

USING REMOTE SENSING IN SOYBEAN BREEDING: ESTIMATING SOYBEAN GRAIN
YIELD AND SOYBEAN CYST NEMATODE POPULATIONS

by

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B.S., Ataturk University, 1996

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

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Abstract

Remote sensing technologies might serve as indirect selection tools to improve phenotyping to differentiate genotypes for yield in soybean breeding program as well as the assessment of soybean cyst nematode (SCN), *Heterodera glycines*. The objective of these studies were to: i) investigate potential use of spectral reflectance indices (SRIs) and canopy temperature (CT) as screening tools for soybean grain yield in an elite, segregating population; ii) determine the most appropriate growth stage(s) to measure SRI's for predicting grain yield; and iii) estimate SCN population density among and within soybean cultivars utilizing canopy spectral reflectance and canopy temperature. Experiment 1 was conducted at four environments (three irrigated and one rain-fed) in Manhattan, KS in 2012 and 2013. Each environment evaluated 48 F₄- derived lines. In experiment 2, two SCN resistant cultivars and two susceptible cultivars were grown in three SCN infested field in Northeast KS, in 2012 and 2013. Initial (Pi) and final SCN soil population (Pf) densities were obtained. Analyses of covariance (ANCOVA) revealed that the green normalized vegetation index (GNDVI) was the best predictive index for yield compared to other SRI's and differentiated genotype performance across a range of reproductive growth stages. CT did not differentiate genotypes across environments. In experiment 2, relationships between GNDVI, reflectance at single wavelengths (675 and 810 nm) and CT with Pf were not consistent across cultivars or environments. Sudden death syndrome (SDS) may have confounded the relationships between remote sensing data and Pf. Therefore, it would be difficult to assess SCN populations using remote sensing based on these results.

Abbreviations: SCN, soybean cyst nematode; SRI, spectral reflectance index; CT, canopy temperature; ANCOVA, analysis of covariance. P_i , initial SCN population density; P_f , final SCN population density; GNDVI, green normalized difference vegetation index; SDS, sudden death syndrome.

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Acknowledgements

I would like to thank my major professor, Dr. William Schapaugh for all his excellent guidance, continuous encouragement, support, caring, and patience. Without his tremendous support this research would not have been possible. I would also like to thank my committee members, Dr. Leigh Murray and Timothy Todd for their valuable inputs and time for this project. I am grateful to Dr. Kevin Price and Dr. Vara Prasad for supplying the equipment and using their lab. I am thankful to Tom Oakley and his entire crew at the Nematology Lab. I appreciated Nicholas Bloedow for his work with the project's statistical analysis. I extended my gratitude Russell Dille who put a lot of work on my experiments from planting to harvest and soybean project crew, Brent Christenson, Nathan Keep, Jacob Peterson and Cheyenne Stephens.

My special thanks go to Diloohi Weerosoriya and Jebril Jebril for their support and friendship during this journey. I also highly acknowledge all the agronomy faculties and staffs who were very nice and helpful throughout my study.

Lastly, I am very thankful to my father and my mother for showing me the path of success in life and my loving husband and my wonderful son for their continuous support, tremendous help, encouragement, and sacrifices.

Dedication

To my husband Huseyin and my son Ali for their love and endless support. Without their help support, I would not able to pursue my master study.

Chapter 1 - Literature View

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most of dynamic crops in the world. It has been used for food, animal feed, and industrial use (Hadley and Fehr, 1982). A soybean seed is comprised of 20% oil and 40% protein (Sun et al., 1999; Wood and Sun, 2005). High protein and oil content makes the soybean the primary source of protein for feeding livestock, and the second most widely used vegetable oil, after palm oil in the world (USDA-ERS, 2012; USDA-FAS, 2013). Soybean proteins and oils have been utilized in non-toxic and environmentally friendly industrial products such as adhesives, plastic, binders, paint, ink, solvent, edible coatings, medical capsules, biodegradable resins, and biodiesel (Sun, et al., 1999; Wood and Sun, 2005).

According to the Foreign Agricultural Service, soybean represented 67.4% of the world's protein meal production, 56.7% of the oilseed production, and 26.5% of the vegetable oil production in 2013 (USDA-FAS, 2013). Soybean represented 90% of the United States oilseed production (USDA-ERS, 2012). In the United States, soybean are the second major agricultural crop planted on 30.9 million hectare (ha), producing 89.5 million metric tons (MMT) with the national average yield of 2.92 MMT/ha in 2013 (USDA-NASS, 2013). The United States is the world's largest soybean producer followed by Brazil, Argentina, China, and India (USDA-ERS, 2012). The United States exported 40.7 MMT of soybeans in the 2012/2013 marketing year which was higher than the preceding year (USDA-FAS, 2013). In 2013, Kansas ranked 10 among the states in soybean production with 1440 hectares planted, 3444 MMT of seed produced, with the state yield average of 2.52 MMT/ha in 2013 (USDA-NASS, 2013).

Remote Sensing Applications

Because of the constantly growing world population, agricultural production must increase 70% by 2050 (Bruinsma, 2009). Soybean production alone must increase by 140% to 515 million tons (excluded biofuel feedstock) to meet the demand of the World population in the year 2050 (Bruinsma, 2009). To support agriculture production, high yielding and quality varieties must be released. To support variety development programs precise and efficient phenotyping is needed (Tester and Langridge, 2010).

For many years, crop breeding has relied mostly on empirical selection criteria for improving grain yield (Araus et al., 2002). Crop yield is determined by many physiological processes with low heritability (Aquah, 2010). If optimum conditions do not exist, genetic gain in grain yield may be low, and empirical selection may not be suitable (Jackson et al., 1996). Physiological and morphological traits can be used as alternative selection criteria for genetic improvement and can be measured using remote sensing technologies in breeding programs (Richard, 1982; Reynolds et al., 1999). Canopy reflectance and thermal characteristics such as canopy temperature (CT) can be captured using these technologies which are non-destructive, rapid and efficient for high-throughput phenotyping (Pinter, et al., 2003; Montes et al., 2007). Breeders would be able to improve selection efficiency by identifying superior lines from large segregating populations in early generations.

Canopy Spectral Reflectance

Canopy spectral reflectance provides information about plant growth and development and assessment of abiotic and biotic stresses (Pinter, et al., 2003). Absorption and reflectance characteristics of photosynthetically active radiation (PAR) in the electromagnetic spectrum can be used to estimate many biophysical parameters such as yield, biomass, leaf area index (LAI), nutrients deficiencies and water status (Ma et al., 2001; Raun et al., 2001; Royo et al., 2003; Babar et al., 2006a; Prasad et. al., 2007; Chang-Hua et al., 2010; Gutierrez et al., 2010). In the visible portion of the electromagnetic spectrum (350-700 nm), leaf reflectance is controlled by pigments such as chlorophyll, β -carotene, and xanthophyll located in the palisade parenchyma cells. Chlorophyll, responsible for photosynthesis, occurs in two forms, chlorophyll a and b (Jensen, 2007). These pigments absorb 70-90% of the light in the blue (430-450 nm) and the red region (650-660 nm), however in the green region (554 nm) absorption is less and reflectance is higher (Campbell and Wynne, 2006; Jensen, 2007). The spongy mesophyll layer is responsible for the leaf reflectance in the near-infrared (NIR) portion of the electromagnetic spectrum (700-1300 nm), and this energy is absorbed less (5-10%) and reflected or transmitted by 40-60% (Jensen, 2007). When a plant matures or is under stress its pigmentation characteristic may change. Reflectance may increase in the red and green region due to low chlorophyll content; in contrast, reflectance may decrease in the NIR (Pinter et al., 2003; Carter, 1993).

In the middle-infrared portion (1300 - 2500 nm) of the electromagnetic spectrum, leaf reflectance is controlled by water content in the spongy mesophyll layer (Campbell and Wynne, 2006; Jensen, 2007). In a typical healthy plant, the incident energy in this region will be mostly absorbed by water and reflected less. If a plant's moisture content decreases, reflectance will increase due to strong scattering in the cellular wall in the mesophyll tissue (Jensen, 2007).

Spectral Reflectance Indices

A spectral reflectance index (SRI) is the ratio derived from given wavelengths in the visible and NIR region of the electromagnetic spectrum. SRI's provide information associated with biophysical characteristics of a plant including yield, biomass, chlorophyll content, plant nutrients and water status (Horler et al., 1983; Boochs et al., 1990; Chappelle et al., 1991; Peñuelas et al., 1997a; Ustin et al., 1998; Aparicio et al., 2000; Ma et al., 2001; Raun et al., 2001; Stimson et al., 2005; Babar et al., 2006; Prasad et. al., 2007; Chang-Hua et al., 2010; Gutierrez et al., 2010). SRI's proved to be a valuable tool for estimating grain yield in winter wheat (Pinter et al., 1981; Rudorff and Batista, 1990; Serrano et al., 2000). A SRI improves sensitivity to these biophysical parameters, and normalizes sun angle, soil, and atmospheric effects (Jensen, 2007). Therefore, external signals can be eliminated from the spectral reflectance data. Numerous spectral indices can be derived for specific traits, for example, a simple ratio (SR) or normalized difference vegetation index (NDVI) for biomass and grain yield, a green normalized difference vegetation index (GNDVI) for chlorophyll content, or a normalized water index (NWI) for water status (Prasad et. al., 2007).

Biomass Estimation

Several studies have reported positive relationships between biomass and grain yield in durum wheat, bread wheat, barley, and rice (Turner, 1982; Ramos et al., 1985; Waddington et al., 1987, Reynolds et al., 2005). Collecting samples for measuring biomass is time consuming, laborious for large breeding populations and destructive. Remote sensing techniques have provided rapid, non-destructive alternatives for estimating biomass and early vigor in wheat (Elliot and Regan, 1993; Bellairs et al., 1996). Thenkabail et al. (1994) reported remotely sensed data from Landsat Thematic Mapper explained 71% and 76% variation in wet and dry biomass for soybean, respectively. The model for corn explained 80% and 66% variation in wet and dry

biomass, respectively. NDVI explained 80% (barley), 81% (wheat), 82% (lentil), 79% (cumin), 95% (chickpeas), and 70% (vetch) of the variation in wet biomass for several crops (Thenkabail et al., 2002). Babar et al. (2006) reported that NDVI and SR distinguished among genotypes for variation in wheat biomass at heading and grain fill under irrigation.

Chlorophyll Content

It was possible to estimate crop nitrogen (N) status for precise nitrogen management by using hyperspectral remote sensing (Hansen et al., 2002; Xue et al., 2004), since chlorophyll content in a plant was highly correlated to the nitrogen status of the plant (Chang-Hua et al., 2010). Reflectance at 550 nm (R550) and 675 nm (R675) were used as non-normalized vegetation indices that were sensitive to changes in chlorophyll content (Curran, 1983). However, spectral reflection indices were developed to improve estimation of chlorophyll content, since R550 and R675 were affected by external factors such as sun and viewing angle (Curran, 1983; Jacquemoud and Barret, 1990; Jensen, 2007). Numerous spectral indices have been used to estimate chlorophyll content such as: NDVI, GNDVI, ratio analysis of reflectance spectra (RARS), the red edge amplitude (REA), the red edge positions (REP), and the red edge symmetry (RES) (Horler et al., 1983; Boochs et al., 1990; Chappelle et al. 1991; Aparicio et al., 2000; Raun et al., 2001; Chang-Hua et al., 2010).

Water Status and Plant Stress

Plant water status can be utilized in breeding programs (Munjal and Dhanda, 2005). Important physiological parameters, related to plant water status include leaf water potential, leaf relative water content, and stomatal conductance. All of these traits can be indirectly measured using spectral reflectance (Peñuelas et al., 1997a; Ustin et al., 1998; Stimson et al., 2005; Gutierrez et al., 2010). Carlson et al. (1971) reported that leaf reflectance was significantly

associated with leaf water content in soybean, corn, and sorghum. Suits (1972) observed reflectance changes in the visible and NIR regions of electromagnetic spectrum with changes in wilting in corn. Cure et al. (1989) stated that reflectance was higher in red (620 nm) and lower in NIR (850 nm) for soybean under drought rather than well-watered conditions. Water stress was measured by using SR in sugarcane and barley under drought conditions (Jackson et al., 1980; Kleman and Fagerlund, 1987). Peñuelas et al. (1997b) observed a positive correlation ($r=0.70$) between a water index (WI) and plant water content in seedlings of *Pinus halepensis*, *Quercus ilex*, *Quercus coccifera*, *Arbutus unedo*, *Cistus albidus*, *Cistus monspeliensis*, *Phillyrea angustifolia*, *Pistacia lentiscus* and *Brachypodium retusum*). Royo et al. (2003) indicated that a WI measured at the milk stage of development was a good yield predictor for durum wheat under Mediterranean environments.

Babar et al. (2006a) observed a significant relationship between a NWI and grain yield in spring wheat under irrigated environments. Prasad et al. (2007) studying $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines of winter wheat (*Triticum aestivum* L.) under rain-fed environments observed that a NWI explained a higher proportion of the variability of the grain yield compared to several other SRIs. NWI was significantly associated with leaf water potential ($R^2=0.56$) and canopy temperature ($R^2=0.42$) in wheat germplasm across water stressed environments (Gutierrez et al., 2010).

Biotic Stress

Remotely sensed data has been used to monitor disease and estimate yield loss for many decades (Bauer, 1972; Heald et al., 1972; Henneberry et al., 1979; Nutter et al., 2002; Zhang et al., 2011). Color infrared photography (CIR) with a multispectral scanner has been used for identifying the spread of southern corn leaf blight diseases (Bauer, 1972). CIR has been used for

monitoring cotton root rot (*Phymatotrichum omnivorum*), and yield forecasting for blackroot (*Rhizoctonia soloni*) disease in sugar beets (Heald et al., 1972; Schneider and Safir, 1975; Henneberry et al., 1979). Toler et al. (1981) reported that high resolution aerial photography was useful for differentiating between healthy and infected *Phymatotrechum* root rot cotton plants. Toler et al. (1981) stated that low resolution aerial photography was cheaper than high resolution, however good quality data could not be obtained for small areas in large infested field.

Nilsson, 1985a and 1985b used an Exotech radiometer with multispectral scanner to investigate the association between barley stripe disease and *Sclerotinia* stem rot in barley and oilseed-rape. He observed that spectral reflectance characteristics of oilseed-rape plants changed due to *Sclerotinia* stem rot effect, and observed a significant relationship between yield and spectral reflectance of infested barley. He concluded spectral reflectance can be utilized to predict yield damage (Nilsson1985b). Sharp et al. (1985) conducted experiments to study spectral response of three susceptible wheat cultivars for stripe rust and stem rust. Stripe rust infected wheat cultivars showed different spectral behavior than control plants, even though disease symptoms were not visible. Spectral response did not differentiate stem rust infestation among cultivars.

A green leaf area index (GLAI) has been used to quantify disease intensity or defoliation; however, sampling is destructive and labor intensive in the field (Nutter, 1989). Spectral reflectance can serve as a rapid and non-destructive tool to estimate GLAI. Nutter (1989) quantified peanut foliar fungal disease, *Cercosporidium personatum*, using a multispectral radiometer. He concluded that reflectance at 810 nm was useful for measuring green leaf area index (GLAI) indirectly, and that GLAI was associated with yield.

Nutter et al. (1990) studied different fungicide applications to control late leaf spot, *Cercosporidium personatum*, in peanut (Flonner), and their effect on spectral reflectance. Visual disease assessment was made to characterize the relationship between spectral reflectance and pod yield. Results showed visual disease assessment and spectral reflectance were significantly related with pod yield. Spectral reflectance explained a larger proportion of yield variability (85-90%) compared to visual assessment methods (77-86%).

Nutter et al. (2002) conducted experiments to quantify soybean cyst nematode (SCN) infestations using different remote sensing platforms (aerial, satellite and ground-based). SCN population density was obtained at planting and harvest. Approximately, 48%, 90%, 14%, and 49% of variation in SCN initial population density, soybean yield, soy oil, and soy protein concentration, respectively, were accounted by reflectance at the 810 nm wavelength. A negative linear relationship was observed between spectral reflectance and SCN initial population density. Grain yield was positively associated with spectral reflectance. Approximately, 33%, 84%, 30%, and 46% of variation for SCN initial population density, soybean yield, soy oil, and soy protein concentration, respectively, were accounted by reflectance at 810 nm (aerial image intensity). A negative relationship was observed between reflectance at 810 nm and SCN initial population. Satellite image intensity (500-900 nm) explained 58% of the variation in SCN initial population. As SCN population density increased, reflectance at 810 nm and 750-900 nm decreased. Bravo et al. (2003) used a spectrograph to differentiate healthy and yellow rust (*Puccinia striiformis*) infested plants in wheat. Total reflectance between 400-900 nm was used with a quadratic discriminating model to explain 96% of the variation between diseased and healthy plants. Vigier et al. (2004) reported plant damage caused by *Sclerotinia sclerotiorum* in soybean was associated with reflectance at 675-685 nm band region of the electromagnetic spectrum.

Kulkarni et al. (2008) used aerial images to identify SCN damage in soybean. No significant relationship was reported between NDVI and GNDVI with SCN population density due to lack of visible SCN symptoms with low disease pressure. Zhang et al. (2011) conducted an experiment to predict rice neck blast (*Pyricularia grisea*) using spectral reflectance in greenhouses. The disease severity index was positively associated with spectral reflectance at 685 nm and negatively associated with reflectance at 711 nm. Approximately, 47% and 58% of the variation for disease damaged was accounted by 685 and 711 nm, respectively.

Grain Yield Estimation

Christenson et al. (2013) studied genetic gain in yield utilizing with spectral reflectance in soybean cultivars released from 1923 through 2010. Significant genetic differences were observed among the genotypes for reflectance at 405-695 nm, 705-725 nm, and 735-1305 nm waveband regions. Recent cultivars showed lower reflectance in the visible and higher reflectance in NIR region of the electromagnetic spectrum than older cultivars.

Five field experiments were conducted using 25 durum wheat genotypes in three different growing environments (low, medium, and high yielding) in Spain (Ferrio et al., 2004). Spectral reflectance data were obtained at growth stages anthesis and milk-grain. A partial least squares regression (PLSR) model was developed to determine the grain yield using 400-500 nm, 700 - 750 nm, 800-900 nm, and 950-1000 nm wavelengths. Regression coefficients from the calibrated models showed yield was negatively associated with reflection at wavelengths 700- 750 nm and 950-1000 nm, and positively associated with yield in the 800- 900 nm region. The yield prediction model performed at milk-grain stage and the medium productive environment showed higher genetic variation in yield compared with anthesis at the low or high productive environments. Weber et al. (2012) studied 300 maize test crosses and obtained leaf and canopy

reflectance data to predict yield under different water regimes. PLSR models were developed to predict yield using wavelengths 495-680 nm, 680-780 nm, 900 nm, 970 nm, 1450 nm, 1150-1260 nm, and 1520-1540 nm. Spectral reflectance explained up to 40% variation in grain yield based on one environment and season.

Thenkabail et al. (1994) used Landsat-5 Thematic Mapper for estimating yield for soybean and corn. The models from remotely sensed data explained 32 -35% variations in grain yield in soybean and corn, respectively. Spectral reflectance indices have been used to estimate grain yield of wheat in numerous experiments, and resulted in models that explained 50 to 83% of yield variability using NDVIs and SRIs (Tucker et al., 1980; Aparicio et al., 2000; Serrano et al., 2000; Raun et al., 2001). Many scientists confirmed NDVI showed higher association with grain yield than single spectral measurements in millet, sorghum, and wheat (Bartholome et al., 1988; Rasmussen, 1992; Smith et al., 1995).

Ma et al. (2001) conducted experiments with 42 soybean cultivars released between 1934 and 1992 from maturity groups (MG) 0, MG 00, and MG 000 to estimate yield from remotely sensed spectral data. A hand-held multispectral radiometer (MSR16, CropScan, Rochester, MN) was used to measure canopy reflectance measurements at growth stage R2 through R5 (Fehr and Caviness, 1977) in two different types of soil with three different plant densities (25, 50, and 75 seeds m⁻²). Results indicated soybean grain yield was negatively correlated with reflectance at wavelengths 500 to 650 nm ($r=-0.70$ to 0.90) and positively correlated at wavelengths 700 to 800 nm ($r= 0.50$ to 0.80). Correlation was higher between grain yield and reflectance at R4-R5 growth stage than R2. NDVI explained from 44 to 80% of the variation in yield at R4-R5. Plant density had no effect on the relationship between NDVI and grain yield.

Aparicio et al. (2000) estimated grain yield of durum wheat genotypes by utilizing spectral reflectance indices in an irrigated and non-irrigated environment in Spain. Under a non-irrigated environment, NDVI, SR, and PRI explained 52 %, 59%, and 39 % of the variability in grain yield, respectively. Under an irrigated environment, NDVI, SR, and PRI explained 28%, 39%, and 26% of the variation in grain yield. They concluded that NDVI, SR, and PRI showed a better relationship under the non-irrigated environment, when LAI was less than three.

Royo et al. (2003) studied durum wheat at nine locations in Spain and France, and estimated grain yield from spectral reflectance indices (WI, NDVI, SR, PRI etc.) and single wavelengths (R550, R680). R680, WI and SR performed better compared to the other spectral reflectance indices for estimating grain yield in durum wheat at the milk stage under Mediterranean conditions.

Canopy Temperature

Thermal infrared remote sensing captures thermal infrared radiation in the 3000 to 14000 nm portion of electromagnetic spectrum and this energy can be used to determine the object's temperature (Jensen, 2007). Remotely sensed infrared canopy temperatures have been widely used as a fast, simple, inexpensive and non-destructive selection tool for screening drought resistance genotypes in plant breeding (Reynolds et al., 2009). Several thermal infrared sensor devices have been developed to measure plant canopy temperature such as an infrared thermometer and a handheld infrared camera (Amani et al., 1996; Mutava et al., 2011). CT is being used as selection criteria by the International Maize and Wheat Improvement Center (CIMMYT) wheat breeding program (Reynolds et al., 1994). It has been reported that 60% of the variation among lines for wheat grain yield was accounted by CT under heat and drought stress environments (Trethowan and Reynolds, 2007). Keep (2013) evaluating soybean cultivars

released from 1920 through 2010 observed significant differences between genotypes for CT, and genotype by environment interaction was non-significant for CT across locations. CT was negatively correlated with grain yield ($r=-0.89$ and $r=-0.79$ in maturity group III and IV cultivars, respectively).

Olivares-Villegas et al. (2007) studied wheat recombinant inbred lines from the cross Seri/Babax (genetically diverse for drought resistance) under different water regimes and rain-fed environments in Mexico and Australia. Results showed CT was 75% negatively correlated grain yield, had a 65% heritability, and explained 74% of the variation in grain yield under drought environments. Rashid et al. (1999) reported that CT decreased as grain yield increased in spring wheat under water stressed conditions.

Some infrared thermometers are able to capture both air temperature and canopy temperature in order to calculate canopy temperature depression (TD). TD is the difference between canopy temperature and air temperature. TD was used to discriminate drought resistance among genotypes by Amani et al. (1996). Many studies reported that TD varied significantly among genotypes in millet, soybean, cotton, alfalfa, and wheat under irrigated environments (Singh and Kanemasu, 1983; Harris et al., 1984; Hatfield et al., 1987; Hattendorf et al., 1990; Pinter et al., 1990; Rashid et al., 1999).

Chaudhuri and Kanemasu, (1982) observed a negative relationship between TD and yield for hybrid sorghum. Stark and Pavek, (1987) observed similar findings for potato. Rashid et al. (1999) and Bellundagi et al. (2013) observed a positive correlation between TD and wheat grain yield under moisture stress. Amani et al. (1996) observed TD to be positively correlated with yield at two growing seasons under an irrigated hot climate. Pinter et al. (1990) observed a similar result, also in wheat.

CT is influenced by a number of environmental factors such as wind speed, vapor pressure deficit (VPD), solar radiation, soil moisture, and relative humidity. Variation among genotypes for this trait is best expressed when water stress, VPD and solar radiation are high, and relative humidity is low (Gardner et al., 1992).

TD has been associated with stomatal conductance, VPD, and a drought susceptibility index (McKinney et al., 1989; Pinter et al., 1990; Stark et al., 1991). Rashid et al. (1999) evaluated the relationship between canopy temperature and a drought susceptibility index (DSI) using 12 spring wheat genotypes under irrigated and water-stressed environments for two years. Results showed that the DSI was positively correlated with CT under water-stress conditions in both growing seasons. Blum et al. (1989) also found that DSI was positively correlated with CT among wheat genotypes under drought, with susceptible genotypes showing low yield and warmer canopy temperatures under stress environments. Mutava et al. (2011) studying 300 sorghum genotypes observed some drought resistance genotypes had higher chlorophyll contents and grain yields despite higher canopy temperatures under a water-stressed environment than drought susceptible genotypes.

CT can also be utilized to identify drought resistant genotypes under well-water environments. Singh and Kanemasu (1993) studied yield performance and stability for pearl millet genotypes under irrigated and non-irrigated environments. Drought resistant genotypes showing high yield under a water-stress environment had higher canopy temperatures under an irrigated environment than drought susceptible genotypes. Hatfield et al. (1987) observed that cotton genotypes with higher CTs under irrigated environments had greater biomass under water stress environments than cotton genotypes with lower CTs.

The relationship between TD and VP has been used for screening drought resistance genotypes in a number of studies (Chaudhuri et al., 1986; Stark et al., 1991). Genotypes that had higher CTs were found to be less sensitive to VPD in sorghum, millet (Chaudhuri et al., 1986) and potato (Stark et al., 1991) than genotypes with lower CTs.

Plant canopy temperature has been shown to be associated with plant health. If a plant is infested with a disease, canopy temperature may be elevated due to deterioration of transpiration vessels (Jackson et al., 1986). Pinter et al. (1979) reported *Pythium omnivorum* infected (moderate) cotton plants were 3.3-5.3 °C warmer than healthy plants; however, there was no significant relationship between CT and disease intensity. Smith et al. (1986) stated that stripe rust lesions in wheat caused stomatal closure, therefore, when the disease progressed, CT increased. Eyal et al. (1989) conducted experiments with wheat germplasm to assess variation in response to *Septoria tritici* blotch as a function of CT. The results indicated that CT was significantly correlated with disease coverage and was significantly correlated with green leaf area. At anthesis, CT explained 72% of variation in percent disease coverage.

Tu and Tan (1985) reported that canopy temperature depression increased significantly with increasing root rot severity in bean. Susceptible cultivars had 2.0-2.3 °C higher TD average than resistant cultivars. They concluded that severe root rot reduced water-uptake and transpiration that resulted in an increase in TD.

Soybean Cyst Nematode

Soybean cyst nematode (SCN), *Heterodera glycines*, is an economically important pathogen of soybean throughout the United States. This nematode caused annual losses of 3.25 MMT in the United States from 1996-2009 (Koenning and Wrather, 2010). Estimated yield loss from SCN was 19530 tonnes in 2005 (Wrather and Koenning, 2006). This nematode causes

\$500 million in losses annually in the United States (Hahn, 2014). During 2004-2013, SCN caused yield loss ranged from 0.7%-4.3% (Jardine, 2014). SCN was first observed in Kansas in Doniphan County in 1985. Approximately, 19% of the fields in Kansas are infested with SCN (Jardine and Todd, 2001; Jardine, 2014).

The infective stage of the SCN is the second stage juvenile (J2) which hatches from an egg within the cyst. The J2 migrates in soil and penetrates the vascular tissue of the soybean root, and starts the feeding process. If the J2 becomes male, they move out from the root to fertilize the female without damaging the root. If the J2 becomes female it becomes immobile and grows with only the head inside the root. When the females die, their bodies turn a brown color and become a protective cyst in the soil. A cyst contains an average of 100-200 eggs. The life cycle of SCN is approximately three weeks under optimum conditions with several generations occurring throughout a growing season (Lauritis et al., 1983).

SCN affects the plant root mechanism by interrupting nutrient and water uptake, and it results in yield loss (SCN Management Guide, 2005). In addition to this, it reduces nitrogen-fixation (Jardine and Todd, 2001). It is difficult to see *H. glycines* visible symptoms in the field, especially in a high yielding soybean production environment and at low disease pressure (Jardine and Todd, 2001; Nutter et al., 2002). It may take several years to observe visible symptoms and dramatic yield loss. Usually, yellow and stunting plants occur in circular or oval shapes in severely SCN infested fields. SCN visible symptoms are similar to potassium deficiencies, iron chlorosis, and herbicide injury (Jardine, and Todd, 2001). SCN interacts with other diseases such as *Pythium*, *Rhizoctonia*, *Phytophthora*, *Fusarium* (sudden death syndrome), and *Macrophomina* (charcoal rot pathogen), after J2 create openings in the root surface, the other pathogens have easier access to the plant to reproduce (SCN Management Guide, 2005). Most of

the time SCN and sudden death syndrome (SDS) are observed together in the same field (Roy et al., 1997; Rupe et al., 1999; Brzostowski et al., 2014).

There is no way to eliminate SCN from the field. However, SCN populations and yield loss can be minimized with the use of SCN resistant varieties and non-host crop rotation (SCN Management Guide, 2005). Some SCN population can still reproduce on resistant varieties, but yield loss is not as great as with susceptible varieties (Chen et al., 2001).

SCN is diagnosed visually and quantitatively by field scouting, and soil and plant collecting (Kulkarni et al., 2008). Soil testing is the most accurate way to quantify the SCN population (Jardine, and Todd, 2001). The farmer should monitor their fields to help keep the SCN population low (Jardine, and Todd, 2001). Remote sensing technologies might be an alternative method to soil sampling, and have been utilized to monitor disease, and estimation of yield loss (Nutter et al., 2002).

Limited research has been conducted applying remote sensing technologies to soybean breeding and genetics. If remote sensing technology is to be used to increase the efficiency of the breeding process we must gain a better understanding of the relationships between spectral data and soybean responses. Therefore, the objectives of this study were to: i) investigate the use of spectral reflectance indices and canopy temperature as a screening tool for soybean grain yield in an elite, segregating population; ii) determine the most appropriate growth stage(s) to measure SRI's for predicting grain yield; and iii) estimate SCN population density among and within soybean cultivars utilizing canopy spectral reflectance and canopy temperature.

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Chapter 2 - Using Remote Sensing as an Indirect Selection Tool for Grain Yield in Soybean

Abstract

To support agriculture production in the future, high yielding and quality varieties need to release through plant breeding. To accomplish this, precise and efficient phenotyping is essential. Canopy reflectance and canopy temperature might serve as indirect selection tools to improve phenotyping to differentiate genotypes for yield in soybean breeding programs. The objectives of study were to: i) investigate potential use of spectral reflectance indices (SRI) and canopy temperature (CT) as screening tools for soybean grain yield; and ii) determine the most appropriate growth stage(s) for each SRI for predicting grain yield. This experiment was conducted at multiple environments (three irrigated and one rain-fed) in Manhattan, KS in 2012 and 2013. The experiment included 48 F₄- derived lines from an elite cross of IA3023 by LG04-5187 planted in four-rows plots, 3.4 m long, spaced 0.76 m apart, in a randomized complete block design with 3 or 4 replications. Reflectance at 550 nm (R550), green normalized difference vegetation index (GNDVI), red normalized difference vegetation index (RNDVI), and a derived water normalized index (NWI) were calculated. ANCOVA was used to determine the relationship between yield and one covariate (SRI or CT) for each environment. Yield was predicted for each genotype using the common-slope model. GNDVI exhibited the best relationship with yield compared to the other SRIs and CT. A specific reproductive growth stage was not more informative than another. SRI's can be considered a valid phenotyping tool for ranking and identifying superior breeding lines for yield from segregating populations in the breeding program.

Abbreviations: SRI, spectral reflectance indices; CT, canopy temperature; R550, reflectance at 550 nm; GNDVI, green normalized difference vegetation index; RNDVI, red normalized difference vegetation index; NWI, water normalized index; ANCOVA, analysis of covariance.

Introduction

To meet the food demand for the world population which is estimated to be 9.6 billion by 2050, agricultural production must increase by 70% (Bruinsma, 2009; United Nations, 2013). Soybean (*Glycine max* (L.) Merr.) production alone must increase by 140% to 515 million tons (excluding biofuel feedstock) by the year 2050 (Bruinsma, 2009). For many years, crop breeding has relied mostly on empirical selection criteria for improving the grain yield (Araus et al., 2001). However, crop yield is determined by many physiological processes, has low heritable characteristics and is highly affected by the environment (Aquah, 2010). To support agriculture production, precise and efficient phenotyping is essential in order to release high yielding and quality varieties through plant breeding (Tester and Langridge, 2010). Physiological and morphological traits have been used as alternative selection criteria for genetic improvement in yield and can be characterized using remote sensing technologies (Richards, 1982; Reynolds et al., 1999). Canopy reflectance and thermal characteristics of a plant such as canopy temperature (CT) can be captured using technologies which are non-destructive, and have the potential to serve as high-throughput phenotyping tools in plant breeding programs (Pinter, et al., 2003; Montes et al., 2007).

Canopy spectral reflectance provides information about plant growth and development and assessment of abiotic and biotic stresses (Pinter, et al., 2003). Absorption and reflectance characteristics of photosynthetically active radiation (PAR) in the electromagnetic spectrum can be used to estimate many biophysical parameters such as yield, biomass, leaf area index (LAI), nutrients deficiencies and water status (Ma et al., 2001; Raun et al., 2001; Royo et al., 2003; Babar et al., 2006a; Prasad et. al., 2007; Chang-Hua et al., 2010; Gutierrez et al., 2010). For instance, in the visible portion of the electromagnetic spectrum (350-700 nm) 70-90% of the light is being absorbed (Araus et al., 2001; Jensen, 2007; Campbell and Wynne, 2011).

Reflectance at wavelengths 550 nm (R550) and 675 nm (R675) have been used to estimate chlorophyll content (Curran, 1983; Jacquemoud and Baret, 1990). In the near-infrared (NIR, 700-1300 nm) and in the middle-infrared (1300 - 2500 nm) regions, spongy mesophyll and plant water content are responsible for spectral properties of the leaf, respectively. Five major atmospheric water absorption bands occur at 970, 1190, 1450, 1940 and 2700 nm in these regions of the electromagnetic spectrum (Jensen, 2007). Plant water status can be assessed utilizing water absorption bands (Peñuelas et al., 1997; Babar et al., 2006a; Prasad et al. 2007; Gutierrez et al., 2010).

Christenson et al. (2013) studied genetic gain in yield in soybean cultivars released from 1923 through 2010 utilizing spectral reflectance. Significant differences were observed among the genotypes for reflectance at 405-695 nm, 705-725 nm, and 735-1305 nm waveband regions. Recent cultivars showed lower reflectance in the visible and higher reflectance in the NIR region of the electromagnetic spectrum than older cultivars. Ma et al. (2001) indicated soybean grain yield was negatively correlated with reflectance at wavelengths from 500 to 650 nm ($r=-0.70$ to 0.90) and positively correlated with reflectance from 700 to 800 nm ($r= 0.50$ to 0.80). Weber et al. (2012) reported that spectral reflectance explained up to 40% of the variation in grain yield in maize.

Spectral reflectance indices (SRIs) are the ratios derived from NIR and visible spectral wavelengths (Jensen, 2007). The most commonly used SRI is the normalized difference vegetative index (NDVI) which has been used to estimate biomass (Elliot and Regan, 1993; Bellairs et al., 1996; Thenkabail et al., 2002; Babar et al., 2006a). NDVI has been associated with biomass in crops such as barley ($R^2=0.80$), wheat ($R^2=0.81$), lentil ($R^2=0.82$), cumin ($R^2=0.79$), chickpeas ($R^2=0.95$), and vetch ($R^2=0.70$) (Thenkabail et al., 2002).

Several experiments have used NDVI to predict grain yield in wheat and corn (Aparicio et al., 2000; Serrano et al., 2000; Shanahan et al., 2001; Raun et al., 2001; Royo et al., 2003; Babar et al., 2006b; Prasad et. al., 2007). Ma et al. (2001) reported that $NDVI = (R_{813} - R_{613}) / (R_{813} + R_{613})$ explained 44% - 80% of the variation in yield among soybean genotypes.

Plant water content and stress can be estimated using NIR-based spectral reflectance indices such as a water index ($WI = R_{970} / R_{900}$), and normalized water indices ($NWI-1 = [R_{970} - R_{900}] / [R_{970} + R_{900}]$, $NWI-2 = [R_{970} - R_{850}] / [R_{970} + R_{850}]$, $NWI-3 = [R_{970} - R_{920}] / [R_{970} + R_{920}]$, and $NWI-4 = [R_{970} - R_{880}] / [R_{970} + R_{880}]$) (Peñuelas et al., 1997; Babar et al., 2006a; Prasad et. al. 2007; Gutierrez et al., 2010). NWI-1 and NWI-2 showed a significant relationship with grain yield in spring wheat under irrigated environments (Babar et al., 2006a). Prasad et al. (2007) found NWI-3 and NWI-4 explained a higher proportion of the variability of the grain yield compared to several other spectral reflectance indices in winter wheat under rain-fed environments. NWI-3 was significantly associated with leaf water potential ($R^2 = 0.56$) and canopy temperature ($R^2 = 0.42$) in wheat germplasm across water stressed environments (Gutierrez et al., 2010). Royo et al. (2003) indicated WI measured at the milk stage was a good predictor for yield in durum wheat.

CT is another physiological parameter that has been widely used as a fast and non-destructive screening tool for identifying drought resistant lines in plant breeding programs (Trethowan and Reynolds, 2007). Many studies have demonstrated that CT has the potential to predict yield in sorghum, wheat, potato, soybean and cotton (Harris et. al., 1984; Hatfield et al., 1987; Stark and Pavek, 1987; McKinney et al., 1989; Rashid et al., 1999; Olivares-Villegas et al., 2007; Mutava et. al., 2011; Keep, 2013).

SRI has been utilized as an indirect selection tool for yield in many breeding programs (Ma et al., 2001; Royo et al., 2003; Babar et al., 2006b; Prasad et al., 2007). However, limited studies have attempted to utilize spectral reflectance as an indirect selection tool for screening soybean genotypes in a segregating population. Therefore, the objectives of the study were to: i) investigate the potential use of spectral reflective indices and canopy temperature as screening tools for soybean grain yield in an elite, segregating population; and ii) determine the most appropriate growth stage(s) for collecting SRI and CT data to predict grain yield.

Materials and Methods

Experimental Materials

The experiment was conducted on three irrigated environments and one rain-fed environment at Ashland Bottoms and Manhattan, KS in 2012 and 2013. In Ashland Bottoms, environment 1 (ENV-1, 39° 7'59" N, 96°37'8" W, 314 m above sea level) had a Bismarckgrove fine-silty, superactive, mesic fluventic hapludoll soil type. The soil type of environment 2 (ENV-2, 39° 8'38" N, 96°37'46" W, 314.6 m above sea level) was a Belvue coarse-silty, mixed, superactive, nonacid, mesic Typic Udifluent. The soil type of environment 3 (ENV-3, 39° 8'29" N, 96°37'44" W, 314.6 m above sea level) was a Eudora coarse-silty, mixed, superactive, mesic fluventic hapludoll. Environment 4 (ENV-4, 39°12'57" N, 96°35'29" W, 320.7 m above sea level) was rain-fed in Manhattan, KS in 2013 and the soil type was a Kahola-fine-silty, mixed, superactive, mesic cumulic hapludoll.

Genotypes evaluated in the experiment consisted of 48 F₄ derived lines from the elite cross of IA3023 by LG04-5187 developed by the soybean breeding program at Kansas State University. Genotypes were planted at a seeding rate of 24 seeds per meter using a ALMACO planter (ALMACO, Nevada, IA). Individual plot size was 3.4 m long x 2.28 m wide, consisting of four rows spaced 0.76 m apart. ENV-1 and ENV-2 were randomized complete block designs with three replications, planted on 16 May 2012 and 4 June 2012, and harvested on 15 October 2012 and 27 October 2012, respectively. ENV-3 and ENV-4 were randomized complete block designs with four replications, planted on 15 May 2013 and 22 May 2013, and harvested on 10 October 2013 and 24 October 2013, respectively. Weeds were controlled with post-emergence herbicide applications and manually as needed during the growing season. In the irrigated plots, flood irrigation was used as necessary throughout the growing season.

Sudden Death Syndrome (SDS) Rating

Sudden death syndrome (SDS), *Fusarium virguliforme*, was present in ENV-3. SDS scores were taken at the R6 growth stage, based on a 0 to 5 scale, where; 0=none to trace, 1= trace to 10% of the plants showing symptoms, 2=11-50% plants showing leaf symptoms, 3=leaf symptoms on more than 50% of plant, 4=severe leaf symptoms but less than 50% dead plants, and 5=severe leaf symptoms and more than 50% dead plants.

Spectral Reflectance Measurements

Spectral reflectance measurements were taken with an ASD FieldSpec® 3 portable spectroradiometer (Analytical Spectral Device, Boulder, CO) on cloudless days between 1000h and 1400h close to solar noon. Data were collected at six reproductive growth stages including beginning bloom (R1), full bloom (R2), beginning pod (R3), full pod (R4), beginning seed (R5), and full seed (R6) (Fehr and Caviness, 1997).

The instrument captured spectral reflectance readings from 350-2500 nm wavelengths with a sampling interval of 1.4 nm between 350 and 1050 nm and 2 nm between 1050 and 2500 nm of the electromagnetic spectrum. The fiber optic sensor of the spectroradiometer was placed with a 25° field of view in a nadir position, yielding in a circular view area of approximately 0.5 m diameter. The distance between the sensor and canopy was approximately 1 meter. Canopy spectral reflectance measurements were taken from the middle rows of each plot. The spectroradiometer was calibrated against a white Spectralon® reference panel, (Labsphere, North Sutton, NH) before collecting canopy spectral reflectance data. The calibration measurement was used to convert radiometric readings to percent reflectance values. Calibrations were made every 20 plots or when necessary (dependent on sky conditions). Each radiometric reading per plot was an average of ten scans. Reflectance data were processed using ViewSpec Pro (ASD Inc., Boulder, CO) software, and outliers also identified and eliminated from raw reflectance data.

Spectral data in the 350-400 nm and 1310-2500 nm regions were removed due to significant noise and atmospheric absorption (Thenkabail et al., 2004). Reflectance readings from 400 to 1310 nm were used for data analysis.

Agronomic Traits

Prior to harvest, plant height, lodging, maturity, and grain yield data were collected. Lodging score was based on a 1 to 5 scale where 1=almost all plants erect, 2=all plants slightly leaning or a few plants down, 3=all plants leaning moderately (45%) or 25 to 50% plants down, 4=all plants leaning considerably or 50 to 80% plants down, and 5=almost all plants down. Height was the average length in cm from the soil surface to the top of the main stem of mature plants. Maturity was the date on which 95% of the pods have ripened. Grain yield was determined by mechanically harvesting two inside rows of each plot and recorded as kilogram per hectare (kg ha^{-1}), adjusted to 13% moisture.

Canopy Temperature

Canopy temperature (CT) was measured using an infrared camera (Flir BCAM, FLIR Systems, Willsonville, OR) on each day that spectral data were collected. Canopy temperature was taken from the middle rows of the plot at a distance 1 m from the edge, approximately 50 cm above the canopy. Readings were made between 1000 and 1400 hours on cloudless days. The average CT was obtained from the entire field of the view of the infrared image using QuickReport (QuickReport1.1, FLIR Systems, Willsonville, OR).

Statistical Analyses

All analyses were conducted using SAS/STAT® software version 9.3 with $\alpha=0.05$ (SAS Institute, 2010). For each environment and growth stage, the basic experimental design was a randomized complete block design with a fixed treatment factor of genotype and random blocks. Genotypes were compared for yield and agronomic traits (for each environment), SRI and CT (for each environment and growth stages) as a randomized complete block design, using Proc MIXED.

Genotype by environment analyses were performed for yield, agronomic traits, SRI, and CT using Proc MIXED. SRI and CT were averaged over growth stages. Genotype and environment were considered fixed effects and block within the environment was treated as a random effect. In addition, a genotype by growth stage analysis was conducted for each environment. Genotype and growth stage were considered fixed effects with random blocks. Pearson's correlation coefficients were determined between SRI, CT and maturity based on replications of genotypes for each environment. For each environment, an analysis of covariance (ANCOVA) was performed using Proc MIXED to determine relationships between the response variable yield and one covariate (SRI or CT) with genotype as the treatment factor. Yield was predicted for each genotype using the common-slope model. The ANCOVA analysis estimated individual genotype intercepts and the common slope for each specified SRI or CT covariate. The Pearson's correlation coefficient between measured and ANCOVA- predicted yield was obtained by Proc CORR.

Results and Discussion

Genotypes Performance for Agronomic Traits

The weather patterns experienced at the three environments are shown in Table 2.1. At Ashland Bottoms in 2012, May and July were dry and hot. The growing season (May-October) average temperature was 1.2 °C higher and total precipitation was 227.4 mm less compared to the 30-year average. The 2013 growing season in Ashland Bottoms was wetter and cooler than the 2012 season. The season average temperature was 0.4 °C warmer and total precipitation was 30.9 mm less compared to the 30-year average. At Manhattan in 2013, July and August were dry, with the average temperature being similar and total precipitation 183.7 mm lower than the 30-year average.

The analysis of variance revealed significant genetic differences among entries ($p \leq 0.01$) for grain yield, height, maturity, and lodging within each environment (Table A.1). Average grain yield of the genotypes was lowest (2173 kg ha⁻¹) in ENV-3 and highest (4134 kg ha⁻¹) in ENV-1 (Table 2.2). ENV-3 was severely affected by SDS. A negative correlation ($r = -0.70^{**}$) was found between grain yield and SDS scores in this field (Table A.2).

Genotypes were significantly different ($p \leq 0.05$) in average yield across four environments (Table 2.3). Genetic variability was not large for grain yield, with genotypes differing by approximately 20% from the lowest to highest average yield (Table 2.4).

Genotype and environment main effects were significant ($p \leq 0.05$) for yield, height, lodging, and maturity across four environments (Table 2.3). The genotype by environment interaction was not significant for grain yield and height across four environments; however, the genotype by environment interaction was significant ($p \leq 0.01$) for lodging and maturity date across four environments.

Genotypes Differences for SRIs and CT

Multiple SRI's were calculated using different combinations of wavelengths from visible and the NIR regions of the spectrum. Vegetation-based SRI's such as SR, GNDVI and RNDVI emphasizing photosynthetically active leaf area (Raun et al., 2001, Aparicio et al., 2000; Gitelson et al., 1996) were calculated along with NIR-based SRI's such as WI, NWI-1, NWI-2, NWI-3, and NWI-4 which characterize plant water status (Peñuelas et al., 1993; Babar et al., 2006a, 2006b; Prasad et al., 2007). In addition to evaluating SRI's, single wavelengths were also tested, such as 550 nm (R550) and 675 nm (R675), to determine if distinction between genotypes could be made with individual wavelengths.

Based on the results of the ANCOVA models for each environment, GNDVI, RNDVI, and NWI and R550 (550 nm wavelength) were selected for detailed analyses (Table 2.5). R550, GNDVI, and RNDVI have been used for predicting yield in wheat (Babar et al., 2000b; Prasad et al., 2007). $NWI = (R_{1000} - R_{925}) / (R_{1000} + R_{925})$ is an index generated in this study that differentiated genotypes. Peñuelas et al. (1995), Thenkabail et al. (2000) and Thenkabail et al. (2002) reported that reflectance at 925 and 1000 nm wavelengths represented moisture sensitive areas. NIR-based indices, $WI = R_{970} / R_{900}$, $NWI-1 = (R_{970} - R_{900}) / (R_{970} + R_{900})$, $NWI-2 = (R_{970} - R_{850}) / (R_{970} + R_{850})$, $NWI-3 = (R_{970} - R_{920}) / (R_{970} + R_{920})$, and $NWI-4 = (R_{970} - R_{880}) / (R_{970} + R_{880})$ did not show significant associations with yield in this study, although these indices were good predictors of yield in the studies of Babar et al. (2000b) and Prasad et al. (2007).

CT and spectral data were not taken at all growth stages and for all environments. CT data has not been taken at R2-R6 growth stages in ENV-1 and ENV-2 due to a malfunction in the infrared camera. In ENV-3, spectral data was not collected at the R5-R6 growth stage because of foliar damage from SDS. In ENV-4, spectral data was not able to be taken due to weather conditions (cloud coverage or rain) during the R3-R4 growth stage.

Significant genetic variation ($p \leq 0.01$) was observed for all SRIs at all growth stages in all environments (Table A.3). CT showed significant genotypic variation ($p \leq 0.01$) in all environments (Table A.3). Similar findings were observed by other researchers working with CT in soybean (Harris et al., 1984; Keep, 2013) and sorghum (Mutava et al., 2011).

Analyses of variance showed that genotype and environment main effects were significant ($p \leq 0.01$) for all SRI's across environments (Table 2.6). The genotype by environment interaction was significant ($p \leq 0.01$) for all SRI's except RNDVI. Babar et al. (2006b) reported similar observations with the genotype by year interaction being significant for several SRI's except RNDVI in spring wheat. Prasad et al. (2007) reported significant genotype by year interactions for SRIs (RNDVI, GNDVI, SR, WI, NWI-1, NWI-2, NWI-3, and NWI-4) in wheat.

There were no genotypic differences observed for CT, nor was the genotype by environment significant, but the environment effect was significant (Table 2.6). Keep (2013) reported that genotypes differed in CT and observed a significant genotype by environment interaction in genetically diverse soybean genotypes. In this study, the lack of genotypic differences in canopy temperature may be due to the diverse and variable environmental factors such as wind speed, vapor pressure deficit (VPD), solar radiation, soil moisture, and relative humidity which could have made it difficult to differentiate genotypes (Gardner et al., 1992).

Analysis of variance was performed to investigate the genotype by growth stage interaction for each environment (Table 2.7). Growth stage main effects were significant for SRI's in all environments except GNDVI in ENV-4. In most cases, the genotype by growth stage interaction was not significant for the SRIs. This may indicate that specific growth stages do not need to be considered when collecting canopy reflectance data in soybean. On the other hand, studies in wheat revealed that the growth stage by genotype interaction was significant (Babar et

al., 2006b; Prasad et al., 2007). The most informative growth stages in wheat were grain yield and heading when taking reflectance measurements (Royo et al., 2003; Babar et al., 2006b; Prasad et al., 2007).

Correlations of SRIs and CT with Maturity

Pearson's correlation coefficients were used to characterize the relationships of the SRI's and CT with maturity (Table 2.8). In most environments, SRI's and maturity were not correlated. However, in ENV-4, GNDVI and RNDVI were positively correlated with maturity, $r=0.38^{**}$ and $r=0.37^{**}$, respectively at the R5-R6 growth stage. In this environment, GNDVI and RNDVI tended to increase in the later maturing genotypes. This may be related to the late maturing genotypes experiencing less leaf senescence than earlier genotypes which could have resulted in more green leaf area and higher photosynthetic rates (Babar et. al, 2006b). NWI tended to decrease with the late maturing cultivars ($r=-0.17^*$ to $r=-0.19^*$) in ENV-2. R550 showed both positive and negative correlations with maturity date. CT was negatively correlated with maturity across several environments ($r=-0.16^*$ to $r=-0.52^{**}$). This may be due to the same reason that later genotypes tended to have higher GNDVI and RNDVI values than the earlier genotypes. The more green leaf area and better hydration of the later maturing genotypes may have resulted in increased transpiration and decreased CT (Oliveras-Villegas et al., 2007).

Relationships between SRIs, Canopy Temperature and Grain Yield

ANCOVA was performed to characterize the relationships between the response variable yield and one covariate (SRIs or CT) for each environment (Table 2.9). Yield was predicted for each genotype using the common-slope model. R550 explained 53-61% ($p \leq 0.05$) of the variation in grain yield in ENV-1 and ENV-4 (Figure A.1). RNDVI showed significant positive relationship ($R^2 = 0.40^*$ to 0.62^{**}) with yield at some growth stages in ENV-1, ENV-3, and ENV-4. GNDVI explained 42-62% ($p \leq 0.05$) of the variation in yield across three of four environments (Figure 2.1, Figure A.2). NWI showed significant negative ($R^2 = -0.53^*$ to -0.58^{**}) relationships with grain yield in ENV-1, ENV-3 and ENV-4 (Figure A.3). The pattern of the relationships between SRIs and grain yield was not consistent for each growth stage in all environments. However, GNDVI exhibited most consistent association with yield across three of the four environments compared to the other SRIs and CT. In ENV-2, no significant relationships were observed between SRI's and yield at any growth stage. The excessive moisture in this field resulted in tall plants that lodged severely. Most plants received lodging scores of 4 or 5. These prostrate plants probably impacted the collection of the spectral and canopy temperature data and may have contributed to the highly variable remotely sensed data collect in this environment.

The study of Royo et al. (2003) showed that the relationship between grain yield and SRIs ($R^2 = 0.17$ to 0.65^{**}) in durum wheat was dependent upon the mean yield and growth stage. In their study, R680, WI, and SR were better at identifying superior genotypes than R550, NDVI, a photochemical reflectance index (PRI), and a structural independent pigment index (SIPI). Babar et al. (2006b) reported that NIR-based spectral reflectance indices (NWI-1, NWI-2, and WI) were superior for predicting grain yield in spring wheat compared to PRI, GNDVI, RNDVI, and SR under irrigated conditions. Prasad et al. (2007) observed similar results; NWI-3 and

NWI-4 explained a higher proportion of the variability for grain yield in winter wheat compared with other SRI's (RNDVI, GNDVI, SR, WI, NWI-1, and NWI-2) under Great Plains conditions.

CT explained 55% ($p \leq 0.05$), 40% ($p \leq 0.05$), and 67% ($p \leq 0.01$) of the variation in grain yield in the ANCOVA model in ENV-1, ENV-3 and ENV-4, respectively (Figure 2.2).

Trethowan and Reynolds (2007) reported CT was significantly related ($R^2=0.60^{**}$) to grain yield in wheat under drought stress. CT explained 74% ($p \leq 0.01$) of the variation in grain yield in wheat evaluated under different water regimes in Mexico and Australia (Olivares-Villegas et al., 2007).

Conclusions

Genotypes in an elite population differed in yield, maturity, lodging, height, and SRI's, however, CT did not differ among genotypes across the environments. GNDVI provided the best predictive index, showing a consistent relationship with yield compared to other SRI's in three of four environments. Since spectral reflectance measurements were not able to be taken at certain growth stages in several environments due to weather conditions, or disease, or intense lodging as observed in ENV-2, there was a lack of information to effectively characterize the informative value of GNDVI across growth stages. In this study, a specific reproductive growth stage was not more informative than another. GNDVI differentiated genotype performance across a range of reproductive growth stages from beginning flowering through full seed. Based on these results, SRI's can be considered a valid phenotyping tool for ranking and identifying superior breeding lines for yield from segregating populations in the breeding program.

Figures and Tables

Table 2.1 Monthly mean, maximum, and minimum temperatures (°C) and total rainfall (mm) for the two growing seasons and average temperatures (°C) and total rainfall (mm) over 30 years.

Parameters	May	June	July	August	Sept.	October	Mean/Sum [†]
Ashland Bottoms 2012							
Max. (°C)	29.0	33.0	37.8	32.1	26.6	20.6	29.9
Min. (°C)	13.7	18.0	21.7	15.9	11.3	4.6	14.2
Mean (°C)	21.3	25.5	29.8	24.0	19.0	12.6	22.0
Total rainfall (mm)	34.1	97.8	25.3	127.9	64	13	362.1
Ashland Bottoms 2013							
Max. (°C)	24.1	30.4	31.6	30.9	30.2	20.3	27.9
Min. (°C)	11.0	17.4	18.8	19.0	14.9	5.4	14.4
Mean (°C)	17.5	23.9	25.2	25.0	22.5	12.9	21.2
Total rainfall (mm)	90.2	80.5	110.0	104.2	78.7	95.0	558.6
30 year average temp. (°C)	17.6	23.2	26.1	25.1	19.9	12.9	20.8
30 year average rainfall (mm)	113.8	129.3	100.8	108.7	80.5	56.4	589.5
Manhattan 2013							
Max. (°C)	23.8	30.0	31.1	30.6	29.7	20.2	27.6
Min. (°C)	11.7	17.8	19.2	19.5	15.6	6.7	15.1
Mean (°C)	17.8	23.9	25.1	25.1	22.7	13.4	21.3
Total rainfall (mm)	99.1	88.4	35.8	24.9	104.6	110.0	462.8
30 year average temp. (°C)	18.4	23.7	26.6	25.6	20.4	13.6	21.4
30 year average rainfall (mm)	129.3	144.8	112.3	104.6	87.1	68.3	646.5

[†]Mean temperature and total rainfall of the growing season (from May to October)

Table 2.2 Descriptive statistics of yield (kg ha⁻¹) for all environments.

Environment	Mean	Standard Error	Minimum	Maximum
ENV-3	2173	105.94	1937	2409
ENV-4	3677	105.91	3431	3903
ENV-2	3722	122.29	3450	3995
ENV-1	4134	122.54	3861	4407

Table 2.3 F-values of analysis of variance for yield, height, lodging, and maturity across four environments.

Source of variation	df	Yield	Height	Lodging	Maturity
Genotype (G)	47	1.45*	15.15**	4.7**	7.96**
Environment (E)	3	60.87**	13.09**	19.47**	58.99**
G x E	141	1.2	1.19	1.33*	2.2**

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

Table 2.4 Mean yield (kg ha⁻¹) of genotypes across four environments.

Genotypes	Yield	Genotypes	Yield
K11-1208	3104	K11-1162	3431
K11-1175	3131	K11-1195	3431
K11-1217	3150	K11-1200	3444
K11-1185	3169	K11-1156	3455
K11-1157	3213	K11-1190	3455
K11-1152	3213	K11-1163	3457
K11-1158	3255	K11-1199	3457
K11-1207	3259	K11-1171	3467
K11-1206	3275	K11-1160	3478
K11-1184	3317	K11-1151	3485
K11-1210	3335	K11-1161	3502
K11-1211	3336	K11-1172	3507
K11-1202	3341	K11-1170	3516
K11-1201	3362	K11-1169	3521
K11-1203	3366	K11-1205	3551
K11-1153	3393	K11-1176	3567
K11-1191	3395	K11-1215	3591
K11-1164	3398	K11-1186	3596
K11-1197	3402	K11-1165	3604
K11-1180	3405	K11-1155	3641
K11-1167	3417	K11-1196	3650
K11-1182	3420	K11-1177	3656
K11-1168	3424	K11-1178	3669
K11-1213	3424	K11-1173	3717

Table 2.5 Description of the spectral reflectance indices used in this study.

Name	Index calculation	Function	References
Simple chlorophyll Index (R550)	R_{550}	Chlorophyll content	Jacquemoud and Barret, 1990
Green normalized difference vegetation index (GNDVI)	$(R_{780}-R_{550})/(R_{780}+R_{550})$	Photosynthetic area	Aparicio et al., 2000
Red normalized difference vegetation index (RNDVI)	$(R_{780}-R_{670})/(R_{780}+R_{670})$	Photosynthetic area	Raun et al., 2001
Normalized water index (NWI)	$(R_{1000}-R_{925})/(R_{1000}+R_{925})$	Water status	Newly developed

† R_n = Reflectance at specific wavelength of the electromagnetic spectrum (in nm).

Table 2.6 F-values from analysis of variance for spectral reflectance indices (SRI's) from R1 through R6 growth stages across four environments.

Source of variation	df	R550	GNDVI	RNDVI	NWI	CT
Genotype (G)	47	3.51**	6.22**	5.35**	2.76**	1.14
Environment (E)	3	28.23**	21.72**	48.58**	22.69**	3248**
G x E	141	1.37**	1.34**	1.15	1.58**	0.95

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†R550, reflectance at 550 nm; GNDVI, green normalized difference vegetation index; RNDVI, red normalized vegetation index; NWI, normalized vegetation index; CT, canopy temperature.

Table 2.7 F-values from analysis of variance for spectral reflectance indices combining all growth stages within each environment.

ENV-1 (R1-R2, R3-R4, R5-R6)					
Source of variation	df	R550	GNDVI	RNDVI	NWI
Genotype (G)	47	0.83	1.9**	1.74**	2.15**
Growth stage (GS)	2	188.8**	288.9**	41.4**	266.7**
G x GS	94	0.6	1.02	0.8	1.0
ENV-2 (R1-R2, R3-R4, R5-R6)					
Source of variation	df	R550	GNDVI	RNDVI	NWI
Genotype (G)	47	1.63**	2.14**	1.6**	0.86
Growth stage (GS)	2	148.5**	20.5**	16.2**	18.6**
G x GS	94	1.1	1.09	0.89	0.8
ENV-3 (R1-R2, R3-R4)					
Source of variation	df	R550	GNDVI	RNDVI	NWI
Genotype (G)	47	1.28	1.59*	1.4*	0.87
Growth stage (GS)	1	81.5**	101.16**	5.28*	5.52*
G x GS	47	0.56	1.11	1.05	0.55
ENV-4 (R1-R2, R5-R6)					
Source of variation	df	R550	GNDVI	RNDVI	NWI
Genotype (G)	47	1.82**	2.4**	1.65**	1.14
Growth stage (GS)	1	123.9**	0.02	12.6**	987.1**
G x GS	47	1.33	1.72**	1.04	0.8

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†R550, reflectance at 550 nm; GNDVI, green normalized difference vegetation index; RNDVI, red normalized vegetation index; NWI, normalized vegetation index; CT, canopy temperature.

Table 2.8 Correlation coefficients between SRI and maturity at different growth stages in four environments.

Indices	ENV-1 (n=140)			ENV-2 (n=144)			ENV-3 (n=191)		ENV-4	
	R1-R2	R3-R4	R5-R6	R1-R2	R3-R4	R5-R6	R1-R2	R3-R4	R1-R2 (n=187)	R5-R6 (n=192)
R550	-0.08	0.03	0.08	0.11	0.12	-0.16*	-0.02	0.16*	0.05	0.24**
GNDVI	-0.09	-0.09	-0.08	0.09	-0.09	0.07	0.01	0.00	0.04	0.38**
RNDVI	-0.12	-0.05	-0.1	0.01	-0.06	-0.01	-0.01	0.04	0.15*	0.37**
NWI	0.08	0.13	0.03	-0.19*	-0.03	-0.17*	-0.06	-0.01	-0.06	-0.12
CT	0.09			-0.24**			0.03	-0.16*	-0.24**	-0.52**

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†R550, reflectance at 550 nm; GNDVI, green normalized difference vegetation index; RNDVI, red normalized vegetation index; NWI, normalized vegetation index; CT, canopy temperature.

Table 2.9 Estimate of slope coefficients for spectral reflectance indices (SRI's) and canopy temperature in an analysis of covariance model to predict yield at each growth stage within environments.

Indices	ENV-1			ENV-2			ENV-3		ENV-4	
	R1-R2	R3-R4	R5-R6	R1-R2	R3-R4	R5-R6	R1-R2	R3-R4	R1-R2	R5-R6
R550	6312*	-11004**	-5199.1	4460.8	-3222.2	-7757.8	-5361.6	2007.7	-9846.6*	-10772**
GNDVI	-1679.4	4132.3*	4850.8*	-4218.0	1479.6	346.4	8078.7**	8788.2**	3635.5*	4286**
RNDVI	-1842.2	5396.6	6896.3*	-7727.8	3185.7	-3414.1	8625**	12145*	1993.2	6748.6**
NWI	-20538**	-17710**	1296.8	-33180.0	-3285.6	-5462.1	-25379*	-23922**	-13094*	-5104.1
CT	-105.4**			110.3	-5462.1		9.2	-77.8*	-33.8	-160.2**

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†R550, reflectance at 550 nm, GNDVI, green normalized difference vegetation index; RNDVI, red normalized difference vegetation index; NWI, normalized vegetation index; CT, canopy temperature.

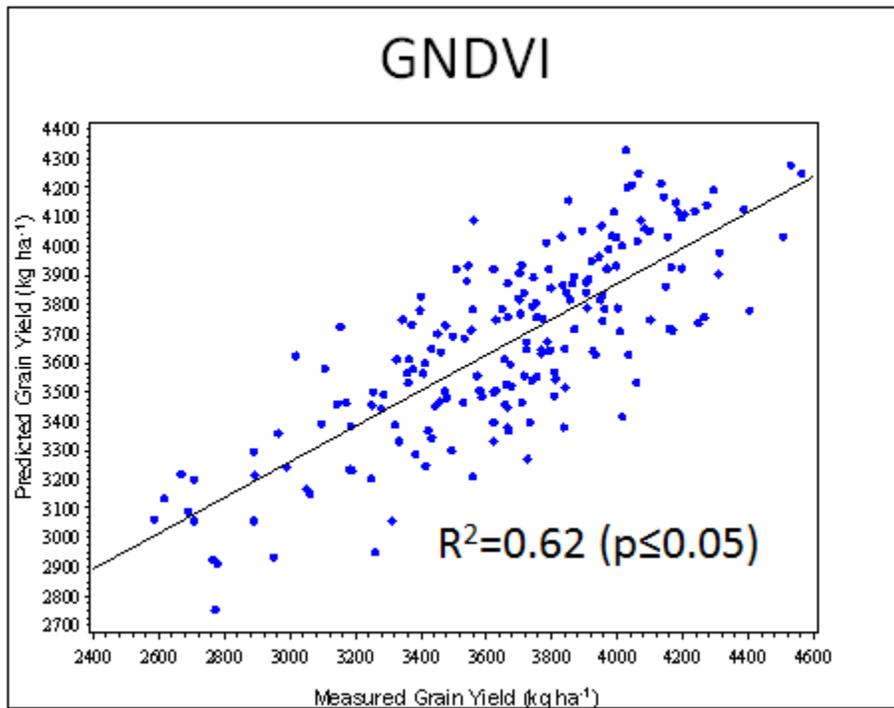


Figure 2.1 Relationship between measured and predicted grain yield based on ANCOVA model using green normalized difference vegetation index (GNDVI) at R5-R6 growth stage in ENV-4.

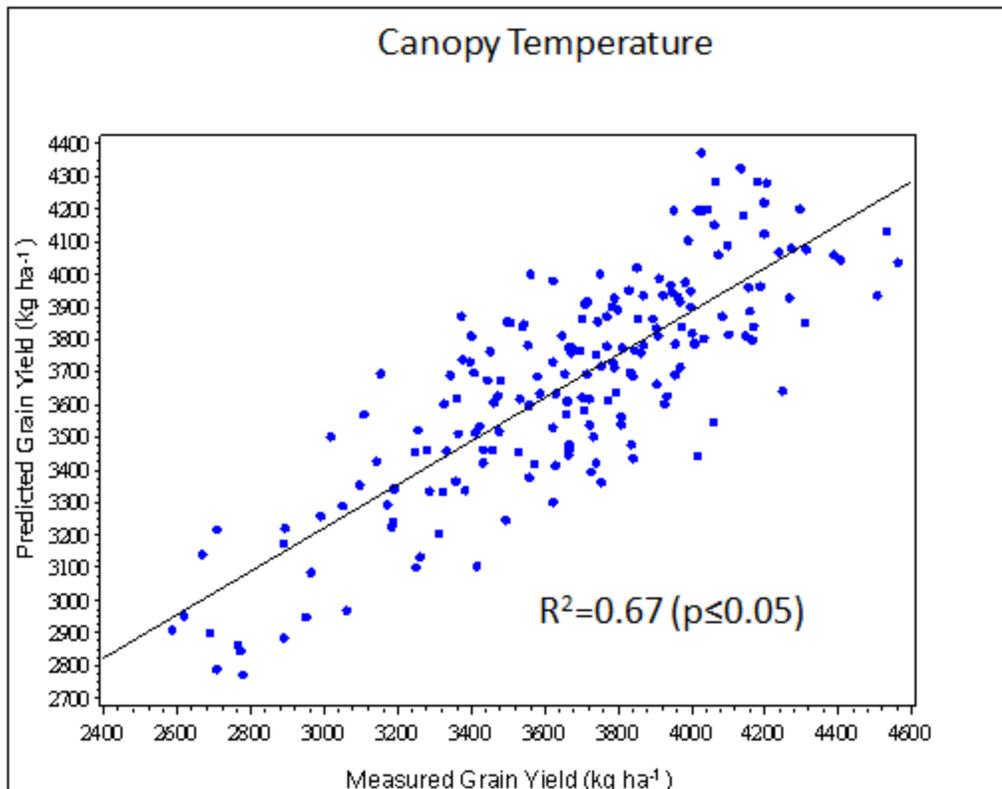


Figure 2.2 Relationship between measured grain yield and predicted grain yield based on ANCOVA model using canopy temperature (CT) in ENV-4.

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Chapter 3 - Using Remote Sensing to Estimate Soybean Cyst Nematode Populations

Abstract

Soybean cyst nematode (SCN), *Heterodera glycines*, is an economically important pathogen of soybean throughout the United States. This nematode caused annual losses of 3.25 MMT in the United States during 1996-2009. Remote sensing technologies may provide a rapid and non-destructive method of identifying and quantifying SCN caused stress at the canopy level. The specific objective of this study was to estimate SCN population density among and within soybean cultivars utilizing canopy spectral reflectance and temperature (CT). The experiment was conducted at three locations in northeast KS in 2012 and 2013. Two SCN resistant cultivars, KS5502N, KS5004N, and two susceptible cultivars 5002T, 5601T were planted in a randomized complete block design with 10 replications. Canopy spectral reflectance and CT were measured from full flowering (R2) through full seed (R6) on sunny days. Initial (P_i) and final SCN population (P_f) densities were obtained on each plot. Relationships between GNDVI, reflectance at single wavelengths (675 and 810 nm) and CT with P_f were not consistent across cultivars or environments. Disease pressure was variable in all environments and cultivars were affected by sudden death syndrome (SDS). SDS likely confounded the relationships between the remotely sensed data and P_f , because of the impact of SDS on the vegetative tissue. Based on these results it would be difficult to assess SCN soil colonization using remote sensing.

Abbreviations: SCN, soybean cyst nematode; R2, full flowering; R6, full seed; CT, canopy temperature; P_i , initial SCN population densities; P_f , final SCN population densities; SDS, sudden death syndrome; GNDVI, green normalized difference vegetation index.

Introduction

Soybean cyst nematode (SCN), *Heterodera glycines*, is an economically important pathogen of soybean throughout the United States. This nematode caused annual losses of 3.25 MMT in the United States during 1996-2009 (Koenning and Wrather, 2010) and \$500 million in yield losses annually in the United States (Hahn, 2014). During 2004-2013, yield loss ranged from 0.7% to 4.3% nationwide. In 2013, 19% of fields in Kansas were estimated to be infested with SCN (Jardine, 2014).

H. glycines is a microscopic worm like organism that penetrates the vascular tissue of the soybean roots, interrupts nutrient and water uptake, that can result in yield loss (SCN Management Guide, 2005). It is difficult to see *H. glycines* visible symptoms in the field, especially in high yielding soybean environments or when disease pressure is low (Jardine, and Todd, 2001; Nutter et al., 2002). Soil testing is the most accurate way to identify SCN. Farmers need to monitor their fields to manage the disease properly (Jardine and Todd, 2001). However, collecting and analyzing soil samples can be laborious and costly for farmers.

In a severely *H. glycines* infested field, yellow and stunting plants may occur in circular or oval shapes (Jardine, and Todd, 2001; Nutter et al., 2002). Ground-, air-, and space-based (satellite) sensors have been used to detect and quantify disease (Pinter, et al., 2003). Aerial images and satellites can be used in large scale, whereas ground-based remote sensing instruments can be useful for research purposes and for small-scale stress assessment (Jackson et al., 1986; Nutter et al., 2002).

In the visible portion of the electromagnetic spectrum (350-700 nm), leaf reflectance is controlled by pigments (Jensen, 2007). These pigments absorb 70-90% of the light in the photosynthetically active (PAR) portion of the spectrum (Campbell and Wynne, 2011; Araus et al., 2001). Wavelengths at 550 nm (R550) and 675 nm (R675) have been used to estimate

chlorophyll content (Curran, 1983; Jacquemoud and Baret, 1990). In the near-infrared (NIR) portion of the electromagnetic spectrum (700-1200 nm), 5-10 % of energy is absorbed and 40-60 % reflected or transmitted (Jensen, 2007).

When plants are under stress, their reflectance characteristics may change. Chlorophyll absorption may decrease and reflection may increase in the visible region of the electromagnetic spectrum (Carter, 1993; Zhang et al., 2011). In contrast, in the near infrared (NIR) region of the electromagnetic spectrum, reflectance may decrease (Pinter et al., 2003; Zhang et al., 2011).

Sharp et al. (1985) differentiated between healthy wheat and wheat infested with stripe rust using spectral reflectance before observing visible symptoms of the disease. Nilsson (1985a) detected *Sclerotinia* stem rot in oilseed-rape plants by using spectral reflectance. Nilsson (1985b) was able to predict yield damage in barley infested with barley stripe disease. Reflectance at wavelengths 706 nm, 760 nm (Dudka et al., 1998), and a narrow band of 675-685 nm (Vigier et al., 2004), were found to be related to *Sclerotinia* stem rot damage in soybean. Zhang et al. (2011) studied rice neck blast (*Pyricularia grisea*) and found that the disease severity was positively associated with spectral reflectance at 685 nm ($R^2=0.47$) and negatively associated with reflectance at 711 nm ($R^2=0.58$). Nutter (1989), Nutter et al. (1990), and Nutter et al. (2002) quantifying fungal disease infestation in peanut and SCN damage in soybean observed associations between reflectance at wavelength 810 nm and disease intensity. Normalized difference vegetation index (NDVI) and green normalized vegetation index (GNDVI) have been used to assess plant stress in various studies (Gitelson et al., 1996; Kulkarni et al., 2008).

Remotely sensed infrared canopy temperature (CT) has been used as a fast, simple, inexpensive and non-destructive tool for assessing plant biotic stress. CT has been used to detect and quantify stress from *Pythium omnivorum* infected cotton plants (Pinter et al., 1979), stripe

rust in wheat (Smith et al., 1986), and root rot severity in bean (Tu and Tan, 1985). CT was found positively associated with disease severity in most cases. Diseases can interfere with root function, which can result in less water uptake and transpiration, resulting in increases in CT.

If SCN population density could be measured with remote sensing, it may be possible to characterize the impact of different varieties on the population. This could benefit both plant breeding programs and cropping systems research. The specific objective of this study was to estimate SCN population density among and within soybean cultivars utilizing canopy spectral reflectance and canopy temperature.

Materials and Methods

Experimental Materials

The experiment was conducted in three SCN infested environments at Rossville, and Ashland Bottoms, Manhattan, Kansas in 2012 and 2013. In 2012, environment 1 (ENV-1) in Rossville, KS (39°6'38.03"N, 95°55'25.91"W, 281 m above sea level) had a Stonehouse, sandy, mixed, mesic typic udifluent soil. In 2013, environment 2 (ENV-2) was conducted at Ashland Bottoms, Manhattan, (39° 8'28.74"N, 96°37'44.29"W, 314.6 m above sea level) on a Eudora coarse-silty, mixed, superactive, mesic fluventic hapludoll soil. Environment 3 (ENV-3) was conducted at Rossville, KS (39° 7'15.67"N, 95°55'29.26"W, 278 m above sea level) in 2013 on a Bismarckgrove fine-silty, superactive, mesic fluventic hapludoll soil. Plots were irrigated with overhead sprinkler (ENV-1 and ENV-3) and flood irrigation (ENV-2) as necessary. The experiment evaluated four determinate, maturity group V (MG-V) cultivars. Susceptible cultivars '5002T' (Pantalone et al., 2004) and '5601T' (Pantalone et al., 2003) were developed by the soybean breeding program at the University of Tennessee, Knoxville. SCN resistant varieties, 'KS5502N' and 'KS5004N', were developed by the soybean breeding program at Kansas State University, Manhattan. KS5502N and KS5004N derived their SCN resistance from 'Hartwig' and 'Peking', respectively. Experiments were planted at a seeding rate of 24 seeds per meter using an ALMACO planter (ALMACO, Nevada, IA). Individual plot size was 3.4 m long x 2.28 m wide, consisting of four rows spaced 0.76 m apart. ENV-1, ENV-2, and ENV-3 were planted on 10 May 2012, 13 May 2013, and 15 May 2013 in a randomized complete block design with ten replications, and harvested on 1 November 2012, 10 November 2013, and 28 October 2013, respectively. Weeds were controlled with herbicides and hand weeding if necessary.

Soil Sample Collection

Soil samples were taken at planting and harvest in the SCN-infested field to determine initial and final egg population densities. A composite soil sample consisting of four cores was taken with a 5-cm diameter soil probe to a 25 cm depth along the middle rows from each plot. The soil samples were stored in a cool room before being processed. One hundred cm³ of soil from each sample was placed in a 3.8 liter jug and filled with water. The soil suspension was poured over a 150-µm-pore sieve with a tea strainer to catch soil particles and debris, and washed into a 50 ml beaker. The sample was washed again into a centrifuge tube and 15 ml of 65% sucrose solution was injected while mixing the solution. The sample was placed in the centrifuge for 30 seconds at 800 rpm and collected on a 150-µm-pore sieve then washed back into 50 ml beaker to view cysts. The sample was placed into a centrifuge tube, and ground for three minutes to release the eggs, which were collected by washing them through 75-µm-pore and 25-µm-pore sieves into the beaker. The sample was filled with 20 ml of water, then eggs and J2 were counted under a microscope at 40x magnification. The nematode population density was expressed as the number of eggs and J2/100 cm³ of soil.

The reproductive factor (Rf) of SCN during the soybean growing season was calculated using the formula: $Rf = Pf/Pi$ where Pf indicates eggs and juveniles per 100 cm³ at harvest and Pi indicates eggs and juveniles per 100 cm³ at planting.

Sudden Death Syndrome (SDS) Rating

Sudden death syndrome (SDS) caused by *Fusarium virguliforme* was present in three environments. SDS scores were taken at the R6 growth stage, based on a 0 to 5 scale, where; 0= none to trace, 1=trace to 10% of the plants showing symptoms, 2=11-50% plants showing leaf symptoms, 3=leaf symptoms on more than 50% of plant, 4=severe leaf symptoms but less than 50% dead plants, and 5=severe leaf symptoms and more than 50% dead plants.

Data Collection for Agronomic Traits at Harvest

Prior to harvest, plant height, lodging, and maturity data were collected. Lodging score was based on a 1 to 5 scale where; 1=almost all plants erect, 2=all plants slightly leaning or a few plants down, 3=all plants leaning moderately (45%) or 25 to 50% plants down, 4 =all plants leaning considerably or 50 to 80% plants down, and 5=almost all plants down. Height was the average length in cm from the soil surface to the top of the main stem of mature plants. Maturity was the date on which 95% of the pods have ripened. Grain yield was determined by mechanically harvesting the two inside rows of each plot and was recorded as kilogram per hectare (kg ha^{-1}) and adjusted to 13% moisture level.

Spectral Reflectance Measurements

Spectral reflectance measurements were taken with an ASD FieldSpec® 3 portable spectroradiometer (Analytical Spectral Device, Boulder, CO) on cloudless days between 1000h and 1400h close to solar noon. The instrument obtained spectral reflectance from 350-2500 nm wavelengths with a sampling interval of 1.4 nm between 350 and 1050 nm and 2 nm between 1050 and 2500 nm of the electromagnetic spectrum. The fiber optic sensor of the spectroradiometer was placed with a 25° field of view in a nadir position, yielding in a circular viewing area of approximately 0.5 m diameter. The distance between the sensor and canopy was approximately 1 meter. Canopy spectral reflectance measurements were taken from the middle rows of each plot. The spectroradiometer was calibrated against a white Spectralon® reference panel, (Labsphere, North Sutton, NH) before collecting canopy spectral reflectance data. Calibration measurement was also used to convert radiometric readings to percent reflectance values. Calibrations were made every 20 plots or when necessary (dependent on sky conditions). Each radiometric reading for per plot was an average of ten scans. Reflectance data were processed using ViewSpec Pro (ASD Inc., Boulder, CO) software, and outliers identified and

eliminated from raw reflectance. Spectral data in the 350-400 nm and 1310-2500 nm regions were removed due to significant noise and atmospheric absorption (Thenkabail et al., 2004). Reflectance readings from 400 to 1310 nm were used for data analysis.

Spectral data were collected at four times during the growing season between beginning bloom (R1) and full seed (R6) (Fehr and Caviness, 1997). Sampling dates for ENV-1 were 25 July 2012, 9 August 2012, 21 August 2012, and 19 September 2012, ENV-2 were 6 August 2013, 18 August 2013, 20 August 2013, and 30 August 2013, ENV-3 were 17 August 2013, 23 August 2013, and 1 September 2013.

Canopy Temperature

Canopy temperature (CT) was measured using an infrared camera (Flir BCAM, FLIR Systems, Willsonville, OR) on each day that spectral data were collected. Canopy temperature was taken from a middle row of each plot at a distance of 1 m from the edge, approximately 50 cm above the canopy. Readings were made between 1000 and 1400 hours on cloudless days. The average CT was obtained from the entire field of the view of the infrared image using QuickReport (QuickReport1.1, FLIR Systems, Willsonville, OR).

Statistical Analysis

All analyses were conducted using SAS/STAT® software version 9.3 with $\alpha=0.05$ (SAS Institute, 2010). Pi, Pf, and Rf were transformed to $\log_{10}(x+1)$ values to reduce heterogeneity of variances.

Cultivar by environment analyses were performed for yield, agronomic traits, Pf, Rf, reflectance at 675 and 810 nm, GNDVI, and CT using Proc MIXED. CT, single wavelengths, and GNDVI were averaged over sampling days. Cultivar and environment were considered as fixed effects and blocks within the environment was treated as a random effect. In addition, a

cultivar by sampling day analysis was conducted for each environment by combining sampling days. Cultivar and sampling day were considered a fixed effect with random blocks. Cultivar means were compared using Tukey's pairwise comparison method for each environment. Pearson's correlation coefficients through Proc CORR were determined between Pf, Rf, CT, GNDVI, SDS, yield, and agronomic traits based on replications of cultivars within each environment. For each environment, an analysis of covariance (ANCOVA) with different slopes for each cultivar was performed using Proc GLM to determine relationships between the response Pf and one covariate (CT, GNDVI, or reflectance at 675 and 810 nm) with cultivars as the treatment factor. To obtain individual R-square values for each cultivar, simple linear regression was performed using Proc REG.

Results and Discussion

Cultivars Performance for Agronomic Traits and SDS

The weather patterns of the environments are shown in Table 3.1. The mean temperature of the growing season (from May to October) at Rossville in 2012 was 0.2 °C higher and total precipitation was 323.3 mm less compared to the 30-year average. The 2013 growing season in Rossville was wetter and cooler than 2012, season average temperature was 0.8 °C lower and total precipitation was 74 mm less compared to the 30-year average. At Ashland in 2013, the season average temperature was 0.4 °C warmer and total precipitation was 30.9 mm less compared to the 30-year average.

Analyses of variance revealed that yield, lodging, and SDS all varied ($p \leq 0.01$) among the cultivars and environments as well as having significant cultivar by environment interactions (Table 3.2). Height differed significantly ($p \leq 0.01$) among cultivars and environments, but the cultivar by environment interaction was not significant (Table 3.2). In ENV-1, maturity date was not taken, since the field died from an early frost and other diseases before plants reached R8. Analysis of variance showed that maturity was significantly different among cultivars, but the relative maturity among cultivars across environments was similar (Table 3.3). Maturity did not differ between both environments.

Cultivar Performance for SCN Population, CT, GNDVI, Reflectance at 810 and 675 nm

Analyses of variance revealed differences in cultivar for Pf, Rf, and CT (Table 3.4). Pi did not vary among the cultivars, but it was significantly different ($p \leq 0.01$) among environments (Table 3.4). Pf and Rf differed ($p \leq 0.01$) among environments, and the cultivar by environment interaction was significant for these traits. CT was significantly different among the cultivars, but not environments, and the cultivar by environment interaction was significant.

Multiple wavelengths from the visible and NIR regions of the spectrum, and spectral reflectance indices such as NDVI and GNDVI were used to estimate SCN population density. Some of these wavelengths and spectral reflectance indices were used in previous disease studies (Vigier et al., 2004; Nutter et al., 2002; Kulkarni et al., 2008). Based on the results of Pearson's correlations for each sampling day between the remotely sensed data and Pf, reflectance at 675 and 810 nm and GNDVI were selected for detailed analyses. Reflectance at 675 nm wavelength has been used for estimation of the chlorophyll content, 810 nm has characterized plant stress, and $GNDVI = (R_{770} - R_{540}) / (R_{770} + R_{540})$ has provided information related to plant growth and health (Nutter, 1989; Nutter et al., 1990, Carter, 1993; Gitelson and Merzlyak, 1996 Nutter et al., 2002; Pinter et al., 2003).

GNDVI and reflectance at 675 nm were significantly ($p \leq 0.01$) different among cultivars and environments, and the cultivar by environments interaction was not significant (Table 3.4). Reflectance at 810 nm was significantly different among cultivars and environments and the cultivar by environment interaction was significant.

Mean Comparison for Agronomic Traits and SDS

Cultivar means for yield, maturity, height, and lodging were compared using Tukey's pairwise comparison method for each environment (Figure 3.1a-d). KS5502N had the highest yield or was not significantly different from the highest yielding entry in two of the three environments. KS5004N had the highest yield among the genotypes in ENV-2. KS5502N was the latest maturing cultivar in the tests. 5002T tended to be the shortest genotype and significantly shorter in height than the other genotypes in ENV-2 and ENV-3. 5601T, KS5004N, and KS5502N were similar in height. Resistant cultivars tended to lodge more in ENV-2 and ENV-3 than susceptible cultivars. 5601T was the most susceptible cultivar to SDS in all

environments (Figure 3.2a). In ENV-1, 5002T, KS5004N, and KS5502N showed similar responses to SDS. KS5004N and KS5502N showed more resistance to SDS compared to the other cultivars in ENV-2; however KS5004N was the second most susceptible cultivar to SDS in ENV-3. The differences among cultivars in SDS response may have been due to variation in disease pressure across environments.

Mean Comparison for Pi, Pf, Rf, CT, GNDVI, Reflectance at 675 and 810 nm

Pi tended to be similar among cultivars across environments, except in ENV-2 where the Pi for 5002T was higher than for 5601T (Figure 3.2b). The mean Pi across cultivars was 303 eggs and J2/100 cm³ soil in ENV-1, 343 eggs and J2/100 cm³ soil in ENV-2, and 2530 egg and J2/100 cm³ soil in ENV-3 (Table B.1). This showed that SCN disease pressure was low in ENV-1 and ENV-2 and relatively high in ENV-3. KS5502N, the genotype which derived its SCN resistance from the cultivar 'Hartwig' tended to have lower Pf and Rf values compared to the other cultivars (Figure 3.2c, Figure 3.2d). Overall, the SCN nematode reproduction on KS5004N was not significantly different than the nematode reproduction on the susceptible cultivars.

The relative differences in CT among genotypes varied across environments. In ENV-1, no differences in CT among entries were noted. In ENV-2 and ENV3, KS5502N exhibited lower CTs than the susceptible cultivars and had the lowest mean CT value across environments, but not consistently lower than KS5004N (Figure 3.3a). Resistant cultivars tended to have higher GNDVI values compared to the susceptible cultivars in ENV-2 and ENV-3 (Figure 3.3b). This may have been the result of the resistant cultivars having more biomass than the susceptible cultivars. Resistant cultivars showed the lowest reflectance at 675 nm in the high disease pressure environment (ENV-3), but they were not significantly different than the susceptible cultivars in the low disease pressure environments (Figure 3.3c). KS5502N and 5002T exhibited

the highest reflectance at 810 nm in the high disease pressure environment (Fig. 3.3d). These two cultivars also had lower SDS ratings than others cultivars in this environment. Less foliar damage from SDS may have contributed to the higher reflectance at 810 nm. Reflectance at 810 nm for KS5004N tended to be the lowest among the cultivars across the three environments.

Cultivars by Sampling Day Interaction

In most cases, analyses of variance showed that there were significant differences for GNDVI, and reflectance at 675 and 810 nm among the cultivars and sampling days (Table 3.5). Genotypic and sampling day variation tended to be greater for reflectance at 810 nm than for reflectance at 675 nm. This may be due to the NIR region of the electromagnetic spectrum being more sensitive to stress than the visible region (Jensen, 2007). Cultivar by sampling day interaction was significant for GNDVI, and 675 nm and 810 nm reflectance values in the high disease pressure environment (ENV-3), but the cultivar by sampling day interaction tended to be a smaller portion of the total phenotypic variation than cultivar.

Correlations between Pf and CT, GNDVI, 675 and 810 nm for Each Sampling Day

Pearson's correlations were calculated based on the ten replications of data for the four cultivars between Pf and CT, GNDVI, and reflectance at wavelengths at 675 and 810 nm for each sampling date and environment (Table 3.6). GNDVI was negatively correlated with Pf in ENV-1 ($r=-0.38^*$), ENV-2 ($r=-0.49^{**}$), and ENV-3 ($r=-0.51^{**}$), on late sampling dates 19 September 2012, 30 August 2013 and 23 August 2013, respectively. When disease pressure increases, reflectance could be increased in the green region and reduced in the NIR region, resulting in a lower GNDVI ratio (Carter, 1993; Prasad et al., 2007; Zhang et al., 2011). Reflectance at 675 nm was positively correlated ($r=0.43^*$) with Pf in ENV-3 (23 August 2013). A possible explanation for this observation could be that the SCN colonization could have

resulted in a reduction in chlorophyll content, a decline in chlorophyll absorption at this wavelength and an increase in reflectance. Zhang et al. (2011) also reported a positive relationship between reflectance in the visible region (685 nm) and neck blast (*Pyricularia grisea*) disease. Reflectance at the 810 nm wavelength in the NIR region was negatively correlated with Pf ($r=-0.34^*$ to $r=-0.36^*$) on the latest sampling days in each environment. On a few sampling dates, canopy temperature showed a positive correlation with Pf, with correlations up to $r=0.47^{**}$. This could be due to SCN limiting water uptake and reducing transpiration. This reduction in transpiration could result in less canopy cooling and greater canopy temperatures. Eyal et al. (1989) also found that CT was positively correlated with disease coverage in wheat germplasm. Pinter et al. (1979) reported *Pythium omnivorum* infected cotton plant's average temperature were 3.3-5.3 °C warmer than healthy plants; even though there was no significant relationship observed between CT and disease intensity.

Correlations Among Pf, Rf, CT, GNDVI, Reflectance at 675 and 810 nm, Yield, and Agronomic Traits

Pearson's correlation coefficients were calculated among Pf, Rf, CT, GNDVI, reflectance at 675 and 810 nm, yield, height, maturity, lodging, and SDS for ENV-1, ENV-2, ENV-3 on 19 September, 30 August 2013, and 23 August 2013, respectively (Table 3.7). These sampling days were considered the best reflectance signature of the cultivars because of increased disease pressure (Table 3.6). There were negative correlations observed between Pf and yield in ENV-1 ($r=-0.65^{**}$), ENV-2 ($r=-0.33^*$), and ENV-3 ($r=-0.58^{**}$). Yield was negatively correlated with Rf in ENV-1 ($r=-0.57^{**}$), ENV-2 ($r=-0.32^*$) and ENV-3 ($r=-0.35^*$). CT was negatively correlated with yield in ENV-1 ($r=-0.29$), ENV-2 ($r=-0.49^{**}$), and ENV-3 ($r=-0.74^{**}$). For example, KS5502, which tended to have the lowest canopy temperature, tended to have the highest yield among the genotypes (Figure 3.1a, Figure 3.3a). Similar results have been observed in wheat and

soybean (Rashid et al. 1999; Olivares-Villegas et al., 2007; Keep, 2013; Mutava et al., 2010).

Yield was positively correlated with GNDVI in ENV-1 ($r=0.34^*$), ENV-2 ($r=0.52^{**}$), and ENV-3 ($r=0.64^{**}$). Similar results were reported for winter and spring wheat (Babar et al., 2006; Prasad et al., 2007).

Yield was negatively correlated with reflectance at 675 nm in ENV-1 ($r=-0.26$), ENV-2 ($r=-0.36^*$) and ENV-3 ($r=-0.32^*$). Lower reflectance at 675 nm may have been the result of higher chlorophyll content. Yield only showed a significant positive correlation ($r=0.33^*$) with reflectance at 810 nm in ENV-3 which had the highest SCN population density.

Maturity date was negatively correlated with Pf in ENV-2 ($r=-0.70^{**}$) and ENV-3 ($r=-0.69^{**}$). Maturity date was positively correlated with yield ($r=0.46^{**}$) in the high disease pressure environment. Positive correlations also were observed between maturity date and GNDVI in ENV-2 ($r=0.40^{**}$) and ENV-3 ($r=0.43^{**}$). The fact that KS5502N was the most SCN resistant and latest-maturing cultivar that maintained more green leaf area later in the season than the other genotypes could have contributed to these relationships between maturity, Pf, yield and GNDVI. There were correlations observed between maturity and CT, reflectance at 675 and 810 nm in ENV-1 and ENV-2. Maturity date was negatively correlated with CT ($r=-0.35^*$) and reflectance at 675 nm ($r=-0.41^{**}$) in the high disease pressure environment. This relationship may also be related to the late maturity of KS5502N which could have resulted in this genotype having better hydration status, more green leaf area and higher photosynthetic activity than the other genotypes at this stage of the growing season. There were no correlations observed between maturity and reflectance at 810 nm in ENV-2 and ENV-3.

Correlation Between SDS and Yield, CT, GNDVI, and Reflectance at 675 and 810 nm

Visual symptoms of SDS were observed in all three environments (Figure 3.2a). SDS rating were positively correlated with Pf in low disease pressure environment (ENV-2, $r=0.43^{**}$) and high disease pressure environment (ENV-3, $r=0.46^{**}$), however no correlation existed between SDS and Pf in ENV-1 (Table 3.7). Previous studies have found a positive correlation between SDS and SCN (Roy et al., 1989; Scherm et al., 1998; Rupe et al., 1993). This soilborne fungus, *F. virguliforme*, has the ability to survive within SCN cysts overwinter and the fungus can enter the opening of root surface created by J2s (Roy et al., 1989; McLean and Lawrence, 1993; Rupe et al., 1993; Scherm et al., 1998).

Yield appeared to be highly affected by SDS. Negative correlations were observed between yield and SDS in the low disease pressure environment (ENV-2, $r=-0.59^{**}$) and high disease pressure environment (ENV-3, $r=-0.78^{**}$). Brzostowski et al. (2014) found a negative correlation between yield and area under the disease progress curve of SDS. Correlations between yield and SDS were higher than the correlations between yield and Pf in both environments. SDS may limited nutrient and water uptake by damaging the plant root system, and result in yield loss (Rupe and Hartman, 1999). CT also showed negative correlations with SDS in the low disease pressure environment (ENV-2, $r=-0.53^{**}$) and high disease pressure environment (ENV-3, $r=-0.82^{**}$). SDS also was negatively correlated with GNDVI in the low disease pressure environment (ENV-2, $r=-0.32^{*}$) and high disease pressure environment (ENV-3, $r=-0.50^{**}$). No correlations were observed between SDS and reflectance at 675 nm in all environments. In the high disease pressure environment, reflectance at 810 ($r=-0.68^{**}$) was negatively correlated with SDS.

Relationship Between Pf and CT, Reflectance at 675 and 810 nm

Analyses of covariance were performed with a different slope for each cultivar to determine the relationship between the response variable, Pf, and one covariate (CT, reflectance at wavelength 675 or 810 nm, or GNDVI) for each environment. The ANCOVA models showed significant ($p \leq 0.05$) associations between reflectance at 675 nm and Pf among the cultivars in a low disease pressure environment (ENV-2) and high disease pressure environment (ENV-3) on 6 August 2013 and 23 August 2013 (Figure 3.4, Figure B.1), respectively. Vigier et al. (2004) confirmed a similar result that plant damage caused by *Sclerotinia sclerotiorum* in soybean was associated with reflectance at the 675-685 nm band region of the electromagnetic spectrum. In the low SCN disease pressure environment, there was no significant associations observed between Pf and reflectance at 675 nm for individual cultivars. In ENV-3, the strongest associations between Pf and reflectance at 675 nm were observed for genotypes KS5004N ($R^2=0.57^{**}$) and KS5502N ($R^2=0.47^{**}$) (Figure 3.4). Reflectance increased as Pf increased in these two genotypes. Zhang et al. (2011) reported similar results, noting that neck blast (*Pyricularia grisea*) in rice was positively associated with spectral reflectance in the red region (685 nm). However, in this study, reflectance at 675 nm increased as Pf decreased in 5002T ($R^2=0.41^*$), and no relationship was observed in 5601T ($R^2=0.13$) between reflectance at 675 nm and Pf. It is difficult to explain why the relationship between Pf and reflectance at 675 nm differed across genotypes, but the response could be related to the stage of plant development at the time of the measurements, and the impact of SDS on plant growth. There was no relationship observed between reflectance at 675 nm and Pf when regression analyses were performed based on the mean across sampling days for each cultivar and environment.

ANCOVA revealed significant relationships between reflectance at 810 nm and Pf in both the low disease pressure environments, ENV-1 (9 August 2012), and ENV-2 (20 August

2013) among the cultivars (Figure 3.5, Figure B.2), but the strength of the relationship varied across genotypes. In ENV-1, the relationship between reflectance at 810 nm and Pf was non-significant for KS5502N, 5002T, and 5601T, while there was a significant ($R^2=0.67^{**}$) relationship between the two variables for KS5004 (Figure B.2). In ENV-2, no associations were observed between reflectance at 810 nm and Pf for cultivars, KS5004N, $R^2=0.35$; 5002T, $R^2=0.09$; and 5601T, $R^2=0.00$; however, there was a negative relationship between Pf and reflectance at 810 nm for KS5502N ($R^2=0.38^*$) (Figure 3.5). Several studies showed *Cercosporidium personatum* in peanut and SCN in soybean was associated at 810 nm band region (Nutter, 1989; Nutter et al., 1990; Nutter et al., 2002), but in this study, the association between reflectance at 810 nm and Pf was minimal, and no relationship was found between reflectance at 810 nm and Pf based on the mean of the sampling days for cultivars in environments.

Changes in GNDVI were associated with changes in Pf among the cultivars in the low disease pressure environment (ENV-2) and high disease pressure environment (ENV-3) on 6 August and 23 August 2013, respectively (Figure 3.6; Figure B.3). In a low disease pressure environment, GNDVI explained 66% ($p \leq 0.01$) of the variation in Pf for KS5502N, but the variables were not related to each other in the other cultivars (Figure B.3). In ENV-3 with high SCN populations, Pf increased with GNDVI in 5002T, decreased with GNDVI in KS5004N, and did not change with variation in GNDVI for 5601T and KS5502N (Fig. 3.6). Kulkarni et al. (2008) reported GNDVI was not associated with SCN population density in an environment with low populations and lack of visible symptoms. The inconsistencies between GNDVI and Pf for the different genotypes are further illustrated by the trends observed across environments. For

5002T, Pf decreased as GNDVI increased in two environments, but measurements of Pf and GNDVI were not related to each other in ENV-3 (Figure 3.7).

ANCOVA revealed that changes in Pf were related to changes in CT in the low disease pressure environment (ENV-2) and high disease environment (ENV-3), on 18 August 2013 and 1 September 2013, respectively (Figure 3.8; Figure B.4). Pf increased as CT increased for KS5502N in both environments. When CT observations averaged across sampling dates, the relationship between Pf and CT was stronger than when examining one sampling day (Figure 3.9). However, CT did not serve as a predictive tool for Pf for any of the genotypes in ENV-1, a low disease pressure environment. Eyal and Blum (1989) reported a positive relationship between CT and *Septoria tritici* blotch in wheat and Tu and Tan (1985) observed a positive association with canopy temperature depression and root rot severity in beans. However, Pinter et al. (1979) did not observe a significant relationship between CT and disease intensity of *Pythium omnivorum* in cotton, because disease pressure was moderate.

Conclusions

Genotypes differing in resistance to SCN produced phenotypes that differed in the range of variables measured in this study. The interaction between the environmental conditions experienced in this study and the genetic backgrounds produced a range of phenotypes, including genetic difference in reflectance, GNDVI and CT. In certain situations, remotely sensed data demonstrated capability to predict SCN final soil populations (Pf). Unfortunately, the relationships between Pf and GNDVI, reflectance at single wavelengths (675 and 810 nm) and CT were not consistent across cultivars or environments. It is not possible to determine why responses were so erratic, but the severity of the SDS symptoms in the fields could have confounded the relationships between the remotely sensed data and Pf. For instance, 5601T was highly susceptible to SDS. For this genotype, no relationships were ever observed between Pf and CT, single wavelengths or GNDVI. In many cases, the physiological responses to SDS may have overshadowed the plants response to SCN soil populations. Therefore, based on these results, it is not possible to assess SCN population density among and within soybean cultivars using canopy spectral reflectance or canopy temperature with any degree of confidence.

Figures and Tables

Table 3.1 Monthly mean, maximum, and minimum temperatures (°C) and total rainfall (mm) for the two growing seasons and average temperatures (°C) and total rainfall (mm) over 30 years.

	May	June	July	August	Sept.	October	Mean/Sum [†]
Rossville 2012							
Max. (°C)	28.3	31.1	35.5	31.7	27.4	20.3	29.1
Min. (°C)	14.3	17.4	20.4	15.4	10.8	4.4	13.8
Mean (°C)	21.3	24.4	28.0	23.4	19.1	12.2	21.4
Total rainfall (mm)	67.6	94.0	21.1	81.3	22.1	27.2	313.2
Rossville 2013							
Max. (°C)	23.6	29.3	30.7	29.4	29.1	19.9	27.0
Min. (°C)	11.2	17.7	18.0	17.9	13.6	4.9	13.9
Mean (°C)	17.4	23.5	24.3	23.6	21.4	12.4	20.4
Total rainfall (mm)	157.5	71.6	62.5	86.1	77.5	107.4	562.6
30 year average temp. (°C)	18.3	23.4	26.1	25.2	20.2	13.7	21.2
30 year average rainfall (mm)	124.7	137.2	97.0	107.7	93.0	77.0	636.5
Ashland Bottoms 2013							
Max. (°C)	24.1	30.4	31.6	30.9	30.2	20.3	27.9
Min. (°C)	11.0	17.4	18.8	19.0	14.9	5.4	14.4
Mean (°C)	17.5	23.9	25.2	25.0	22.5	12.9	21.2
Total rainfall (mm)	90.2	80.5	110.0	104.2	78.7	95.0	558.6
30 year average temp. (°C)	17.6	23.2	26.1	25.1	19.9	12.9	20.8
30 year average rainfall (mm)	113.8	129.3	100.8	108.7	80.5	56.4	589.5

[†]Mean temperature and total rainfall of the growing season (from May to October).

Table 3.2 F-values from analyses of variance for yield, height, lodging, and Sudden Death Syndrome (SDS) across the three environments.

Source of variation	d.f.	Yield	Height	Lodging	SDS [†]
Cultivar (C)	3	46.59**	21.62**	15.28**	74.80**
Environment (E)	2	113.75**	67.98**	60.48**	33.27**
C x E	6	9.36**	1.15	2.98**	9.24**

** indicates significance at the 0.01 alpha level.

[†]Yield (kg ha⁻¹); height (cm); SDS, sudden death syndrome.

Table 3.3 F-values from analyses of variance for maturity across two environments (ENV-2 and ENV-3).

Source of variation	d.f.	Maturity
Cultivar (C)	3	340.6**
Environment (E)	1	0.18
C x E	3	1.46

** indicates significance at the 0.01 alpha level.

Table 3.4 F-values from analyses of variance for SCN population densities at harvest, reproductive factor, canopy temperature, green normalized differences vegetation index, reflectance at 675 nm and 810 nm across the three environments.

Source of variation	d.f.	Pi	Pf	Rf	680 nm	810 nm	GNDVI
Cultivar (C)	3	1.47	66.57**	22.26**	9.14**	29.74**	13**
Environment (E)	2	31.25**	17.69**	17.01**	20.32**	32.18**	18.65**
C x E	6	0.8	7.77**	2.97**	0.68	3.72**	0.73

** indicates significance at the 0.01 alpha level.

[†]Pi, soybean cyst nematode population density at planting (log₁₀ egg and J2 /100 cm³ soil); Pf, soybean cyst nematode population density at harvest (log₁₀ egg and J2 /100 cm³ soil); Rf, reproductive factor (Pf/Pi); CT, canopy temperature (°C); GNDVI, green normalized difference vegetation indices; reflectance at 675 nm and 810 nm.

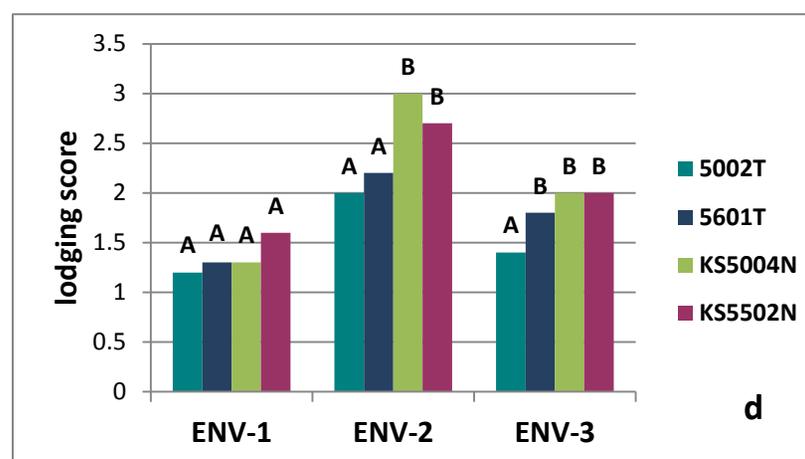
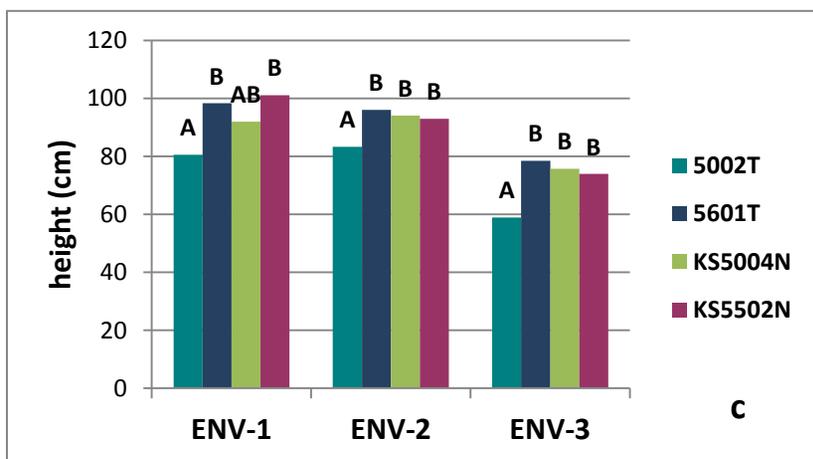
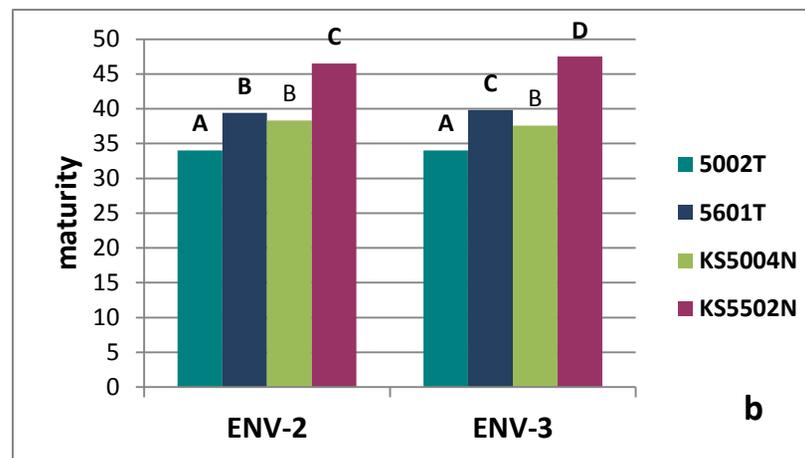
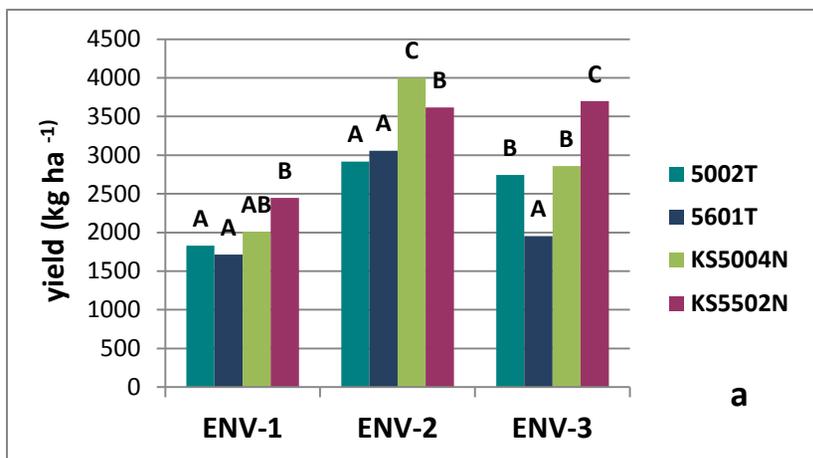


Figure 3.1 Mean comparison of yield (a), maturity (b), height (c), and lodging score (d) for each cultivar within each environment.

†Cultivars not significantly different from one another share the same or common letter.

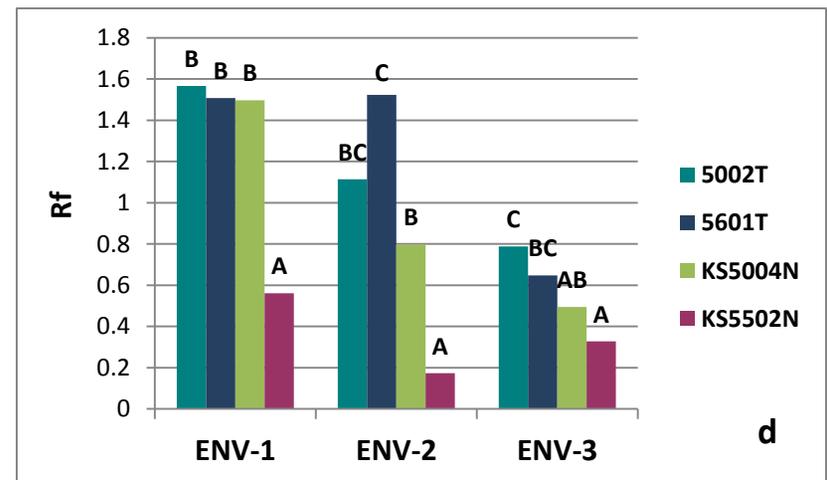
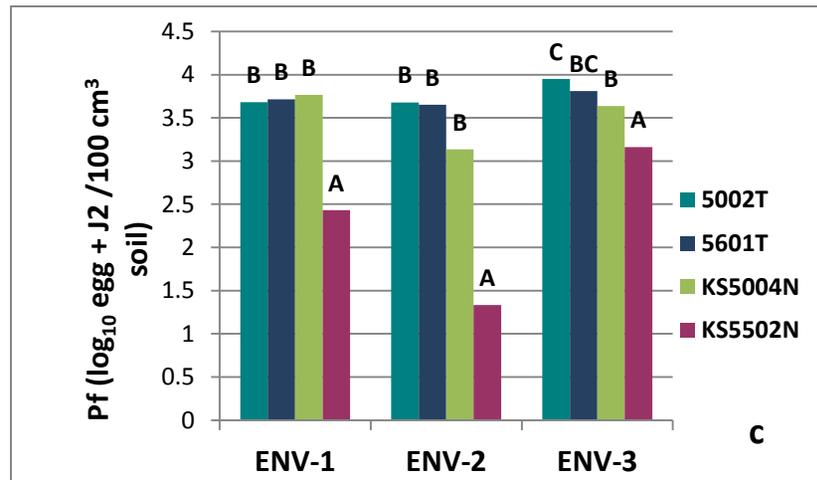
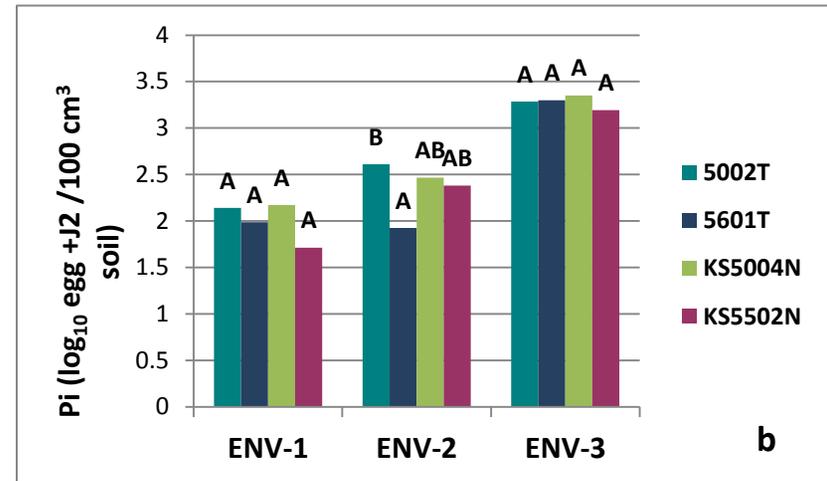
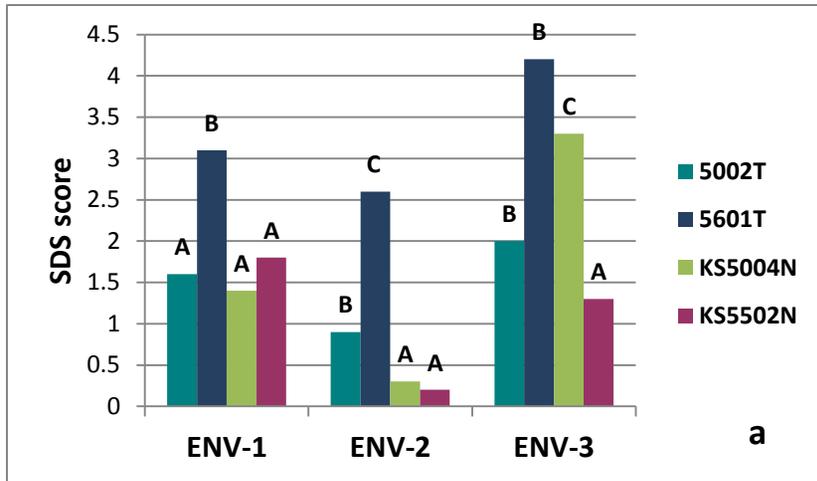


Figure 3.2 a) Effect on soybean cultivars on sudden death syndrome (SDS) b) SCN population density at planting (Pi). c) Effect on soybean cultivars on SCN population density at harvest (Pf) d) Reproductive factor (Rf=Pf/Pi) for each environment.

†Cultivars not significantly different from one another share the same or common letter.

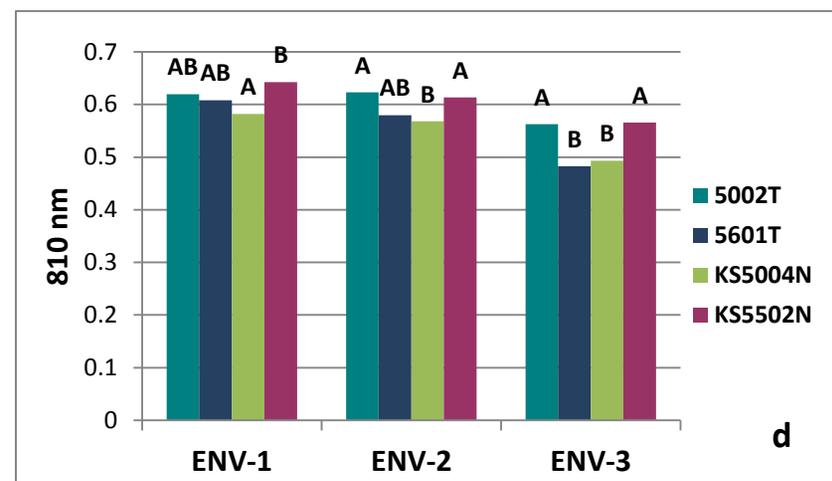
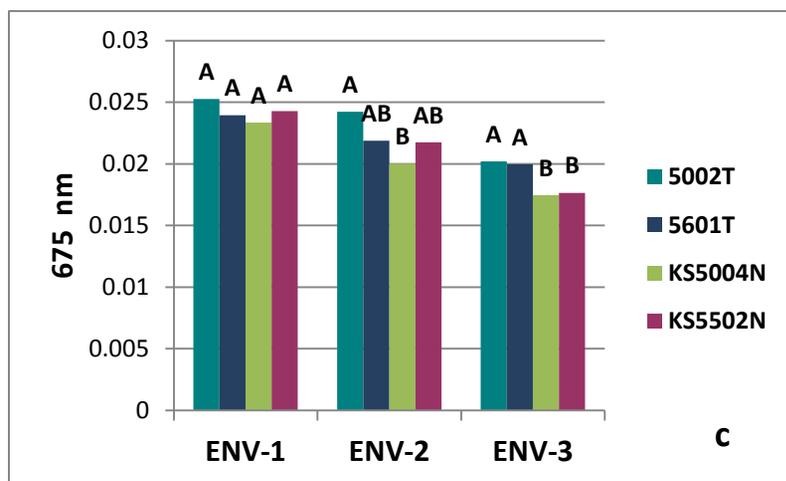
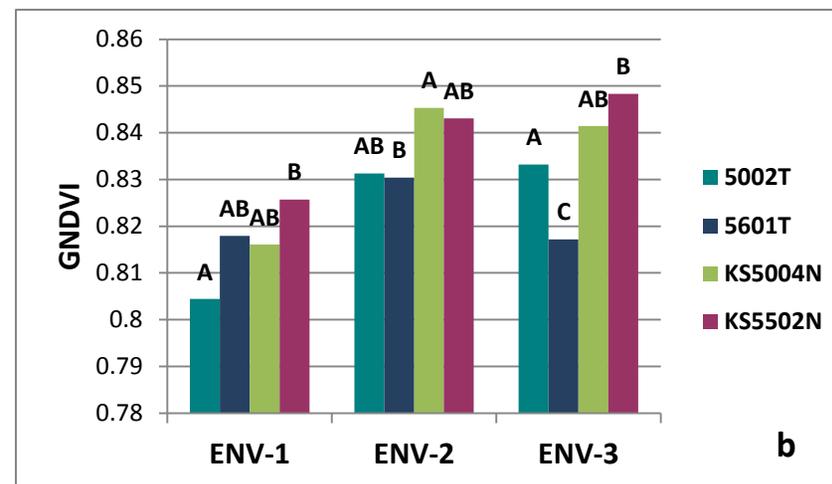
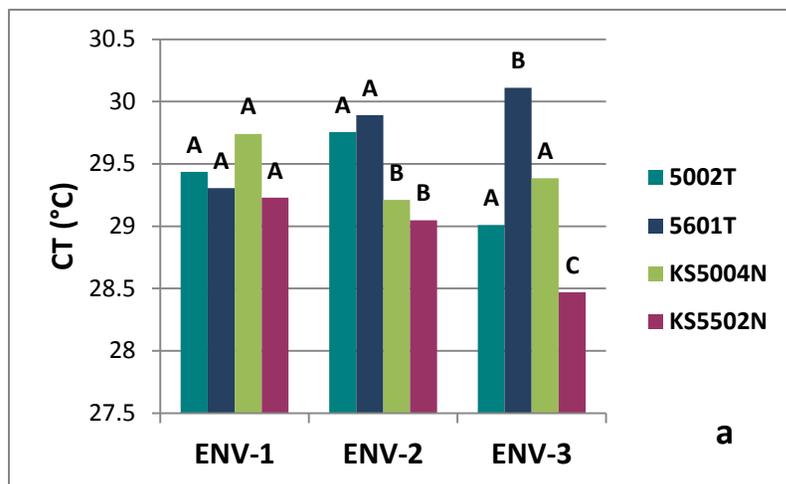


Figure 3.3 Mean comparison of CT, canopy temperature (a), GNDVI, green normalized difference vegetation index (b), reflectance at 675 nm (c), and 810 nm (d) for each cultivar within each environment.

†Cultivars not significantly different from one another share the same or common letter.

Table 3.5 F-values from analyses of variance for green normalized difference vegetation index, reflectance at 675 nm and 810 nm across the sampling days for each environment.

ENV-1				
Source of variation	d.f.	GNDVI [†]	675 nm	810 nm
Cultivar (C)	3	3.39*	0.46	6.63**
Sampling Day (SD)	3	253.85**	72.07**	365.68**
C x SD	9	2.1*	0.32	1.19
ENV-2				
Source of variation	d.f.	GNDVI	675 nm	810 nm
Cultivar (C)	3	4.04**	2.86*	5.07**
Sampling Day (SD)	3	3.38*	3.37*	40.62**
C x SD	9	1.86	1.29	0.28
ENV-3				
Source of variation	d.f.	GNDVI	675 nm	810 nm
Cultivar (C)	3	15.13**	5.53**	18.84**
Sampling Day (SD)	3	21.68**	4.66**	34.23**
C x SD	9	5.04**	2.51**	2.61**

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

[†]GNDVI, green normalized difference vegetation index; reflectance at 675 nm; reflectance at 810 nm.

Table 3.6 Pearson's correlations coefficient between SCN population densities at harvest and canopy temperature, green normalized vegetation differences, 675 nm, and 810 nm for each sampling day (based on replications).

2012				
ENV-1				
	25 July (n=40)	9 August (n=38)	21 August (n=40)	19 September (n=38)
CT	0.1	0.36*	-	0.25
GNDVI	-0.12	-0.18	0.05	-0.38*
675 nm	0.0	0.01	-0.18	0.09
810 nm	-0.35*	-0.13	-0.1	-0.35*
2013				
ENV-2				
	6 August (n=37)	18 August (n=40)	20 August (n=40)	30 August (n=40)
CT	0.34*	0.35*	0.04	0.25
GNDVI	-0.11	-0.18	-0.16	-0.49**
675 nm	0.03	0.05	0.11	0.08
810 nm	-0.04	-0.35*	-0.28	-0.34*
ENV-3				
	1 August (n=40)	17 August (n=40)	23 August (n=40)	1 September (n=40)
CT	0.12	0.45**	0.47**	0.33*
GNDVI	-0.05	-0.3	-0.51**	-0.30
675 nm	0.1	0.28	0.43**	-0.03
810 nm	-0.22	-0.2	0.07	-0.36*

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†CT, canopy temperature (°C); GNDVI, green normalized difference vegetation index; reflectance at 675 nm; reflectance at 810 nm.

Table 3.7 Pearson's correlations coefficients among SCN population densities at harvest, canopy temperature, reflectance at 675 and 810 nm, green normalized vegetation differences, yield, height, lodging, sudden death syndrome for each environment on a certain sampling day (based on replications).

ENV-1 (n=38, 19 September 2012)										
	Pf [†]	Rf	CT	675 nm	810 nm	GNDVI	Yield	Height	Lodging	SDS
Rf	0.72**									
CT	0.25	0.302								
675 nm	0.08	-0.07	0.25							
810 nm	-0.35*	-0.15	-0.07	0.04						
GNDVI	-0.38*	-0.16	-0.32*	-0.77**	0.43**					
Yield	-0.65**	-0.57**	-0.29	-0.26	0.18	0.34*				
Height	-0.45**	-0.35*	-0.04	0.05	0.07	0.112	0.61**			
Lodging	-0.39*	-0.37*	-0.2	0.16	-0.06	0.04	0.34*	0.45**		
SDS	-0.16	-0.28	-0.029	0.24	-0.17	-0.23	0.008	0.50**	0.36*	
ENV-2 (n=40, 30 August 2013)										
Rf	0.76**									
CT	0.25	0.17								
675 nm	-0.08	0.008	-0.027							
810 nm	-0.34*	-0.25	-0.17	0.60**						
GNDVI	-0.49**	-0.32*	-0.19	-0.72**	0.02					
Yield	-0.33*	-0.32*	-0.49**	-0.36*	-0.16	0.52**				
Height	-0.21	-0.16	0.15	-0.32*	-0.27	0.14	0.08			
Lodging	-0.17	-0.35*	-0.14	-0.3	-0.34*	0.29	0.50**	0.41**		
SDS	0.43**	0.50**	0.53**	-0.08	-0.22	-0.32*	-0.59**	0.24	-0.25	
Maturity	-0.70**	-0.51**	-0.13	-0.07	0.22	0.40**	0.28	0.36*	0.41**	-0.15

continued

Table 3.7 (continued) Pearson's correlations coefficients among SCN population densities at harvest, canopy temperature, reflectance at 675 and 810 nm, green normalized vegetation differences, yield, height, lodging, sudden death syndrome for each environment on a certain sampling day (based on replications).

		ENV-3 (n=40, 23 August 2013)								
	Pf [†]	Rf	CT	675 nm	810 nm	GNDVI	Yield	Height	Lodging	SDS
Rf	0.64**									
CT	0.46**	0.27								
675 nm	0.42**	0.16	0.13							
810 nm	0.07	0.03	-0.51**	0.58**						
GNDVI	-0.51**	-0.29	-0.59**	-0.71**	0.05					
Yield	-0.58**	-0.35*	-0.74**	-0.32*	0.33*	0.64**				
Height	-0.33*	-0.29	0.27	-0.67**	-0.70**	0.21	0.02			
Lodging	-0.39*	-0.27	-0.03	-0.44**	-0.21	0.37*	0.31*	0.49**		
SDS	0.46**	0.18	0.82**	0.00	-0.68**	-0.50**	-0.78**	0.36*	0.03	
Maturity	-0.69**	-0.37*	-0.35*	-0.41**	0.00	0.43**	0.46**	0.35*	0.46**	-0.32*

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†Pf, soybean cyst nematode (SCN) population density at harvest (\log_{10} egg and J2 /100 cm³ soil); Rf, reproductive factor (SCN population density at harvest/SCN population density at planting); CT, canopy temperature (°C); reflectance at 810 nm; reflectance at 675 nm; GNDVI, green normalized difference vegetation index; yield (kg ha⁻¹); height (cm); SDS, sudden death syndrome.

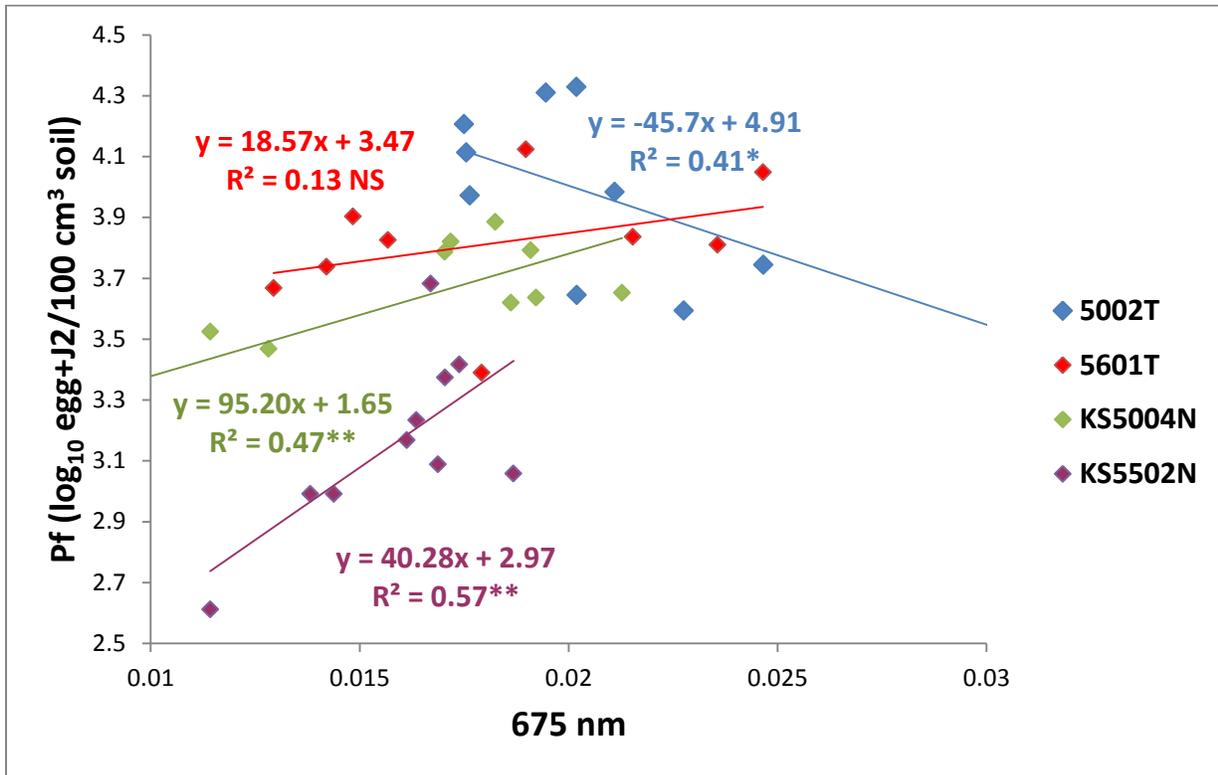


Figure 3.4 Relationship between reflectance at 675 nm and SCN soil population at harvest (Pf) among cultivars in ENV-3 (23 August 2013).

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

§NS, not significant

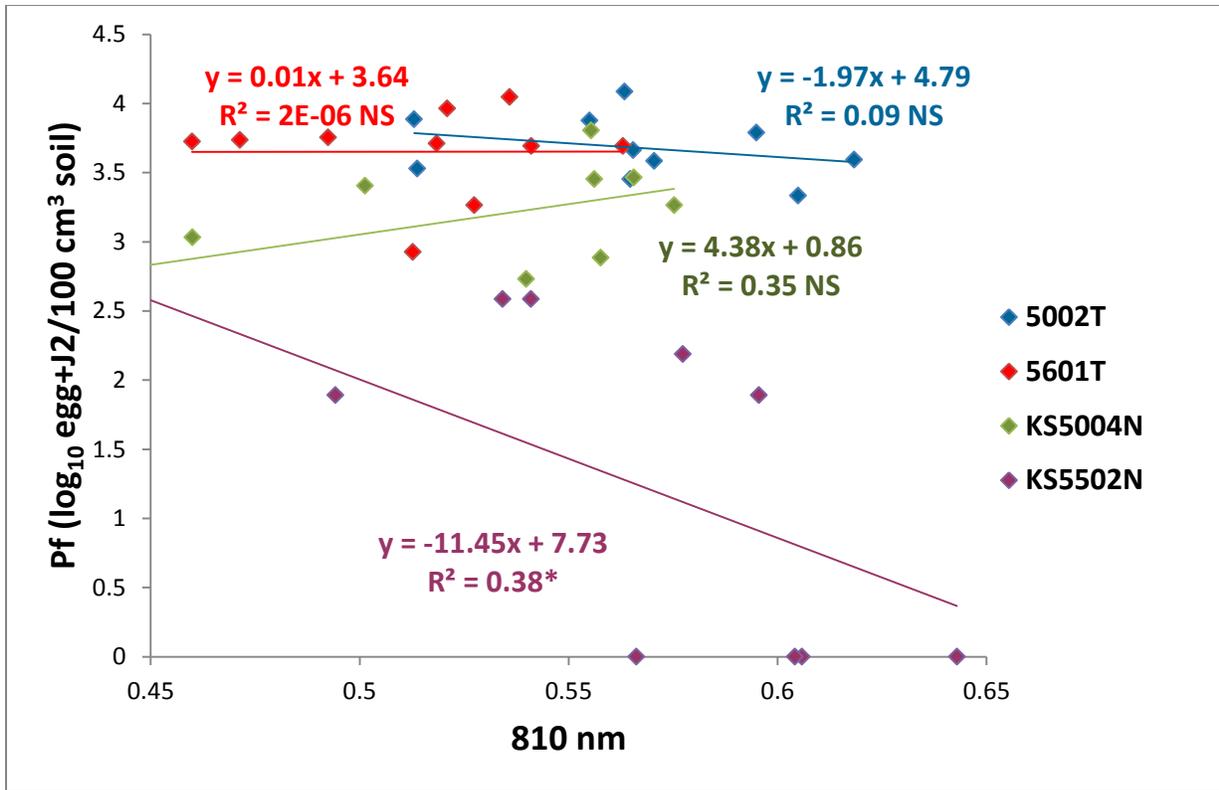


Figure 3.5 Relationship between reflectance at 810 nm and soil SCN population density at harvest (Pf) in ENV-2 (20 August 2013).

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

§NS, not significant

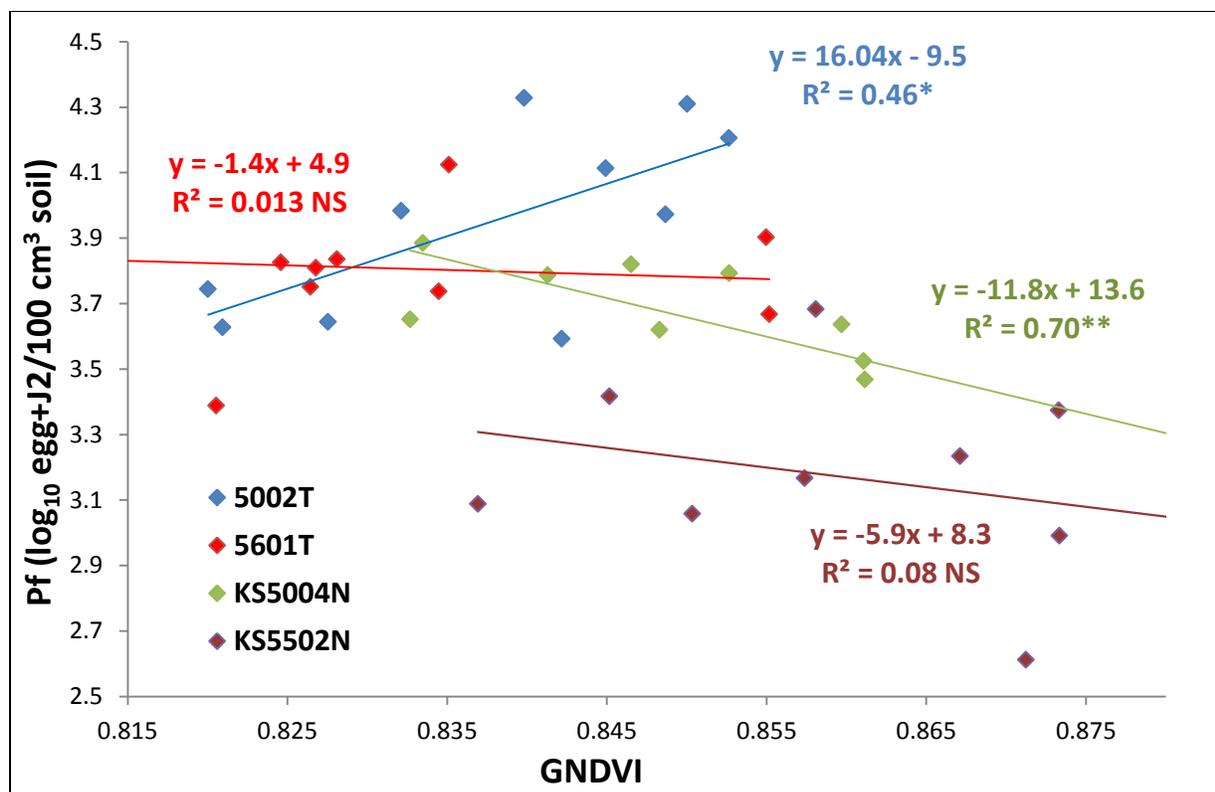


Figure 3.6 Relationship between green normalized difference vegetation index (GNDVI) and SCN soil population at harvest (Pf) among cultivars in ENV-3 (23 August 2013).

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

§NS, not significant

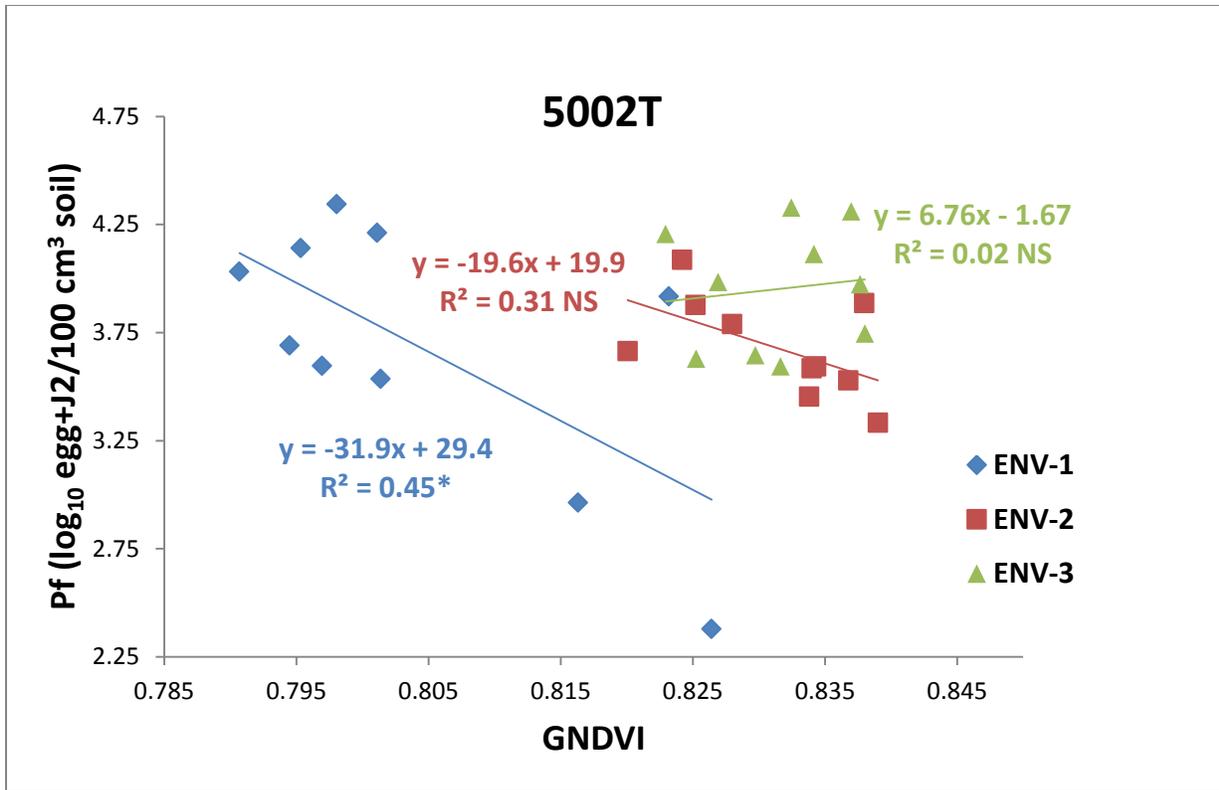


Figure 3.7 Relationship between green normalized difference vegetation index (GNDVI) and SCN soil population density at harvest (Pf) for 5002T based on average sampling days of environments.

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

[§]NS, not significant

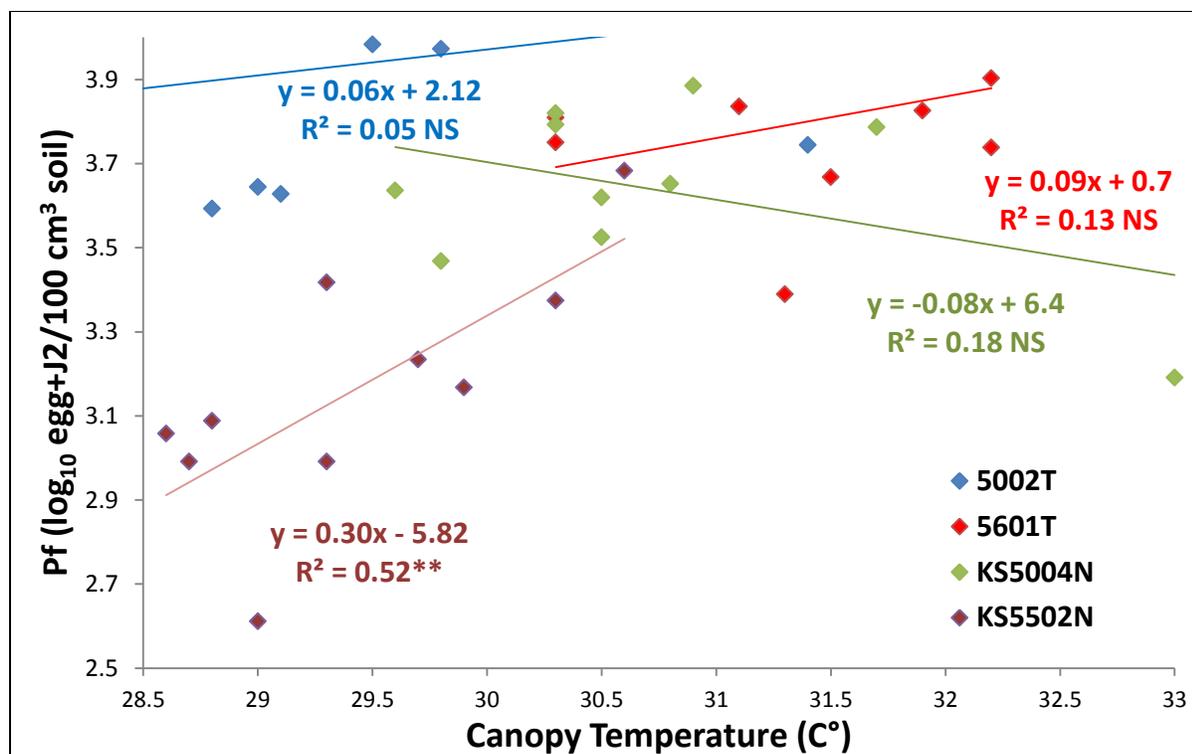


Figure 3.8 Relationship between canopy temperature (CT) and SCN soil population density (Pf) on 1 September 2013 in ENV-3.

* ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.
 §NS, not significant

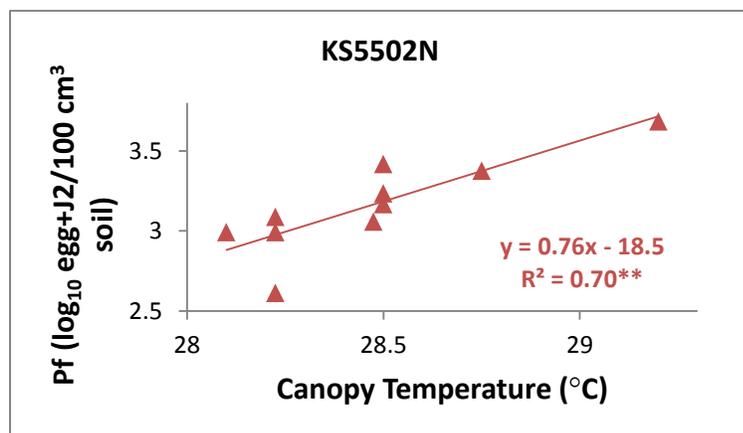


Figure 3.9 Relationship between canopy temperature (CT) and soil SCN population density at harvest (Pf) sampling days for KS5502N in ENV-3.

** indicates significance at the 0.01 alpha level.

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Appendix A - Supplementary Data

Table A.1 F-values of analysis of variance for agronomic traits in four environments.

Environment	df	Yield	Height	Maturity	Lodging
1	48	54.6**	33.1**	651.8**	29.2**
2	48	15.3**	21.1**	61.9**	13.3**
3	48	15.8**	113.7**	248.5**	27.7**
4	48	18.2**	134.8**	21.8**	129.7**

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

Table A.2 Pearson's correlation coefficients among agronomic traits in four environments.

	Yield	Height	Lodging	Maturity
ENV-1 (n=140)				
Height	0.29**			
Lodging	0.16*	0.55**		
Maturity	-0.11	0.1	0.1	
ENV-2 (n=144)				
Height	0.49**			
Lodging	0.45**	0.60**		
Maturity	0.54**	0.58**	0.51**	
ENV-3 (n=191)				
Height	0.20**			
Lodging	-0.05	0.14		
Maturity	0.36**	0.22*	-0.07	
SDS [†]	-0.70**	-0.06	0.02	-0.23**
ENV-4 (n=192)				
Height	0.27**			
Lodging	0.06	0.42**		
Maturity	0.56**	0.29**	0.09	

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

[†]Sudden death syndrome, SDS

Table A.3 F-values of analysis of variance for spectral reflectance indices (SRIs) and canopy temperature (CT) in four environments.

Indices	ENV-1			ENV-2			ENV-3		ENV-4	
	R1-R2	R3-R4	R5-R6	R1-R2	R3-R4	R5-R6	R1-R2	R3-R4	R1-R2	R5-R6
R550	6.4**	2.5**	8.4**	27.3**	63.0**	54.0**	148.5**	7.8**	76.9**	39.9**
GNDVI	1112.0**	802**	2307.6**	3963.0**	1251**	4501.0**	8129.7**	4706.9**	1659.0**	439.8**
NDVI	4444**	3827.5**	2802**	14327.8**	16253.6**	20727.5**	8484.0**	31006.0**	926.8**	1447.0**
NWI	5.0**	5.7**	24.0**	120.0*	188.0**	84.0**	32.0**	5.4**	9.8**	20.0**
CT	188.0**	-	-	11158.7**	-	-	490.7**	223.4**	147.7**	95.0**

*, ** indicate significance at the 0.05 and 0.01 alpha levels, respectively.

†R550, reflectance at 550 nm; GNDVI, green normalized difference vegetation index; RNDVI, red normalized difference vegetation index; NWI, normalized vegetation index.

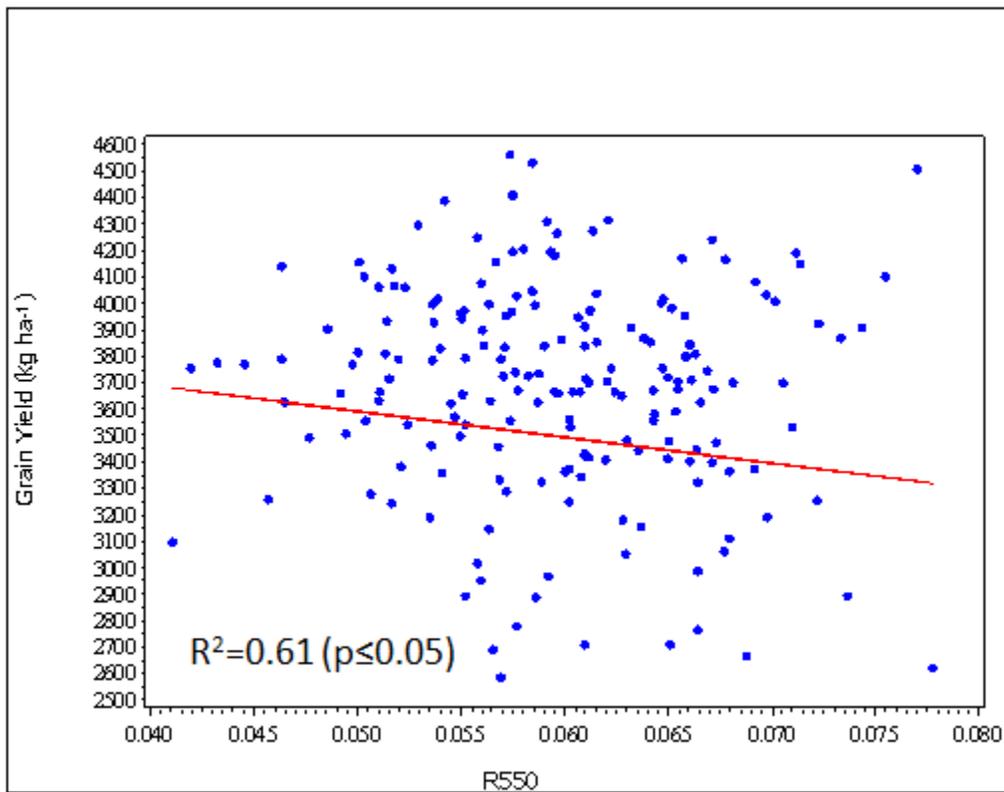


Figure A.1 Relationship between reflectance at wavelength 550 nm (R550) and yield (kg ha⁻¹) at R1-R2 growth stage in ENV-4.

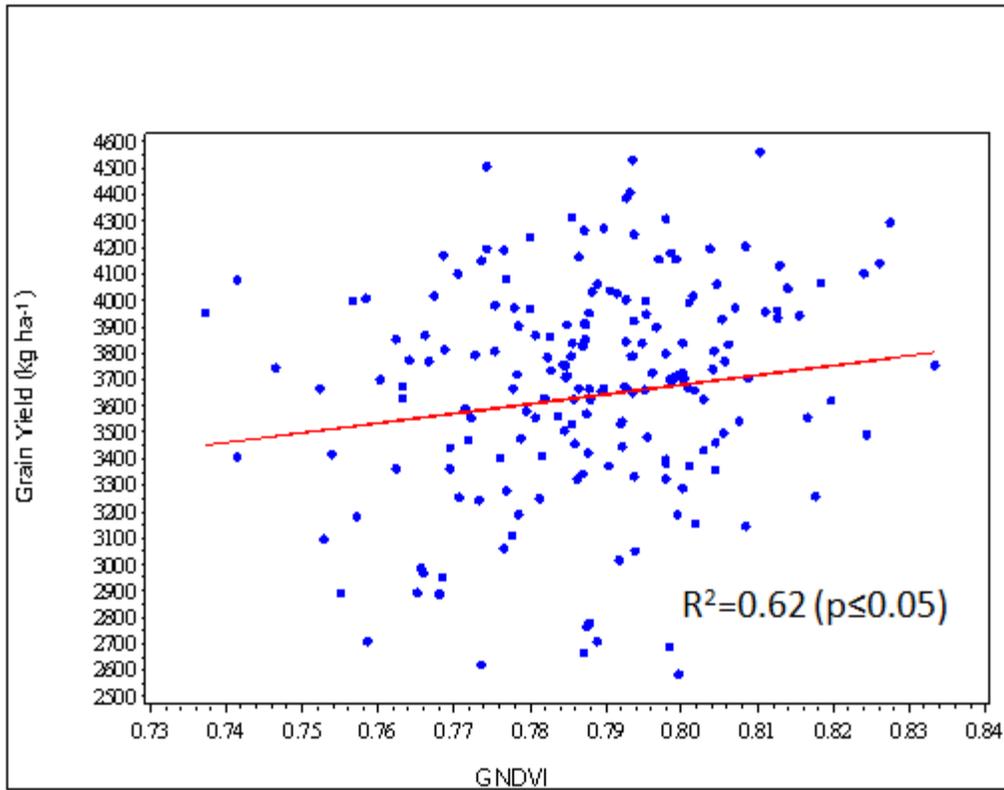


Figure A.2 Relationship between green normalized difference vegetation index GNDVI and yield (kg ha⁻¹) at growth stage R1-R2 in ENV-4.

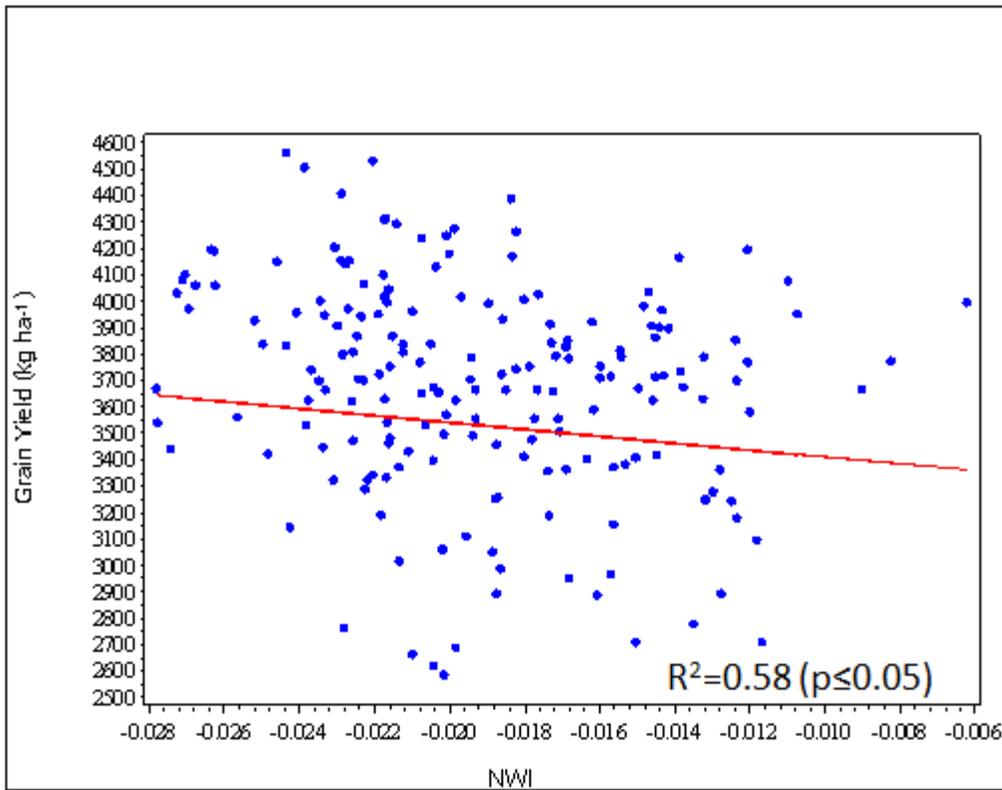


Figure A.3 Relationship between normalized water index (NWI) and yield (kg ha-1) at growth stage R1-R2 growth stage in ENV-4.

Table A.4 Mean value of yield, height, lodging, maturity, and canopy temperature (CT) for genotypes in ENV -1.

Genotypes	Yield	Height	Lodging	Maturity	CT
K11-1151	4080.7	107.5	3	35	33.3
K11-1152	4003.4	113.5	3	33	33.5
K11-1153	4365.0	120.2	3	34	34.3
K11-1155	3641.6	110.9	3	34	34.2
K11-1156	3848.3	111.8	2	32	33.2
K11-1157	3951.8	120.2	3	33	32.7
K11-1158	3564.3	109.2	3	33	34.0
K11-1160	4468.3	109.2	3	33	33.6
K11-1161	4003.4	119.4	3	34	34.1
K11-1162	3951.6	115.1	4	35	34.1
K11-1163	4532.7	111.8	3	33	33.3
K11-1164	4365.0	123.6	4	35	34.2
K11-1165	4520.1	113.5	2	31	33.9
K11-1167	4339.2	117.7	3	33	33.8
K11-1168	4184.1	128.7	3	34	34.7
K11-1169	4390.8	120.2	3	32	34.1
K11-1170	4468.3	110.1	2	33	33.0
K11-1171	4313.2	123.6	3	35	34.2
K11-1172	4209.9	132.9	3	35	34.5
K11-1173	4442.3	127.0	3	34	34.6
K11-1175	4080.7	122.8	3	34	34.0
K11-1176	4442.5	126.2	3	32	34.0
K11-1177	4364.7	101.6	2	32	34.0
K11-1178	4364.7	135.5	3	33	33.5
K11-1180	4287.6	116.0	3	34	34.3
K11-1182	3796.7	109.2	2	31	33.0
K11-1184	3796.9	110.1	3	32	33.9
K11-1185	3822.7	121.1	3	32	34.8
K11-1186	4080.7	112.6	3	31	33.6
K11-1190	4068.0	100.3	3	34	34.6
K11-1191	4235.9	119.4	3	35	33.4
K11-1195	4287.4	108.4	2	33	33.2

Table A.4 (continued) Mean value of yield, height, lodging, maturity, and canopy temperature (CT) for genotypes in ENV -1.

Genotypes	Yield	Height	Lodging	Maturity	CT
K11-1196	4080.7	110.9	2	31	34.1
K11-1197	4003.4	125.3	3	32	35.0
K11-1199	4261.6	119.4	3	33	34.0
K11-1200	4003.4	135.5	3	33	33.9
K11-1201	4313.4	122.8	3	33	33.8
K11-1202	4235.9	107.5	2	32	32.8
K11-1203	3900.1	136.3	3	33	34.0
K11-1205	4055.0	117.7	3	33	33.2
K11-1206	3719.1	124.5	3	34	33.6
K11-1207	4339.2	111.8	3	31	34.6
K11-1208	3900.1	121.1	3	34	35.0
K11-1210	3951.6	115.6	3	33	33.6
K11-1211	4442.3	116.0	3	32	33.3
K11-1213	3900.1	110.9	3	34	33.6
K11-1215	4132.3	95.7	2	33	34.8
K11-1217	3977.4	110.9	2	34	33.5

Yield (kg ha⁻¹), height (cm), CT, canopy temperature (C°).

Table A.5 Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -1.

Genotypes	R1-R2				R3-R4			
	R550	GNDVI	RNDVI	NWI	R550	GNDVI	RNDVI	NWI
K11-1151	0.07211	0.78138	0.91403	-0.03372	0.06160	0.82021	0.92366	-0.03526
K11-1152	0.07530	0.79915	0.91978	-0.02906	0.05159	0.82141	0.92220	-0.03757
K11-1153	0.08352	0.77392	0.91213	-0.03495	0.04971	0.81130	0.91881	-0.04042
K11-1155	0.06913	0.78372	0.91350	-0.02852	0.04334	0.84145	0.93286	-0.04361
K11-1156	0.08630	0.76797	0.90643	-0.03350	0.05136	0.83199	0.92156	-0.04194
K11-1157	0.07866	0.78343	0.91708	-0.02453	0.04133	0.83834	0.93105	-0.04523
K11-1158	0.07882	0.78098	0.91284	-0.02779	0.05271	0.82965	0.92438	-0.03491
K11-1160	0.08155	0.77807	0.90885	-0.03073	0.05762	0.81271	0.91552	-0.03740
K11-1161	0.07657	0.78376	0.91416	-0.03147	0.05709	0.82338	0.92024	-0.03382
K11-1162	0.07344	0.79147	0.91807	-0.03311	0.06425	0.80188	0.91076	-0.03509
K11-1163	0.06146	0.81693	0.92758	-0.03535	0.05596	0.81675	0.92069	-0.03832
K11-1164	0.08362	0.77376	0.90059	-0.03220	0.04304	0.80457	0.90256	-0.03000
K11-1165	0.08300	0.78476	0.92274	-0.02982	0.06285	0.81309	0.92146	-0.03882
K11-1167	0.07204	0.77305	0.90341	-0.03135	0.05543	0.82449	0.92184	-0.03850
K11-1168	0.07451	0.77267	0.90286	-0.02991	0.06273	0.80727	0.90799	-0.03709
K11-1169	0.07734	0.78395	0.90804	-0.03437	0.06240	0.81306	0.91206	-0.03998
K11-1170	0.07536	0.77815	0.90947	-0.02815	0.05746	0.82690	0.92124	-0.03637
K11-1171	0.06654	0.79959	0.91767	-0.03706	0.05761	0.81642	0.91340	-0.03926
K11-1172	0.07203	0.79845	0.91936	-0.02888	0.04802	0.81638	0.91664	-0.03806
K11-1173	0.08509	0.77190	0.90534	-0.03157	0.05887	0.80467	0.90458	-0.04225
K11-1175	0.07392	0.78095	0.90967	-0.03472	0.05261	0.82737	0.92461	-0.04308
K11-1176	0.07658	0.77001	0.90143	-0.02716	0.05871	0.81951	0.91644	-0.03907
K11-1177	0.07885	0.78800	0.91272	-0.03128	0.06099	0.82193	0.91998	-0.03837

Table A.5 (continued) Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -1.

Genotypes	R1-R2				R3-R4			
	R550	GNDVI	RNDVI	NWI	R550	GNDVI	RNDVI	NWI
K11-1178	0.06633	0.80298	0.92006	-0.03423	0.05684	0.81181	0.91091	-0.03819
K11-1180	0.08945	0.77497	0.91540	-0.02902	0.05738	0.81445	0.91415	-0.03964
K11-1182	0.07573	0.79110	0.92056	-0.03045	0.05783	0.80990	0.91570	-0.03503
K11-1184	0.08226	0.77013	0.90820	-0.02613	0.05610	0.83385	0.92471	-0.04135
K11-1185	0.07745	0.78655	0.90966	-0.03050	0.05780	0.83408	0.92106	-0.03698
K11-1186	0.07367	0.78703	0.91552	-0.03448	0.06812	0.81115	0.91507	-0.03299
K11-1190	0.05415	0.81788	0.92494	-0.02234	0.06358	0.83716	0.92233	-0.03056
K11-1191	0.08130	0.77200	0.90981	-0.03004	0.04813	0.83602	0.92484	-0.04492
K11-1195	0.07060	0.80509	0.91974	-0.02945	0.06031	0.83040	0.92397	-0.03321
K11-1196	0.07269	0.79928	0.92160	-0.03154	0.05026	0.84317	0.92881	-0.03713
K11-1197	0.06723	0.79720	0.91930	-0.03183	0.07029	0.80370	0.90636	-0.03132
K11-1199	0.06271	0.78854	0.91269	-0.03539	0.05418	0.82950	0.91552	-0.03901
K11-1200	0.08276	0.77237	0.90734	-0.03714	0.05424	0.82135	0.91297	-0.04196
K11-1201	0.07757	0.77204	0.90494	-0.02480	0.07143	0.80081	0.90748	-0.03371
K11-1202	0.06966	0.81091	0.93059	-0.03068	0.05464	0.82186	0.92132	-0.03707
K11-1203	0.07622	0.77455	0.90274	-0.03384	0.05975	0.80900	0.91190	-0.03815
K11-1205	0.09554	0.76848	0.90437	-0.02421	0.06632	0.80206	0.90715	-0.03445
K11-1206	0.07772	0.78605	0.90942	-0.03243	0.05162	0.82229	0.91438	-0.03863
K11-1207	0.07653	0.79049	0.91593	-0.03112	0.06263	0.82217	0.91664	-0.03517
K11-1208	0.07392	0.77727	0.91639	-0.03331	0.05882	0.81118	0.91484	-0.03503
K11-1210	0.07956	0.75485	0.90251	-0.02452	0.06449	0.81270	0.91253	-0.03692
K11-1211	0.06698	0.79440	0.91962	-0.03439	0.06221	0.82625	0.92211	-0.03211
K11-1213	0.08474	0.77827	0.91227	-0.02927	0.06941	0.81444	0.91711	-0.03035
K11-1215	0.06752	0.80571	0.92478	-0.03499	0.05015	0.83533	0.92551	-0.04276
K11-1217	0.07748	0.79586	0.92267	-0.02876	0.05544	0.80852	0.91568	-0.03649

Table A.5 (continued) Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -1.

Genotypes	R5-R6			
	R550	GNDVI	RNDVI	NWI
K11-1151	0.05014	0.83213	0.92062	-0.04406
K11-1152	0.04288	0.84059	0.92775	-0.04583
K11-1153	0.04345	0.83323	0.93148	-0.04925
K11-1155	0.04706	0.82779	0.92628	-0.04518
K11-1156	0.03699	0.85535	0.93534	-0.05019
K11-1157	0.04370	0.83428	0.92955	-0.04440
K11-1158	0.05022	0.82095	0.91880	-0.04548
K11-1160	0.05388	0.82301	0.92008	-0.04217
K11-1161	0.04791	0.83206	0.92182	-0.04162
K11-1162	0.05211	0.81363	0.91563	-0.04059
K11-1163	0.04031	0.84433	0.93285	-0.05091
K11-1164	0.04652	0.82753	0.92076	-0.04949
K11-1165	0.05447	0.82105	0.92485	-0.04554
K11-1167	0.04318	0.84311	0.92730	-0.04411
K11-1168	0.05441	0.80656	0.91194	-0.03950
K11-1169	0.04780	0.83285	0.92245	-0.04597
K11-1170	0.04276	0.84051	0.92902	-0.04491
K11-1171	0.04828	0.82530	0.91387	-0.04347
K11-1172	0.04530	0.82912	0.92593	-0.04250
K11-1173	0.04112	0.84022	0.92620	-0.04760
K11-1175	0.03993	0.84252	0.93004	-0.05368
K11-1176	0.04143	0.84265	0.93831	-0.04978
K11-1177	0.04803	0.83196	0.92389	-0.04765
K11-1178	0.03694	0.85132	0.92977	-0.05363
K11-1180	0.04066	0.84952	0.93127	-0.05176
K11-1182	0.04867	0.83020	0.92437	-0.04637
K11-1184	0.04722	0.82442	0.91946	-0.04462
K11-1185	0.04815	0.83587	0.92498	-0.04224
K11-1186	0.04820	0.82360	0.92136	-0.04288
K11-1190	0.04573	0.85045	0.92851	-0.04084
K11-1191	0.04006	0.85455	0.92871	-0.04337
K11-1195	0.04600	0.84789	0.93090	-0.04028

Table A.5 (continued) Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -1.

Genotypes	R5-R6			
	R550	GNDVI	RNDVI	NWI
K11-1196	0.04526	0.83812	0.92648	-0.04186
K11-1197	0.04354	0.83031	0.91533	-0.04661
K11-1199	0.04136	0.83650	0.91455	-0.05392
K11-1200	0.03561	0.84901	0.93024	-0.05652
K11-1201	0.04344	0.83915	0.92251	-0.04618
K11-1202	0.03886	0.85481	0.93500	-0.04876
K11-1203	0.04232	0.85207	0.93125	-0.04808
K11-1205	0.04536	0.83819	0.92359	-0.04252
K11-1206	0.03794	0.85082	0.92761	-0.05300
K11-1207	0.04799	0.82378	0.91459	-0.04548
K11-1208	0.04781	0.81748	0.91728	-0.04248
K11-1210	0.04202	0.83720	0.92860	-0.03996
K11-1211	0.04272	0.85057	0.93474	-0.04152
K11-1213	0.05209	0.82574	0.92235	-0.03703
K11-1215	0.04011	0.84910	0.93503	-0.04994
K11-1217	0.04808	0.83187	0.92738	-0.04302

Table A.6 Mean value of yield, height, lodging, maturity, and canopy temperature (CT) for genotypes in ENV -2.

Genotypes	Yield	Height	Lodging	Maturity	CT
K11-1151	3792.0	121.9	3	37	29.0
K11-1152	3194.6	120.2	3	34	29.7
K11-1153	3220.6	123.6	3	37	28.8
K11-1155	4022.4	127.8	4	39	29.6
K11-1156	3766.0	125.3	3	37	28.4
K11-1157	3921.8	122.8	3	38	30.3
K11-1158	3714.0	120.2	3	38	29.8
K11-1160	3298.6	111.8	3	37	30.0
K11-1161	3714.2	129.5	4	39	28.5
K11-1162	3532.4	123.6	4	37	29.7
K11-1163	3324.6	116.8	4	37	29.4
K11-1164	3428.2	119.4	4	37	30.2
K11-1165	3870.0	119.4	3	36	29.7
K11-1167	3921.8	132.1	4	37	30.1
K11-1168	3714.2	130.4	3	37	29.5
K11-1169	4103.6	128.7	4	37	29.0
K11-1170	3870.0	127.0	3	36	29.6
K11-1171	3688.2	123.6	3	37	29.0
K11-1172	4077.8	131.2	4	38	29.4
K11-1173	4259.4	132.9	4	37	29.1
K11-1175	3220.6	121.9	4	35	30.1
K11-1176	3973.6	129.5	4	37	29.6
K11-1177	4233.4	116.8	3	36	29.6
K11-1178	4129.6	131.2	3	36	29.8
K11-1180	3584.2	118.5	4	38	28.7
K11-1182	3688.0	116.8	3	36	29.7
K11-1184	3480.4	131.2	3	37	29.0
K11-1185	3168.8	124.5	3	37	30.0
K11-1186	4103.6	114.3	3	35	29.3
K11-1190	3584.2	121.1	3	39	29.8
K11-1191	3714.0	124.5	3	37	29.4
K11-1195	3584.2	121.9	3	37	29.0

Table A.6 (continued) Mean value of yield, height, lodging, maturity, and canopy temperature (CT) for genotypes in ENV -2.

Genotypes	Yield	Height	Lodging	Maturity	CT
K11-1196	4675.0	123.6	4	35	29.6
K11-1197	3791.8	128.7	4	37	29.0
K11-1199	3765.8	133.8	4	38	29.5
K11-1200	3218.8	127.8	4	37	30.2
K11-1201	3558.2	121.1	3	38	29.1
K11-1202	3376.4	116.8	4	36	29.3
K11-1203	4285.2	141.4	4	39	28.7
K11-1205	4129.4	120.2	3	38	29.5
K11-1206	3636.0	138.9	3	38	29.1
K11-1207	3610.2	114.3	3	33	29.5
K11-1208	3220.4	126.2	4	36	29.4
K11-1210	3895.8	129.5	4	38	29.2
K11-1211	3506.2	116.0	4	34	29.1
K11-1213	3558.2	125.3	3	39	29.3
K11-1215	4103.8	105.8	3	36	29.6
K11-1217	3428.2	116.8	3	38	28.8

Table A.7 Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -2.

Genotypes	R1-R2				R3-R4			
	R550	GNDVI	RNDVI	NWI	R550	GNDVI	RNDVI	NWI
K11-1151	0.07290	0.81914	0.93005	-0.04064	0.05673	0.82720	0.93649	-0.03831
K11-1152	0.07944	0.81179	0.92245	-0.03709	0.05577	0.82162	0.92687	-0.03810
K11-1153	0.07696	0.81426	0.92977	-0.04303	0.06215	0.80873	0.92792	-0.03768
K11-1155	0.07293	0.82137	0.93162	-0.03681	0.05930	0.81988	0.93551	-0.03125
K11-1156	0.08745	0.79852	0.92160	-0.03773	0.04220	0.85241	0.94593	-0.04344
K11-1157	0.08713	0.78445	0.91118	-0.04307	0.05946	0.81240	0.92688	-0.03989
K11-1158	0.07791	0.80920	0.92761	-0.04349	0.05742	0.82332	0.93266	-0.03852
K11-1160	0.08095	0.81636	0.92730	-0.04000	0.05684	0.82868	0.93432	-0.03819
K11-1161	0.07321	0.82285	0.92991	-0.03751	0.05684	0.82275	0.92971	-0.03750
K11-1162	0.07478	0.80322	0.92287	-0.04263	0.05791	0.81444	0.92932	-0.03979
K11-1163	0.07882	0.81514	0.92858	-0.03753	0.05907	0.82372	0.93213	-0.03515
K11-1164	0.07958	0.80084	0.91758	-0.04379	0.06308	0.81087	0.92196	-0.03597
K11-1165	0.07027	0.81953	0.93239	-0.04445	0.04939	0.84389	0.94093	-0.03890
K11-1167	0.07427	0.82839	0.93109	-0.03895	0.05595	0.82680	0.93530	-0.03405
K11-1168	0.06733	0.80961	0.91902	-0.04567	0.05332	0.82246	0.93023	-0.03660
K11-1169	0.07618	0.81418	0.92315	-0.04306	0.06088	0.81129	0.92311	-0.03592
K11-1170	0.07243	0.82792	0.93213	-0.03906	0.05426	0.82820	0.93511	-0.04015
K11-1171	0.07845	0.81569	0.92291	-0.03817	0.05580	0.82371	0.93464	-0.03893
K11-1172	0.07123	0.80862	0.92756	-0.04425	0.05884	0.81114	0.92567	-0.03399
K11-1173	0.07291	0.81630	0.92548	-0.04055	0.05787	0.80984	0.92303	-0.03647
K11-1175	0.07559	0.81295	0.92845	-0.04189	0.05669	0.81360	0.92994	-0.04109
K11-1176	0.08252	0.81011	0.92593	-0.04001	0.06021	0.82091	0.92922	-0.03473
K11-1177	0.07772	0.81618	0.92315	-0.04298	0.05120	0.84304	0.93856	-0.03999

Table A.7 (continued) Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -2.

Genotypes	R1-R2				R3-R4			
	R550	GNDVI	RNDVI	NWI	R550	GNDVI	RNDVI	NWI
K11-1178	0.06916	0.81005	0.91811	-0.03895	0.05322	0.81931	0.93453	-0.03770
K11-1180	0.08185	0.80978	0.92459	-0.04023	0.06043	0.82214	0.93487	-0.03787
K11-1182	0.07341	0.81632	0.92684	-0.04008	0.05396	0.82956	0.93349	-0.03353
K11-1184	0.06757	0.82882	0.93374	-0.04616	0.05728	0.82426	0.93676	-0.03643
K11-1185	0.08209	0.80279	0.92321	-0.03904	0.04915	0.83318	0.93706	-0.03753
K11-1186	0.07149	0.81772	0.92387	-0.03987	0.05522	0.82340	0.92951	-0.03745
K11-1190	0.07436	0.82221	0.92890	-0.04048	0.05410	0.82787	0.93312	-0.03553
K11-1191	0.07834	0.81026	0.92346	-0.04157	0.04967	0.82925	0.93349	-0.04172
K11-1195	0.06839	0.82601	0.93457	-0.04518	0.05526	0.83369	0.94028	-0.03536
K11-1196	0.06785	0.82800	0.92751	-0.04245	0.04168	0.85791	0.94626	-0.04025
K11-1197	0.06484	0.82873	0.93387	-0.03955	0.04000	0.85864	0.94509	-0.04367
K11-1199	0.05794	0.84367	0.93626	-0.04509	0.05659	0.82455	0.92265	-0.03699
K11-1200	0.07182	0.82429	0.93210	-0.04219	0.06130	0.81868	0.92255	-0.03659
K11-1201	0.07178	0.81674	0.91861	-0.04430	0.06516	0.80418	0.92612	-0.03396
K11-1202	0.07375	0.82134	0.92373	-0.03925	0.04740	0.85288	0.94498	-0.03988
K11-1203	0.06717	0.82305	0.93036	-0.04409	0.05798	0.82243	0.92954	-0.03691
K11-1205	0.06905	0.82213	0.92866	-0.04356	0.06090	0.80775	0.92308	-0.03515
K11-1206	0.07739	0.81209	0.92487	-0.04192	0.05191	0.82100	0.92946	-0.03757
K11-1207	0.07537	0.82215	0.92435	-0.04160	0.04869	0.84760	0.94073	-0.03639
K11-1208	0.07275	0.81624	0.93099	-0.03971	0.05159	0.83428	0.93598	-0.03483
K11-1210	0.07083	0.81621	0.92640	-0.04062	0.05370	0.82922	0.93473	-0.03876
K11-1211	0.07781	0.81290	0.92645	-0.03650	0.06001	0.82414	0.93401	-0.03433
K11-1213	0.08474	0.79686	0.92439	-0.03991	0.05011	0.82981	0.93989	-0.04255
K11-1215	0.07607	0.82103	0.92895	-0.03942	0.05285	0.83517	0.93731	-0.03836
K11-1217	0.08099	0.80276	0.92370	-0.03935	0.06100	0.81469	0.92870	-0.03630

Table A.7 (continued) Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -2.

Genotypes	R5-R6			
	R550	GNDVI	RNDVI	NWI
K11-1151	0.06303	0.82302	0.92436	-0.03883
K11-1152	0.05251	0.84374	0.93680	-0.04033
K11-1153	0.06978	0.80510	0.92273	-0.03969
K11-1155	0.07567	0.79357	0.92115	-0.03218
K11-1156	0.05583	0.82927	0.92596	-0.04334
K11-1157	0.06042	0.81807	0.92441	-0.04244
K11-1158	0.05151	0.84458	0.93377	-0.04134
K11-1160	0.06933	0.80134	0.92152	-0.03723
K11-1161	0.05650	0.84137	0.92568	-0.03950
K11-1162	0.06435	0.82668	0.92588	-0.03773
K11-1163	0.05253	0.83931	0.93437	-0.04296
K11-1164	0.07467	0.81498	0.91905	-0.04124
K11-1165	0.06528	0.80705	0.92456	-0.04491
K11-1167	0.05135	0.84688	0.93631	-0.04642
K11-1168	0.06573	0.81569	0.92121	-0.04099
K11-1169	0.07061	0.80520	0.91409	-0.04019
K11-1170	0.06553	0.82139	0.92478	-0.03746
K11-1171	0.05839	0.83747	0.93101	-0.04164
K11-1172	0.06342	0.81259	0.92154	-0.04114
K11-1173	0.05536	0.83837	0.92747	-0.04069
K11-1175	0.07933	0.80692	0.91326	-0.03593
K11-1176	0.07449	0.80289	0.92094	-0.04187
K11-1177	0.06087	0.82775	0.92534	-0.04270
K11-1178	0.05725	0.83091	0.93206	-0.03708
K11-1180	0.05276	0.84503	0.93476	-0.04505
K11-1182	0.05167	0.84521	0.93558	-0.04146
K11-1184	0.05176	0.84466	0.93601	-0.03978
K11-1185	0.07094	0.81116	0.92102	-0.03594
K11-1186	0.06110	0.81433	0.92412	-0.03815
K11-1190	0.05298	0.84856	0.93468	-0.04076
K11-1191	0.05025	0.85341	0.93895	-0.04032
K11-1195	0.06003	0.83925	0.93253	-0.03700

Table A.7 (continued) Mean value of spectral reflectance indices for genotypes at R1-R2, R3-R4 and R5-R6 in ENV -2.

Genotypes	R5-R6			
	R550	GNDVI	RNDVI	NWI
K11-1196	0.05684	0.83715	0.93267	-0.04001
K11-1197	0.05163	0.84871	0.93432	-0.03864
K11-1199	0.05749	0.83569	0.92402	-0.03757
K11-1200	0.05031	0.83979	0.93263	-0.04584
K11-1201	0.05023	0.84358	0.92943	-0.04269
K11-1202	0.04578	0.86408	0.94529	-0.04403
K11-1203	0.05863	0.83766	0.93095	-0.03861
K11-1205	0.06649	0.80496	0.91664	-0.03788
K11-1206	0.04407	0.85721	0.93695	-0.04737
K11-1207	0.05545	0.83400	0.92931	-0.04135
K11-1208	0.05649	0.83619	0.93339	-0.04295
K11-1210	0.06124	0.83237	0.93291	-0.03711
K11-1211	0.05657	0.83068	0.92787	-0.04382
K11-1213	0.05563	0.83244	0.93097	-0.04833
K11-1215	0.06672	0.81960	0.92935	-0.03624
K11-1217	0.05291	0.83870	0.93437	-0.03811

Table A.8 Mean value of yield, height, lodging, maturity, and sudden death syndrome (SDS) for genotypes in ENV -3.

Genotypes	Yield	Height	Lodging	Maturity	SDS
K11-1151	2084.8	94.6	3	27	4
K11-1152	2142.6	101.0	3	21	4
K11-1153	2239.3	106.0	3	25	3
K11-1155	3028.1	109.2	3	26	2
K11-1156	2248.3	109.9	3	22	3
K11-1157	1810.2	105.4	2	24	4
K11-1158	2317.4	108.0	3	25	3
K11-1160	2513.8	91.4	2	22	3
K11-1161	2430.8	111.1	2	26	3
K11-1162	2401.0	113.7	2	28	3
K11-1163	2188.7	97.8	3	24	4
K11-1164	2452.4	106.0	3	25	4
K11-1165	2277.3	97.2	3	23	4
K11-1167	1687.5	101.0	3	26	4
K11-1168	2031.3	109.2	3	24	4
K11-1169	2021.2	111.1	3	21	4
K11-1170	2042.2	96.5	2	25	4
K11-1171	2352.2	106.7	3	26	4
K11-1172	1846.2	113.0	3	24	4
K11-1173	2257.4	108.6	2	24	4
K11-1175	1720.4	103.5	3	24	4
K11-1176	2112.2	113.7	3	23	4
K11-1177	2400.5	88.3	2	24	3
K11-1178	2165.8	112.4	3	24	4
K11-1180	2255.1	101.0	2	26	4
K11-1182	2331.7	95.9	3	22	3
K11-1184	2318.3	108.0	3	24	3
K11-1185	2050.3	110.5	3	25	4
K11-1186	2340.1	98.4	3	23	3
K11-1190	1997.8	91.4	2	22	4
K11-1191	2207.6	101.0	2	25	4
K11-1195	2049.9	95.3	2	25	3

Table A.8 (continued) Mean value of yield, height, lodging, maturity, and sudden death syndrome (SDS) for genotypes in ENV -3.

Genotypes	Yield	Height	Lodging	Maturity	SDS
K11-1196	2278.1	102.9	3	22	3
K11-1197	2190.3	113.0	3	24	4
K11-1199	2207.8	101.6	3	26	4
K11-1200	2602.7	110.5	3	24	3
K11-1201	1971.4	108.6	2	23	4
K11-1202	2470.9	92.1	3	21	3
K11-1203	1776.9	115.6	3	22	4
K11-1205	2339.3	109.9	2	24	3
K11-1206	2225.1	120.7	2	25	3
K11-1207	1503.9	91.4	3	16	5
K11-1208	1926.0	101.6	3	24	4
K11-1210	1624.3	99.1	3	23	4
K11-1211	2156.5	99.1	3	25	3
K11-1213	2403.2	104.1	3	26	3
K11-1215	2477.0	87.0	2	24	3

Table A.9 Mean value of spectral reflectance indices and canopy temperature (CT) for genotypes at R1-R2 and R3-R4 in ENV -3.

Genotypes	R1-R2					R3-R4				
	R550	GNDVI	RNDVI	NWI	CT	R550	GNDVI	RNDVI	NWI	CT
K11-1151	0.05778	0.83214	0.93404	-0.03039	26.3	0.04714	0.83545	0.93598	-0.03103	30.5
K11-1152	0.05862	0.82561	0.93516	-0.03366	27.4	0.04859	0.84487	0.93842	-0.03321	30.3
K11-1153	0.06187	0.81624	0.93082	-0.03260	27.0	0.05872	0.82400	0.92915	-0.03130	30.7
K11-1155	0.06166	0.81038	0.93001	-0.03276	27.6	0.05166	0.84023	0.93605	-0.03136	29.9
K11-1156	0.05539	0.82849	0.93466	-0.03399	27.2	0.04642	0.84380	0.93461	-0.03361	30.1
K11-1157	0.05993	0.81550	0.93136	-0.03265	27.5	0.05066	0.83602	0.93084	-0.03395	31.4
K11-1158	0.05099	0.82687	0.93186	-0.03249	27.0	0.05412	0.83753	0.93560	-0.03222	29.6
K11-1160	0.05555	0.82974	0.93478	-0.03254	27.4	0.04335	0.83655	0.93472	-0.02930	31.0
K11-1161	0.05691	0.80675	0.91891	-0.02753	26.9	0.05042	0.83362	0.93420	-0.03235	30.1
K11-1162	0.06189	0.80774	0.92611	-0.03197	27.2	0.05187	0.83622	0.93439	-0.03237	30.2
K11-1163	0.05871	0.81582	0.93241	-0.03195	26.7	0.04835	0.83319	0.93569	-0.02966	30.6
K11-1164	0.06037	0.81592	0.92803	-0.03243	26.9	0.05397	0.83017	0.93339	-0.03070	30.2
K11-1165	0.06562	0.81445	0.93296	-0.03307	26.7	0.05310	0.83359	0.93875	-0.03294	30.0
K11-1167	0.05339	0.83628	0.93836	-0.03233	27.4	0.05033	0.83638	0.93071	-0.03027	30.9
K11-1168	0.06313	0.80128	0.92155	-0.03109	26.9	0.04833	0.81587	0.92308	-0.02350	30.2
K11-1169	0.05396	0.82929	0.93086	-0.03420	26.4	0.04471	0.83019	0.92978	-0.03105	30.0
K11-1170	0.05565	0.83204	0.93825	-0.03288	25.9	0.04514	0.83835	0.93466	-0.03077	30.5
K11-1171	0.05545	0.82604	0.92928	-0.03304	26.3	0.05551	0.84013	0.93405	-0.03540	29.8
K11-1172	0.05148	0.81785	0.93079	-0.03145	27.0	0.04457	0.82793	0.93170	-0.02755	30.0
K11-1173	0.05672	0.82138	0.92652	-0.02947	27.1	0.05310	0.83466	0.92817	-0.03196	30.3
K11-1175	0.05555	0.82474	0.93337	-0.03131	27.3	0.04975	0.82452	0.93348	-0.02828	30.4
K11-1176	0.06274	0.81874	0.93502	-0.03540	26.1	0.05522	0.83009	0.93492	-0.03363	30.6
K11-1177	0.05740	0.82627	0.93530	-0.03264	26.7	0.04242	0.82761	0.92787	-0.02936	31.0

Table A.9 (continued) Mean value of spectral reflectance indices and canopy temperature (CT) for genotypes at R1-R2 and R3-R4 in ENV -3.

Genotypes	R1-R2					R3-R4				
	R550	GNDVI	RNDVI	NWI	CT	R550	GNDVI	RNDVI	NWI	CT
K11-1178	0.06247	0.80779	0.92120	-0.03126	27.0	0.05182	0.82265	0.92629	-0.02828	31.1
K11-1180	0.05693	0.82811	0.93301	-0.03134	26.1	0.04933	0.83652	0.93567	-0.03552	30.4
K11-1182	0.05731	0.82598	0.93396	-0.03192	27.6	0.04607	0.84156	0.93498	-0.02980	30.6
K11-1184	0.05599	0.82346	0.93195	-0.03248	27.5	0.04712	0.83912	0.93488	-0.03502	30.1
K11-1185	0.05911	0.82195	0.92735	-0.03133	27.1	0.04893	0.83968	0.93429	-0.03131	31.4
K11-1186	0.05196	0.82852	0.93369	-0.03372	27.4	0.04780	0.83712	0.93269	-0.03007	31.1
K11-1190	0.05072	0.84510	0.94218	-0.03190	27.6	0.04755	0.83713	0.93673	-0.02854	30.4
K11-1191	0.04872	0.81647	0.91244	-0.02757	26.2	0.04692	0.84835	0.94168	-0.03229	31.6
K11-1195	0.05439	0.83559	0.93559	-0.03112	27.4	0.05453	0.83601	0.93440	-0.02996	30.4
K11-1196	0.05720	0.82567	0.93483	-0.03105	26.7	0.04421	0.84431	0.93365	-0.03326	30.1
K11-1197	0.05558	0.82585	0.93719	-0.03364	26.5	0.05083	0.83267	0.93000	-0.03131	30.7
K11-1199	0.05691	0.82149	0.92395	-0.03180	27.4	0.05036	0.82787	0.92449	-0.02933	30.9
K11-1200	0.05677	0.82503	0.93148	-0.03253	26.7	0.04693	0.83395	0.92879	-0.03226	30.4
K11-1201	0.06105	0.82243	0.92933	-0.03255	26.4	0.04765	0.84015	0.93250	-0.03085	31.1
K11-1202	0.05749	0.82588	0.93149	-0.03177	26.5	0.04415	0.84339	0.93720	-0.03045	30.6
K11-1203	0.05241	0.83053	0.93454	-0.03601	27.3	0.04950	0.81965	0.92767	-0.03019	30.3
K11-1205	0.05925	0.81910	0.93192	-0.03247	26.7	0.05444	0.82883	0.92847	-0.03044	30.5
K11-1206	0.05819	0.81659	0.92080	-0.03620	26.5	0.05310	0.83008	0.92541	-0.03456	30.3
K11-1207	0.05824	0.82609	0.92981	-0.02962	27.2	0.04568	0.83954	0.93204	-0.02945	31.5
K11-1208	0.05569	0.81823	0.93255	-0.03115	27.0	0.04911	0.84203	0.93786	-0.02815	30.5
K11-1210	0.05978	0.82158	0.93672	-0.03299	27.2	0.05227	0.83160	0.93530	-0.03155	31.2
K11-1211	0.05095	0.82755	0.93016	-0.03065	27.2	0.04021	0.85177	0.94235	-0.02685	30.7
K11-1213	0.06155	0.82145	0.93327	-0.02965	26.6	0.05082	0.83819	0.93838	-0.03092	30.0
K11-1215	0.05262	0.83646	0.93825	-0.03276	27.9	0.04512	0.85351	0.94128	-0.03198	29.8
K11-1217	0.05705	0.82255	0.93536	-0.03439	26.7	0.06102	0.82969	0.93243	-0.03003	30.9

Table A.10 Mean value of yield, height, lodging, and maturity for genotypes in ENV -4.

Genotypes	Yield	Height	Lodging	Maturity
K11-1151	3981.9	111.1	3	31
K11-1152	3512.0	107.3	3	29
K11-1153	3747.8	108.6	3	32
K11-1155	3873.8	107.3	3	34
K11-1156	3957.2	104.8	3	32
K11-1157	3168.0	107.3	3	28
K11-1158	3497.7	106.7	3	26
K11-1160	3630.0	99.7	3	29
K11-1161	3860.3	114.3	3	31
K11-1162	3839.3	113.0	3	36
K11-1163	3853.8	102.9	3	28
K11-1164	3345.9	113.0	3	27
K11-1165	3750.2	105.4	3	28
K11-1167	3720.6	108.6	3	32
K11-1168	3765.5	118.7	3	35
K11-1169	3566.4	110.5	3	29
K11-1170	3684.6	106.7	3	28
K11-1171	3570.3	106.7	3	34
K11-1172	3894.6	122.6	3	35
K11-1173	3910.8	114.9	3	33
K11-1175	3502.7	111.1	3	32
K11-1176	3740.8	116.2	3	26
K11-1177	3623.4	99.1	3	28
K11-1178	4017.3	118.1	3	29
K11-1180	3494.3	102.9	3	35
K11-1182	3864.0	101.6	3	29
K11-1184	3673.9	109.9	3	31
K11-1185	3633.3	109.9	3	28
K11-1186	3858.3	104.1	3	28
K11-1190	4120.6	101.0	3	32
K11-1191	3424.4	105.4	3	28
K11-1195	3804.0	99.7	2	31

Table A.10 (continued) Mean value of yield, height, lodging, and maturity for genotypes in ENV -4.

Genotypes	Yield	Height	Lodging	Maturity
K11-1196	3565.4	104.8	3	27
K11-1197	3621.6	117.5	3	28
K11-1199	3594.2	114.3	3	34
K11-1200	3952.1	114.9	3	29
K11-1201	3606.1	113.7	3	31
K11-1202	3282.0	92.7	2	25
K11-1203	3503.1	111.8	3	31
K11-1205	3679.6	111.1	3	32
K11-1206	3521.4	120.0	3	28
K11-1207	3583.8	101.0	3	27
K11-1208	3370.1	109.9	3	29
K11-1210	3817.4	111.1	3	34
K11-1211	3239.6	99.7	3	29
K11-1213	3836.3	111.1	3	35
K11-1215	3649.7	91.4	2	28
K11-1217	3314.6	98.4	3	30

Table A.11 Mean value of spectral reflectance indices and canopy temperature (CT) for genotypes at R1-R2 and R5-R6 in ENV -4.

Genotypes	R1-R2					R5-R6				
	R550	GNDVI	RNDVI	NWI	CT	R550	GNDVI	RNDVI	NWI	CT
K11-1151	0.06158	0.79623	0.91686	-0.02105	32.6	0.05234	0.79861	0.90819	-0.03322	25.5
K11-1152	0.06099	0.78762	0.90086	-0.01964	33.5	0.05744	0.76582	0.89062	-0.03088	26.1
K11-1153	0.06636	0.79163	0.91347	-0.02395	33.3	0.06019	0.76918	0.89090	-0.03278	26.2
K11-1155	0.05999	0.78122	0.89671	-0.01464	33.7	0.04728	0.79680	0.90227	-0.03480	25.4
K11-1156	0.05737	0.79382	0.90943	-0.01838	33.3	0.04956	0.80029	0.90740	-0.03371	25.9
K11-1157	0.06132	0.79052	0.90814	-0.01918	33.0	0.05769	0.76210	0.88247	-0.03290	26.4
K11-1158	0.06067	0.77994	0.89513	-0.01848	33.0	0.04585	0.78144	0.89154	-0.03372	25.7
K11-1160	0.06315	0.79032	0.90650	-0.02009	34.5	0.05391	0.77324	0.88401	-0.04128	25.9
K11-1161	0.06357	0.79922	0.91486	-0.01935	33.3	0.04700	0.80318	0.90178	-0.03670	26.9
K11-1162	0.06263	0.77902	0.89958	-0.01796	33.2	0.05234	0.78518	0.89964	-0.03619	25.5
K11-1163	0.06338	0.78622	0.90426	-0.02335	33.1	0.04793	0.79231	0.90428	-0.03627	25.9
K11-1164	0.05678	0.80114	0.91144	-0.02222	33.1	0.05593	0.75501	0.87512	-0.03664	27.4
K11-1165	0.06308	0.79095	0.91551	-0.02263	33.8	0.05180	0.78514	0.90833	-0.03708	26.4
K11-1167	0.05616	0.80424	0.90863	-0.01930	33.7	0.05134	0.79465	0.89823	-0.03448	25.7
K11-1168	0.06944	0.76396	0.89577	-0.01976	32.9	0.05410	0.75929	0.88447	-0.03287	26.1
K11-1169	0.05740	0.78908	0.90452	-0.02115	33.3	0.04653	0.78003	0.89078	-0.03780	25.7
K11-1170	0.05785	0.78190	0.89384	-0.01914	32.9	0.05491	0.78550	0.89721	-0.03577	27.1
K11-1171	0.06544	0.77938	0.90018	-0.01828	34.1	0.04721	0.79122	0.89285	-0.03342	25.9
K11-1172	0.06009	0.77968	0.89738	-0.01758	33.6	0.04990	0.79156	0.90030	-0.03520	25.6
K11-1173	0.05426	0.80113	0.90802	-0.02025	33.3	0.04949	0.79089	0.89672	-0.03130	25.1
K11-1175	0.06366	0.78809	0.90483	-0.01781	33.9	0.05268	0.78151	0.90061	-0.03616	25.7
K11-1176	0.06378	0.77356	0.89861	-0.01799	33.8	0.06215	0.74815	0.88967	-0.03053	26.8
K11-1177	0.06097	0.79560	0.91079	-0.02043	33.3	0.05672	0.77475	0.88259	-0.03450	26.5

TableA.11 (continued) Mean value of spectral reflectance indices and canopy temperature (CT) for genotypes at R1-R2 and R5-R6 in ENV -4.

Genotypes	R1-R2					R5-R6				
	R550	GNDVI	RNDVI	NWI	CT	R550	GNDVI	RNDVI	NWI	CT
K11-1178	0.06746	0.78023	0.90155	-0.02272	33.0	0.04816	0.80518	0.90528	-0.03564	25.5
K11-1180	0.06012	0.79381	0.90254	-0.02117	34.5	0.05025	0.79488	0.89984	-0.03781	26.6
K11-1182	0.06098	0.78060	0.90175	-0.01944	34.6	0.05260	0.78528	0.89871	-0.03914	26.3
K11-1184	0.05255	0.78318	0.89007	-0.01673	33.3	0.05007	0.78861	0.90166	-0.03755	25.9
K11-1185	0.05440	0.78388	0.89088	-0.01776	34.2	0.05687	0.76060	0.87474	-0.03269	25.9
K11-1186	0.05642	0.80164	0.91382	-0.01741	33.8	0.05379	0.77467	0.89449	-0.03162	26.3
K11-1190	0.05287	0.80741	0.91253	-0.02069	33.7	0.04191	0.83739	0.92493	-0.03425	26.0
K11-1191	0.05940	0.79041	0.90432	-0.01726	33.4	0.04581	0.80844	0.90399	-0.03518	26.6
K11-1195	0.06047	0.78248	0.90021	-0.01912	34.1	0.04626	0.80387	0.90216	-0.03125	26.2
K11-1196	0.05733	0.79267	0.90553	-0.02166	33.9	0.05150	0.79130	0.90183	-0.03145	26.2
K11-1197	0.05511	0.80006	0.91242	-0.02074	32.6	0.04490	0.80921	0.90535	-0.03569	25.6
K11-1199	0.05615	0.78753	0.89661	-0.01744	33.6	0.05166	0.78966	0.89112	-0.03238	25.9
K11-1200	0.05374	0.76873	0.88657	-0.01474	32.7	0.05540	0.78132	0.89213	-0.03517	26.5
K11-1201	0.06144	0.78128	0.89834	-0.01972	33.1	0.05522	0.78511	0.89214	-0.03404	26.5
K11-1202	0.05703	0.78916	0.89642	-0.01553	34.2	0.05028	0.79891	0.90040	-0.03498	26.5
K11-1203	0.06295	0.77137	0.88583	-0.02131	33.7	0.04892	0.80006	0.89767	-0.03559	25.9
K11-1205	0.05870	0.77488	0.89394	-0.01898	34.2	0.04885	0.80422	0.90425	-0.03369	25.8
K11-1206	0.05461	0.80072	0.90067	-0.01957	33.3	0.04407	0.80673	0.89901	-0.03947	26.0
K11-1207	0.05848	0.79162	0.89947	-0.01698	33.1	0.05603	0.77446	0.88583	-0.03314	26.7
K11-1208	0.06120	0.78256	0.89917	-0.01612	33.6	0.05073	0.78448	0.89617	-0.03483	26.3
K11-1210	0.06209	0.78015	0.90465	-0.02230	33.3	0.05676	0.78664	0.90136	-0.03288	26.0
K11-1211	0.05146	0.80003	0.90441	-0.01798	34.2	0.05380	0.77696	0.89143	-0.03321	26.2
K11-1213	0.05931	0.79150	0.90230	-0.01781	33.8	0.05818	0.78992	0.90289	-0.03057	25.8
K11-1215	0.05696	0.78702	0.89322	-0.01656	33.5	0.06164	0.77315	0.89469	-0.03057	27.0
K11-1217	0.06122	0.77462	0.89389	-0.01854	34.1	0.04901	0.79282	0.90109	-0.03169	25.9

Appendix B - Supplementary Data

Table B.1 Mean SCN soil population density (egg and J2/100 cm³ soil) at planting for four cultivars and environment.

Environment	5002T	5601T	KS5004N	KS5502N	Mean
ENV-1	224	425	323	241	303
ENV-2	506	230	331	304	343
ENV-3	2530	2387	2941	2264	2530

Table B.2 Mean SCN soil population density at planting (egg and J2/100 cm³ soil) for four cultivars and environment.

Environment	5002T	5601T	KS5004N	KS5502N	Mean
ENV-1	8459	7735	10142	320	6664
ENV-2	5430	5445	1981	123	3244
ENV-3	10788	7067	4741	1771	6091

Table B.3 Mean value of yield (kg ha⁻¹) for each cultivar within environment.

Environment	5002T	5601T	KS5004N	KS5502N	Mean
ENV-1	1831.2	1714.3	2010.2	2446.6	2000.6
ENV-2	2916	3058.7	4001.0	3615.7	3397.9
ENV-3	2742	1951.8	2860.3	3700	2813.5

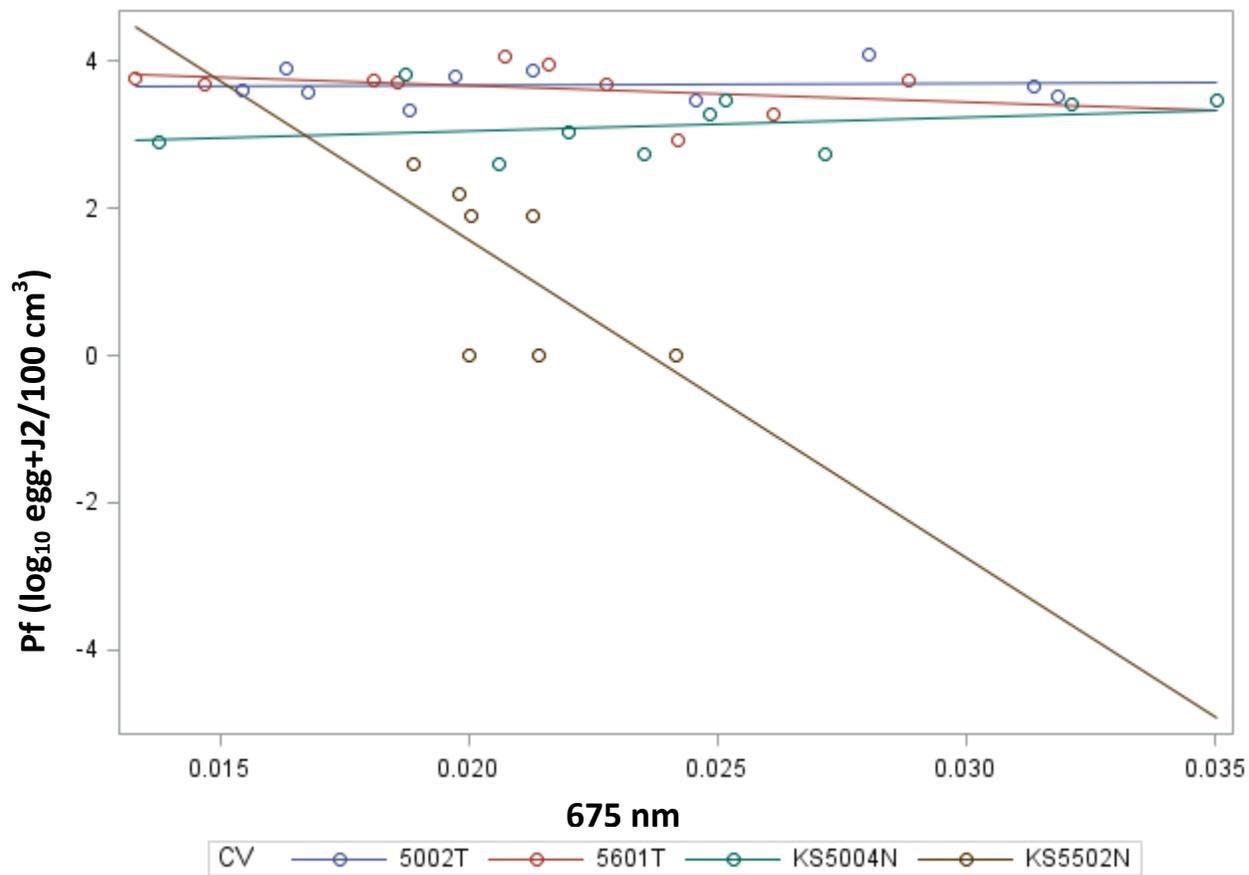


Figure B.1 Relationship between reflectance at 675 nm and SCN soil population density at harvest (Pf) in ENV-2 (6 August 2013).

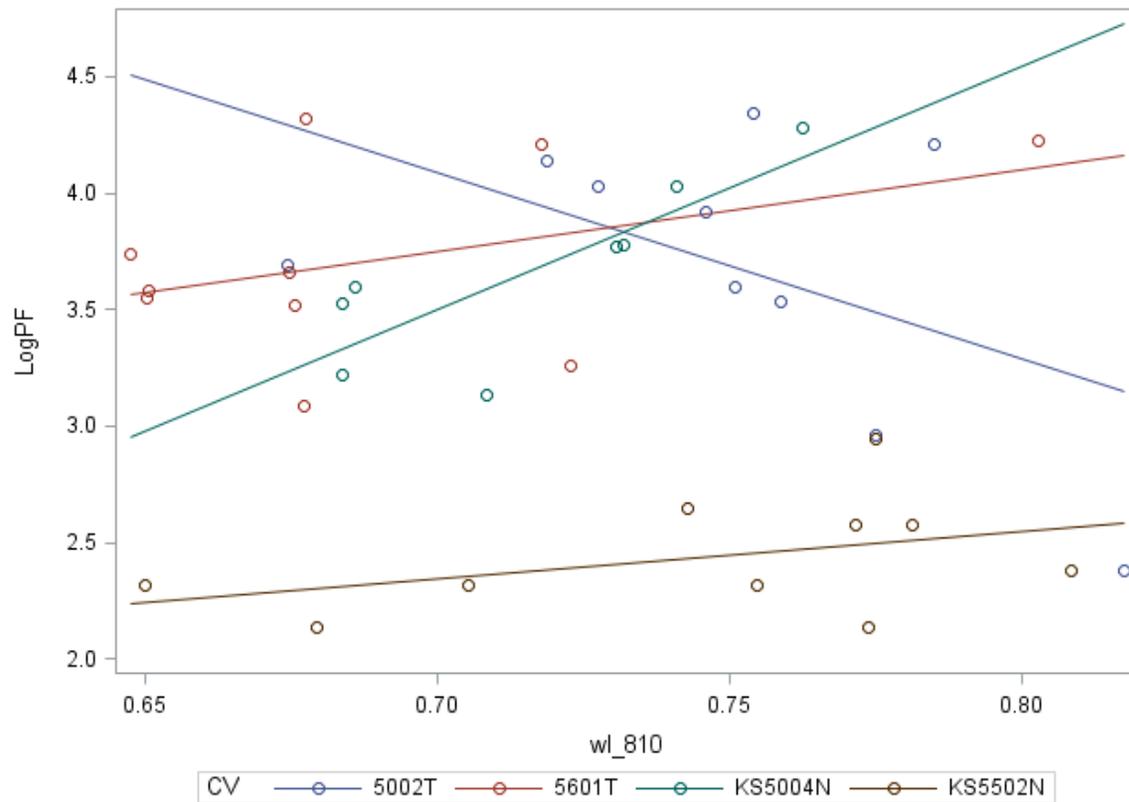


Figure B.2 Relationship between reflectance at 810 nm and SCN soil population at harvest (Pf) among cultivars in ENV-1 (9 August 2012).

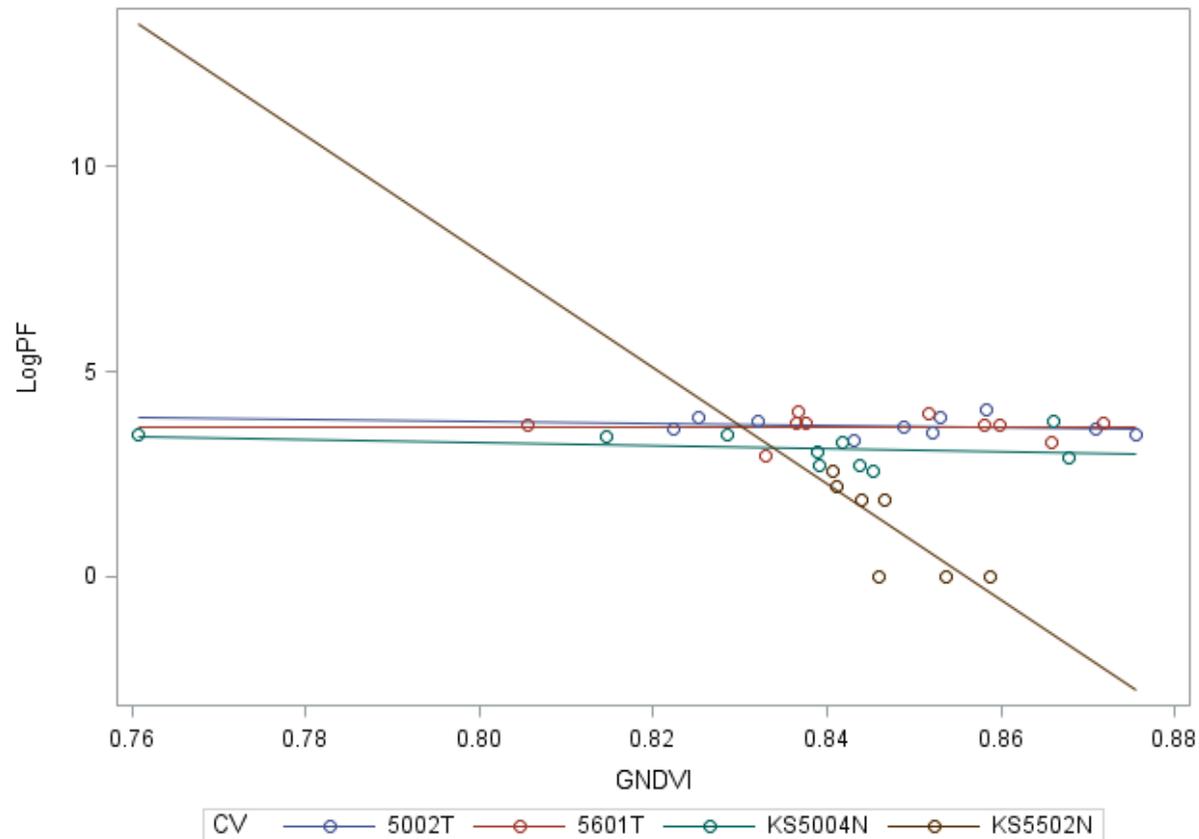


Figure B.3 Relationship between green normalized difference vegetation index (GNDVI) and SCN soil population at harvest (Pf) among cultivars in ENV-2 (6 August 2013).

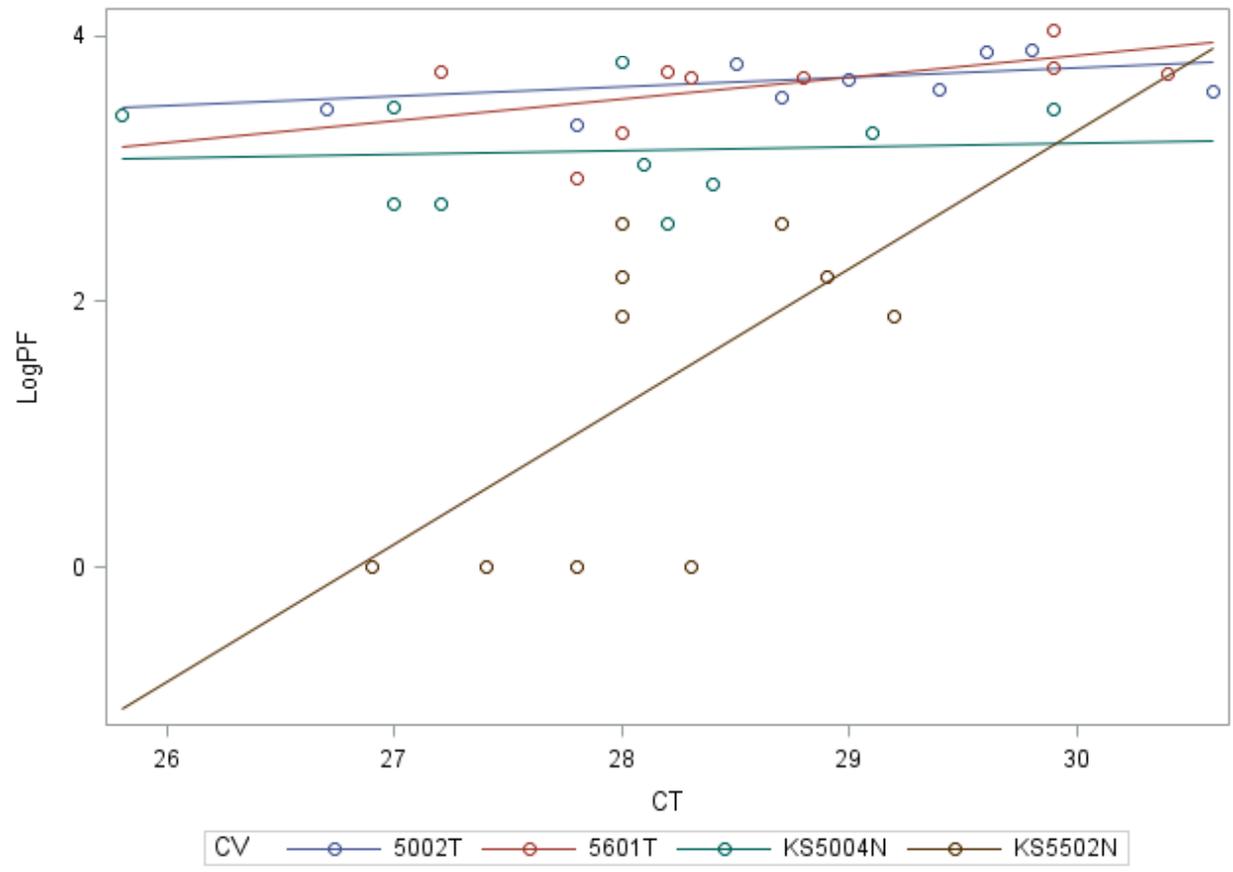


Figure B.4 Relationship between canopy temperature (CT) and SCN soil population density (Pf) on 18 August 2013 in ENV-2.

Table B.4 Mean of GNDVI, reflectance at 675 and 810 nm for each cultivar on each sampling day in ENV-1 (2012).

GNDVI				
Cultivars	July 25	August 9	August 21	September 19
5002T	0.8124	0.8626	0.8675	0.6751
5601T	0.8241	0.8576	0.8701	0.7063
KS5004N	0.8252	0.8689	0.8777	0.6924
KS5502N	0.8277	0.8684	0.8657	0.7409
675 nm				
Cultivars	July 25	August 9	August 21	September 19
5002T	0.0297	0.0237	0.0146	0.0370
5601T	0.0312	0.0224	0.0129	0.0309
KS5004N	0.0298	0.0185	0.0121	0.0296
KS5502N	0.0293	0.0229	0.0152	0.0335
810 nm				
Cultivars	July 25	August 9	August 21	September 19
5002T	0.7665	0.7507	0.5627	0.3984
5601T	0.7903	0.6896	0.5260	0.3619
KS5004N	0.7503	0.5728	0.4950	0.3445
KS5502N	0.8298	0.7441	0.5561	0.4411

Table B.5 Mean of GNDVI, reflectance at 675 and 810 nm for each cultivar on each sampling day in ENV-2 (2013).

GNDVI				
Cultivars	6 August	18 August	20 August	30 August
5002T	0.8481	0.8294	0.8204	0.8275
5601T	0.8457	0.8328	0.8096	0.8335
KS5004N	0.8346	0.8554	0.8467	0.8447
KS5502N	0.8473	0.8404	0.8344	0.8516
675 nm				
Cultivars	6 August	18 August	20 August	30 August
5002T	0.0234	0.0279	0.0265	0.0226
5601T	0.0218	0.0240	0.0252	0.0195
KS5004N	0.0253	0.0200	0.0198	0.0181
KS5502N	0.0153	0.0248	0.0229	0.0207
810 nm				
Cultivars	6 August	18 August	20 August	30 August
5002T	0.7188	0.6648	0.5143	0.5421
5601T	0.6666	0.6047	0.5587	0.5008
KS5004N	0.6710	0.6047	0.5190	0.4764
KS5502N	0.4773	0.6895	0.5587	0.5533

Table B.6 Mean of GNDVI, reflectance at 675 and 810 nm for each cultivar on each sampling day in ENV-3 (2013).

GNDVI				
Cultivars	1 August	17 August	23 August	1 September
5002T	0.8207	0.8445	0.8379	0.8232
5601T	0.8288	0.8334	0.8305	0.7971
KS5004N	0.8419	0.8663	0.8518	0.8119
KS5502N	0.8263	0.8565	0.8613	0.8472
675 nm				
Cultivars	1 August	17 August	23 August	1 September
5002T	0.0208	0.0215	0.0220	0.0223
5601T	0.0183	0.0190	0.0189	0.0227
KS5004N	0.0174	0.0156	0.0171	0.0221
KS5502N	0.0190	0.0175	0.0166	0.0211
810 nm				
Cultivars	1 August	17 August	23 August	1 September
5002T	0.5694	0.6051	0.5901	0.5535
5601T	0.5443	0.4945	0.4795	0.4142
KS5004N	0.5375	0.5034	0.4854	0.4596
KS5502N	0.5907	0.5871	0.5598	0.5610

Table B.7 HG type determinations for field populations of *Heterodera glycines*.

Location	Cysts on Lee	Female Index							HG Type
		PI 548402	PI 88788	PI 90763	PI 437654	PI 20932	PI 89772	PI 548316	
Ashland	622	5.1	18.1	1.6	0.4	25.2	1.7	40.6	2.5.7
Rossville	430	10.9	54.3	2.7	0.2	63.0	3.0	59.5	1.2.5.7