a difference in minimum and maximum temperature of 15.98˚C.

RH varied across all locations. The average minimum RH was between 35% and 38% for all locations. The maximum RH was similar for Manhattan, Pittsburg and, Salina at 60%. McCune's RH was at 72% which was higher than the other locations. VPD for Manhattan had a 26 kPa difference between the minimum and maximum temperatures. McCune saw the lowest minimum temperature and the biggest difference between the minimum and maximum temperature of 28.89˚C. Salina also had a large difference between the minimum and maximum temperature of 28.65˚C. The max DP was fairly consistent across Manhattan, McCune, and Pittsburg, ranging around 14˚C. Salina had a higher DP than the other than the other locations at 16.81˚C. McCune and Pittsburg both had negative minimum DP's, which means that the temperature was low enough that for there to be any moisture it would have to be in frozen form.

Soil temperature had the biggest range in Manhattan at 57.16°C. The max being 80.13°C and the min being at 22.97°C. Both of these temperatures were higher than the other three locations, which all had minimum temperatures between 17°C and 18°C and maximum temperatures between 25°C and 30°C. SR varied across locations but the overall mean for all the locations was 702.83 w ms^{-1}. Manhattan, Pittsburg, and Salina all had similar minimum and maximum SR values. McCune experienced the lowest minimum and maximum SR readings at 384.63 w ms^{-1} and 780.63 w ms^{-1}. In examining the relationships between the variance components for the sources of variation of error and entry, each environment had 9 entries for each location, due to missing weather data in two locations, the observations from which to base a correlation. .. With this limitation, the size of the correlation coefficient (r) needed to be fairly large to be significantly different from zero. SR and ST were the only weather variables that was significantly correlated with the components of variance for NDVI (Table 3.4). As SR increased, the error variance decreased (r = -0.72**) and as ST increased the entry variance decreased (r = -0.65) in Manhattan. In McCune, SR decreased as the error variance decreased (r = -0.70*), while the entry variance decreased at Salina (r = -0.69**). A bigger difference seen between the minimum and the maximum amount of solar radiation seen in Manhattan, McCune, and Salina compared to Pittsburg. Other variables also saw major ranges between the minimum and the maximum, but did not affect the NDVI.

Several weather variables were significantly correlated with the components of variance for CT (Table 3.5). As RH increased at Pittsburg, entry variance decreased (r = -0.96*). As WS increased, entry variance decreased at Manhattan (r = -0.86*) and McCune (r = -0.83*). As ST increased, entry variance decreased at McCune (r = -0.80*).

Manhattan saw the second largest difference between the minimum and maximum for wind speed compared to the other locations, it also saw the largest difference in soil temperatures, and saw the third largest difference between the solar radiation. The wind speed observed in McCune showed to have the biggest differences between the minimum and maximum speeds, and saw the second biggest range in soil temperatures. Pittsburg showed the biggest difference between the minimum and the maximum relative humidity as well. There were also some big correlations seen between the VPD and the variation entry and the variation error and solar radiation, that were not significant at the p-val $<.01$ level, but were significant at the p-val $<.10$ level, indicating that these may affect them, but may not have a major effect. Salina did not see any correlations between the variance components and the weather variables. There were some big correlations observed here for example there was a non-significant correlation between the variation entry and the VPD. Salina saw some variation between all the minimum and maximums of all the weather variables, but were not big enough to notice any changes between entries or increase or decrease the error.

Conclusion

The idea that weather can affect how well vegetative indices and canopy temperature perform when trying to predict yield and other parameters. Based on the above data weather is different between all locations. Each location showed correlations between the weather variables and the variance components. All of the significant correlations were strong correlations. While the correlations between the environments was not consistent, weather is not consistent between environments and different varieties perform differently in each environment it is placed in. The data presented shows that weather can impact the data being taken. Very little research has been done looking at this information, making it difficult to compare the data seen here to other data. This suggests that there is a need to look into how weather parameters affect the spectral readings being collected. A better way of examining the impact of weather on capturing this type of data would be to use infield data loggers to get the precise weather information for that environment, this may show stronger correlations or give a better view on how weather is affecting the spectral readings taken at that time.

Figures and Table

Table 3.1 Environmental analysis of variance statistics and weather variables at each sampling day when NDVI and CT were taken

Environment	Ventry ($x10^{-4}$)	Verror ($x10^{-3}$)	AT† ◦C	RH† %	DP† ◦C	VPD† ◦C	WS† m s-1	ST†	SR†
Manhattan	0	0.3297	30.85	0.53	10.96	21.3	3.5	25.35	653.5
Manhattan	0.1826	0.3042	31.15	0.5	11.25	11.63	3.1	25.45	600
Manhattan	0.3445	0.4183	23.8	0.6	3.92	21.44	1.7	23.5	603.55
Manhattan	0.4665	0.2098	29.1	0.4	9.18	37.63	2.85	24.95	907
Manhattan	0.5565	0.1420	30.05	0.43	10.14	24.35	2.9	25.9	755.15
Manhattan	1.1120	0.0786	27.13	0.5	7.23	18.1	2.57	22.97	819.37
Manhattan	0.3842	0.1620	31.53	0.46	11.62	25.16	3.67	24.5	779.53
Manhattan	0	0.2380	32.57	0.47	12.66	26.06	2.9	25.17	766.13
Manhattan	0.0368	0.1931	34.03	0.38	14.11	33.05	4.13	27.1	800.03
McCune	0	0.2149	30.27	0.59	10.39	17.54	5.43	26.03	702.57
McCune	0	0.1590	32.77	0.51	12.87	24.61	6.07	28.17	780.63
McCune	0	1.5900	26.57	0.72	6.71	9.69	4.53	25.33	384.63
McCune	0.0467	0.3692	34.5	0.38	14.58	33.67	7.1	28.85	727.35
McCune	0	0.1590	11.43	0.65	-8.44	4.78	2.87	17.07	573.13
McCune	0	0.1590	17.2	0.46	-2.71	10.55	2.5	19.3	759.95
McCune	0.0271	0.2863	22.15	0.65	2.28	9.28	1.75	19.6	454.95
McCune	0	0.2681	26.2	0.48	6.3	17.63	3	21.7	674.25
McCune	0.0271	0.2863	27.5	0.5	7.6	18.3	1.25	21.5	667.8

† AT, Air Temperature; RH, Relative Humidity; VPD, Vapor Pressure Defficit; DP, Dew Point; WS, Wind Speed; ST, Soil Temperature; SR, Solar Radiation

Table 3.1 Environmental analysis of variance statistics and weather variables at each sampling day when NDVI and CT were taken

Environment	Ventry ($x10^{-4}$)	Verror ($x10^{-3}$)	AT† ◦C	RH† %	DP† ◦C	VPD† ◦C	WS† m s-1	ST†	SR†
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Manhattan	0.1826	0.3042	31.15	0.5	11.25	11.63	3.1	25.45	600
Manhattan	0.3445	0.4183	23.8	0.6	3.92	21.44	1.7	23.5	603.55
Manhattan	0.4665	0.2098	29.1	0.4	9.18	37.63	2.85	24.95	907
Manhattan	0.5565	0.1420	30.05	0.43	10.14	24.35	2.9	25.9	755.15
Manhattan	1.1120	0.0786	27.13	0.5	7.23	18.1	2.57	22.97	819.37
Manhattan	0.3842	0.1620	31.53	0.46	11.62	25.16	3.67	24.5	779.53
Manhattan	0	0.2380	32.57	0.47	12.66	26.06	2.9	25.17	766.13
Manhattan	0.0368	0.1931	34.03	0.38	14.11	33.05	4.13	27.1	800.03
McCune	0	0.2149	30.27	0.59	10.39	17.54	5.43	26.03	702.57
McCune	0	0.1590	32.77	0.51	12.87	24.61	6.07	28.17	780.63
McCune	0	1.5900	26.57	0.72	6.71	9.69	4.53	25.33	384.63
McCune	0.0467	0.3692	34.5	0.38	14.58	33.67	7.1	28.85	727.35
McCune	0	0.1590	11.43	0.65	-8.44	4.78	2.87	17.07	573.13
McCune	0	0.1590	17.2	0.46	-2.71	10.55	2.5	19.3	759.95
McCune	0.0271	0.2863	22.15	0.65	2.28	9.28	1.75	19.6	454.95
McCune	0	0.2681	26.2	0.48	6.3	17.63	3	21.7	674.25
McCune	0.0271	0.2863	27.5	0.5	7.6	18.3	1.25	21.5	667.8

† AT, Air Temperature; RH, Relative Humidity; VPD, Vapor Pressure Defficit; DP, Dew Point; WS, Wind Speed; ST, Soil Temperature; SR, Solar Radiation

Table 3.2 Shows the overall mean and averages for each location for the weather variables

	AT† ◦C		
Environment	**Mean**	**Min**	**Max**
Manhattan	30.02	23.8	34.03
McCune	25.4	11.43	34.5
Pittsburg	26.61	14.87	34.65
Salina	26.89	20.75	36.73
Overall	27.21	11.43	36.73
	RH† %		
Environment	**Mean**	**Min**	**Max**
Manhattan	0.46	36	0.6
McCune	0.55	38	0.72
Pittsburg	0.49	35	0.59
Salina	0.45	36	0.59
Overall	0.48	35	0.72
	VPD† ◦C		
Environment	**Mean**	**Min**	**Max**
Manhattan	24.3	11.63	37.63
McCune	16.23	4.78	33.67
Pittsburg	19.07	8.12	36.09
Salina	20.57	10.82	39.47
Overall	20.07	4.78	39.47
	DP† ◦C		
Environment	**Mean**	**Min**	**Max**
Manhattan	10.12	3.92	14.11
McCune	5.51	-8.44	14.58
Pittsburg	6.71	-5.03	14.72
Salina	6.98	0.84	16.81
Overall	7.31	-8.44	16.81

Table 3.2 (continued) Shows the overall mean and averages for each location for the weather variables

	WS† m s-1		
Environment	**Mean**	**Min**	**Max**
Manhattan	3.45	1.7	5.83
McCune	3.83	1.25	7.1
Pittsburg	1.9	0.45	3.3
Salina	2.27	1.5	3.35
Overall	2.86	0.45	7.1
	ST† ◦C		
Environment	**Mean**	**Min**	**Max**
Manhattan	34.88	22.97	80.13
McCune	23.06	17.07	28.85
Pittsburg	20.68	17.73	24.6
Salina	24.53	18.7	29.77
Overall	26.18	17.07	29.77
	SR† w ms-1		
Environment	**Mean**	**Min**	**Max**
Manhattan	737.87	600	907
McCune	636.14	384.63	780.63
Pittsburg	685.3	538.8	825.95
Salina	736.71	502.9	962.1
Overall	702.83	384.63	962.1

† AT, Air Temperature; RH, Relative Humidity; VPD, Vapor Pressure Deficit; WS, Wind Speed; ST, Soil Temperature; SR, Solar Radiation

Table 3.4. Pearson's correlation coefficients (r) between the weather variables and the variance components for NDVI at each location.

Weather variable	Manhattan (n=11)		McCune (n=9)		Pittsburg (n=9)		Salina (n=11)	
	VENTRY†	VERROR†	VENTRY	VERROR	VENTRY	VERROR	VENTRY	VERROR
AT†	-0.56	-0.30	0.37	0.14	-0.41	-0.63	0.31	-0.45
RH†	0.13	0.57	-0.42	0.53	-0.46	0.36	0.41	0.15
DP†	-0.56	0.30	0.37	0.14	0.41	-0.63	0.31	-0.45
VPD†	-0.15	-0.23	0.54	-0.19	0.61	-0.64	0.06	-0.40
WS†	-0.33	-0.24	0.09	0.15	0.27	-0.31	0.60	0.27
ST†	-0.07	-0.11	0.23	0.25	0.38	-0.49	0.28	-0.28
SR†	0.32	-0.72**	0.01	-0.70*	0.03	-0.32	-0.69**	0.08

* Significant at .05 probability level

** Significant at .01 probability level

† NDVI, Normalized Differential Vegetative Index; AT, Air Temperature; RH, Relative Humidity; VPD, Vapor Pressure Deficit; WS, Wind Speed; ST, Soil Temperature; SR, Solar Radiation; VENTRY, Entry variance; VERROR, Error variance

Table 3.5 Pearson's correlation coefficient for the weather variables and the variance components for each location for CT

Location	Manhattan (n=4)		McCune (n=6)		Pittsburg (n=4)		Salina (n=7)	
Traits	VENTRY†	VERROR†	VENTRY	VERROR	VENTRY	VERROR	VENTRY	VERROR
AT†	-0.41	0.14	-0.76	-0.08	0.58	0.05	0.65	-0.07
RH†	0.58	0.67	-0.19	-0.26	-0.96*	-0.51	-0.64	-0.03
DP†	-0.41	-0.14	-0.76	-0.08	0.58	0.49	0.65	-0.07
VPD†	-0.50	-0.42	-0.52	0.12	0.91	0.29	0.86	-0.09
WS†	-0.86	-0.49	-0.83*	-0.03	0.66	-0.23	-0.15	-0.82
ST†	-0.41	-0.46	-0.80*	-0.06	0.52	-0.06	0.58	-0.07
SR†	0.03	-0.70	0.22	0.50	0.35	0.82	0.32	-0.04

* Significant at .05 probability level

** Significant at .01 probability level

† NDVI, Normalized Differential Vegetative Index; AT, Air Temperature; RH, Relative Humidity; VPD, Vapor Pressure Deficit; WS, Wind Speed; ST, Soil Temperature; SR, Solar Radiation; VENTRY, Variation Entry; VERROR, Variation Error

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