CHARACTERIZATION OF PHYSIOLOGICAL PARAMETERS IN SOYBEAN WITH GENETIC IMPROVEMENT IN SEED YIELD

by

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Abstract

Recent results from a genetic gain study have illustrated the contribution of plant breeding to the improvement in seed yield of soybean (Glycine max (L.) Merr.). The objective of this research was to characterize the changes in several physiological parameters that have occurred in the released cultivars with improvement of seed yield. Sixty maturity group III and 54 maturity group IV cultivars, released from the 1920's through 2010, were evaluated in dryland and irrigated environments at Manhattan, KS in 2010 and 2011. Genotypes were planted in four-row plots, 3.4 m long, spaced 76 cm apart, arranged in a randomized complete block design with four replications. Genotypes were evaluated for canopy temperature, leaf chlorophyll content, pollen germination, leaf chlorophyll fluorescence, leaf antioxidants, and yield components. Canopy temperature measurements were captured between 1000h and 1400h using an infrared camera multiple times from R1 continuing through R6. Leaf chlorophyll content was measured using a SPAD meter several times from R1 through R6. In vitro pollen germination was measured using incubation temperatures of 28 and 34° C, beginning at late R1 through the end of flowering. Leaf chlorophyll fluorescence was measured beginning at R1 through R6. Leaf antioxidants were analyzed for total antioxidant capacity and electrolyte leakage by collecting leaves from the top 3rd to 5th trifoliate at R4 and R6. Yield components were analyzed from a hand harvested 0.33 m section of one border row. Genotypes differed significantly for canopy temperature, leaf chlorophyll content, pollen germination, and yield components. No significant differences were found for leaf chlorophyll fluorescence or leaf antioxidants. Seed yield increased with year of release. Canopy temperature was negatively correlated and leaf chlorophyll content was positively correlated with year of release in both maturity groups. No significant correlation with year of release was found for in vitro pollen germination or

electrolyte leakage. Leaf chlorophyll fluorescence, yield components, and total antioxidant capacity was positively correlated in the maturity group IV genotypes with year of release. Evaluation of these parameters may serve as a basis to select for seed yield, or to assess the abiotic stress tolerance of a genotype.

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Thanks to my family and friends for their support throughout my life and college.

Dedication

This work is dedicated to my family, for their love and support. All praise and glory belongs to my Heavenly Father, for it is through Him that all things are possible.

CHAPTER 1 - Literature Review

Introduction

Food production and security of food for the global population is one of the top challenges that face the agriculture industry. The United Nations expects the world population to increase to about 10 billion by year 2050 (United Nations, 2011). The increasing population places more pressure on resources for food production and a reliance on agricultural research to increase the efficiency and output of production agriculture. Soybeans (*Glycine max* (L.) Merr.) are used in a wide range of products from animal and human food products to industry oils and solvents. Worldwide, soybeans are the largest source of high protein feed for animals and the second largest source of vegetable oil (USDA-ERS, 2012). Recent worldwide grain production, from 2010/2011, consisted of 2,197.1 million metric tons (MMT) of total grains and 456.5 MMT of all oilseeds (FAS, 2012). Of all the oilseeds, soybeans represented 264.7 MMT with the United States as the leading producer at 90.61 MMT, followed by Brazil, Argentina, China, and India. As shown in Figure A.1, the United States averaged 2,791 kilograms of soybeans per hectare in 2011 and 2,925 kilograms per hectare in 2010, which were both slightly below the highest national average set in 2009 of 2,959 kilograms per hectare (USDA-NASS, 2012). In 2010, Kansas ranked 10th out of all states in soybean production with 3.76 MMT produced with a state average yield of 2,186 kilograms per hectare (Kansas Farm Facts, 2011). Figure A.1 also illustrates that between 1924 and 2011, soybean seed yield increased an average of 23.4 kilograms per hectare per year.

Genetic Improvement

Cox et al. (1988) illustrated the value of evaluating cultivars released over time in a common environment for the best estimate of genetic gain and reported an increase in grain yield of wheat per year from 0.4% under drought to 0.6% in high production environments. Voldeng et al. (1997) found an increase of 0.5% of soybean seed yield and harvest index per year since 1930 on short season cultivars in Canada and an improvement of 0.7% per year when looking at cultivars from 1976 on. Green et al. (2012) found the rate of genetic yield improvement per year to range from 0.56% to 1.4% between the high and low yielding controlled environments in soft red winter wheat. In evaluating regional performance nurseries of winter wheat, Graybosch and Peterson (2010) found genetic yield improvement of 0.79% to 1.1% per year for grain yield.

Physiological Parameters

There have been many studies that link changes in physiological traits to the improvement found from increased seed yield. Morrison et al. (1999) found a positive relationship with photosynthesis, stomatal conductance, and chlorophyll concentration with an increase in seed yield of soybean. Jin et al. (2010) found a positive correlation between soybean seed yield and year of release with an average increase of 0.58% annually and a 0.59% increase per year in photosynthesis of cultivars. Reduced plant height and lodging have also been noted in soybeans with improvement in seed yield (Jin et al., 2010). Lopes et al. (2012) found genetic yield gains of spring wheat to be 0.7% per year in Mexico and a strong relationship with physiological traits, such as cooler canopy temperatures at grain filling, increased stay-green, earlier heading, and increased thousand kernel weight. Xiao et al. (2012) found an increase of 62 kg per hectare per year of genetic gains in grain yield of winter wheat in China with

improvements in physiological traits such as chlorophyll content, canopy temperature depression, and photosynthetic rate.

Pollen Germination

Soybeans tend to abort a large percentage of flowers and pods throughout the growing season (van Schaik and Probst, 1958; Jiang and Egli, 1993). These reports have led to suggestions that the seed yield for soybeans can be enhanced by reducing factors that limit pod and seed set (Hansen and Shibles, 1978). Improving pollen germination may have a positive impact on pod set and seed yield.

One of the first procedures for a simple and reproducible medium for in vitro pollen germination for soybeans was reported by Gwata et al. (2003) in a study that examined pollen germination in nodulating and nonnodulating soybean genotypes. Comparisons were made between a nodulating wild-type soybean, "Bragg", and an EMS-derived mutant Bragg line, Nod 139, which was nonnodulating. Significant genotypic differences were found with the nodulating genotype having 20% higher pollen germination than the nonnodulating genotype. No other agronomic or physiological measurements were presented.

In vitro pollen germination was used in a study by Koti et al. (2004) to show the response of enhanced ultraviolet-B radiation to soybean pollen. Six soybean genotypes were grown in pots placed within soil-plant-atmosphere research (SPAR) chambers and treated with four intensities of UV-B radiation. Flowers were collected from plants in the SPAR chambers and allowed to air dry for 2 hours. Pollen was extracted from the flowers and placed on a growing medium modified from Gwata et al. (2003). Pollen was incubated at 30°C and analyzed under a microscope to determine germination percentages and pollen tube length. Pollen germination and pollen tube length showed that there were significant reductions ($P<0.001$) at high levels of UV-

B radiation, but genotypes also differed significantly ($P<0.001$) in the amount of reduction to UV-B radiation. Pollen germination ranged from 72 to 92% and pollen tube lengths ranged from 187 to 329 µm among treatments. Pollen number per anther was reduced at a significant level ($P<0.001$) compared to the control plants, showing that pollen production is decreased with increased UV-B radiation. This study suggests that UV-B radiation damages reproductive efficiency and will have a direct effect on fruit set in sensitive varieties. With significantly different sensitivity to UV-B radiation among genotypes, it allows the opportunity to select for UV-B tolerant genotypes for breeding purposes and proves the successful use of the Gwata et al. (2003) medium and procedure on soybean.

Kakani et al. (2005) evaluated twelve diverse cotton cultivars grown in a field for pollen germination and pollen tube elongation. The flowers were collected from the first fruiting position on ten plants per cultivars. Three flowers were used to sprinkle pollen on three petri dishes of growth medium for each genotype at each of eight different temperature treatments. Pollen germination was measured through a microscope by counting germinated pollen grains, grains that have a pollen tube equal to or greater than the grain diameter that were in the view scope and divided that by the total number of pollen grains. Pollen tube length was measured from 20 pollen tubes per petri dish by an ocular micrometer attached to the microscope. Pollen germination differed significantly among cultivars and ranged from 33 to 60%, with a mean of 44%. Pollen tube length showed significant differences among cultivars at optimum temperatures and ranged from 605 µm to 903 µm, with an average of 778 µm. It was suggested that in vitro pollen germination could be used to screen cultivars for high-temperature tolerance, although more work would be needed in order to verify the performance of these selections in high temperature environments (Kakani et al., 2005).

Salem et al. (2007) conducted a study to screen soybean pollen for high temperature tolerance. Forty-four soybean cultivars in maturity groups III to VI were grown in pots at an outdoor facility. Pollen germination and pollen tube lengths were measured under eight different temperature treatments ranging from 15 to 50 ± 0.2ºC at 5 ºC intervals. They observed significant variation among genotypes with pollen germination ranging from 70% up to 93%.

Kakani et al. (2002) examined pollen germination in 21 diverse genotypes of groundnut. Pollen samples were subjected to a total of sixteen temperature treatments ranging from 10 to 47.5°C at 2.5°C intervals. Measurements were made every 45 minutes up until 240 minutes after germination to show the growth rate. Genotypic differences in pollen germination ranged from 35% to 73% with an average of 56%. Pollen tube maximum length ranged from 410 μm to >1400 μm with growth rates of 4.5 μm min^{-1} to > 25 μm min^{-1}. These studies provided evidence for genotypic variability in pollen viability and pollen tube growth rate subjected to different temperature regimes.

Canopy Temperature

Early work with infrared thermometers has proven successful in monitoring evapotranspiration rates in crops (Stone and Horton, 1974) and in the observation of daily crop temperatures (Blad and Rosenberg, 1976). Stone and Horton used an infrared thermometer to measure canopy temperature of sorghum. The Bartholic-Namken-Wiegand method, compared to traditional methods, was evaluated with the use of canopy temperature, air temperature, soil heat flux, and net radiation to provide estimates of crop evapotranspiration rates for large areas and showed promising potential (Stone and Horton, 1974).

Blad and Rosenberg (1976) compared crop surface temperatures measured with an infrared thermometer to plant temperatures measured with leaf thermocouples throughout the

course of a day. Results found that measurements performed by infrared thermometers produced accurate readings when the canopy was completely full. When canopy coverage was not full, heat from the exposed soil affected the readings of the plant surfaces. Leaf thermocouples were not accurate enough due to the large number of thermocouples needed to get an accurate average temperature for the plant. In evaluating the temperatures over the day, the surface temperature of alfalfa was shown to remain cooler than the air temperature during the afternoons, and sometimes all day, by as much as 7°C. Measurements of corn was also similar, but was usually warmer than the air temperature except during late afternoon hours.

Hatfield et al. (1984) evaluated methods for estimating evapotranspiration rates over multiple locations and on several crops with the use of remotely sensed canopy temperature. Their findings show that surface energy balance models can use canopy temperatures as an input to provide a method for measuring actual evapotranspiration rates from crops (Hatfield et al., 1984).

Breeding programs have been evaluating possible uses of infrared thermal sensing for decades. Leaf and canopy temperatures have been fast and easy to measure with infrared thermometers and have been related to plant water stress. Blum et al. (1982) evaluated barley strains and wheat cultivars for leaf temperatures along with leaf water potential measurements. Measurements were made during vegetative growth stages in October on three different days as water stress increased. Leaf temperatures were measured with Barnes “Instatherm” infrared thermometer aimed at each plot’s canopies with a target area diameter of 30 cm. The results showed significant variations in leaf temperatures and a significant correlation, across strains, between leaf temperature and leaf water potential. This study indicates that infrared thermal

sensing of canopy temperatures, in wheat and barley, can be used for screening entries for dehydration avoidance when analyzed under soil moisture stress (Blum et al., 1982).

Putting canopy temperature to use for detecting genetic diversity in field grown soybeans, Harris et al. (1984) used a hand-held infrared thermometer to evaluate 20 soybean genotypes by measuring leaf canopy temperatures. A diverse group of genotypes were selected with maturity groups ranging from groups III to V consisting of both indeterminate and determinate growth habits. Canopy temperature differentials were found by subtracting the air temperature from the canopy temperature and were used in the analysis. Significant differences in the average canopy temperature differentials were found across years. Significant correlation was found between air temperature and canopy temperature during both growing seasons. Significant differences between varieties occurred on almost half of the days measured in both years of the study. A negative correlation between canopy temperature differentials and seed yield were found to be significant for both irrigated and dryland plots for the first year, 1980. In the second year, 1981, there were no significant correlations detected between canopy temperature differentials and seed yield due to the wetter and cooler than normal temperatures that occurred that growing season. Results from this study show that when evaporative demands are relatively high measurements of canopy temperature differentials will tend to be more highly correlated with seed yield, compared to years when evaporative demands are relatively low. With the proper set up and growing season climate, infrared thermometers may be successfully implemented into breeding programs for selection purposes.

McKinney et al. (1989a) evaluated the use of canopy temperature for selections in a soybean breeding program, within six soybean populations by using a hand-held infrared thermometer. Measurements on single plants of early generations, F_1 and F_2 planted in hill plots

and F_3 grown in non-replicated rows, showed low heritability estimates and only one population was significant in the F_2 and F_3 generations. Of the $F_{3:4}$, which were grown in non-replicated rows, and $F_{3:6}$, which were grown in rows replicated three times, showed three of the six populations were significant in heritability estimates. The weather was warmer and drier than normal for the growing seasons and did not create any difficulties in the measurements. The results suggest that replicated tests are more effective than using non-replicated rows or single plants for measuring canopy temperature. In another test by McKinney et al. (1989b), evaluations were made on thirty elite soybean lines and cultivars replicated three times where the five warmest and five coolest lines were selected and used in the following two years. Measurements were made with a hand-held infrared thermometer for canopy temperature and an aspirated psychrometer for vapor pressure deficit (VPD). Significant differences were found for mean canopy temperature differentials among the genotypes and a significant negative correlation with seed yield of -0.72 from across all environments. VPD only showed genetic differences when the highest 5 days were selected for evaluation. VPD had low correlation with canopy temperature differentials and yield. The results show that using canopy temperature could be used to indirectly select for yield but not for selection on drought tolerance.

Kashiwagi et al. (2008) conducted a study to evaluate the use of thermal imagery system in evaluating field scale experiments for temperature differences of plant canopies fast and accurately. A diverse chickpea germplasm of sixteen accessions were used that were grown at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in the post rainy season under rainfed conditions. Thermal images were captured from early flower until late pod fill stage with an infrared camera between 1400 and 1430 hours. From the images, the sequential color gradients were extracted to obtain the numerical thermal data and were analyzed by image

analysis software WinRhizo (Regent Instruments Inc, Canada) for the ratio of plant canopy area occupied by each color to the total plant canopy area. The areas of the canopy that were measured as relatively cool and relatively hot, showed significant differences among genotypes. A significant correlation of 60% was found in the relationship between the relatively cool canopy area and the seed yield. Suggestions from this study include relating the higher transpiration rates to stronger root systems supplying a better source of water for increased yields and use for drought tolerance. This study shows the potential for more work to be done with large field scale germplasm screenings in looking for efficiency in transpiration later in the reproductive stage, at seed fill.

Fletcher et al., (2007) ran experiments to test slow-wilting soybean genotypes against commercial genotypes on maximum transpiration rate with the use of canopy temperature as part of one trial. Infrared temperatures of the complete soybean canopies for a slow-wilting and a commercial genotype were taken on plants grown in a SPAR growth chamber. Canopy temperature was measured with an industrial infrared radiation thermometer (Omega Engineering, Stamford, CT) every 5 minutes. These measurements were shown as an indication of differences in transpiration rate. A high canopy temperature would mean a lower transpiration rate within that canopy. Results showed that the canopy of the slow-wilt genotype was warmer than the commercial genotype with a 1.5°C difference at 0800 hours and increasing, along with atmospheric vapor pressure deficit, to a 3.0°C difference around 1500 hours. With the slow-wilting genotype's canopy warmer, it suggests that the transpiration rate is lower than the rate of the commercial genotype.

In a more in-depth slow-wilting soybean study, Ries et al. (2012) measured canopy temperature, among many other traits on three slow-wilting, one intermediate wilting, and five

fast-wilting genotypes in an effort to relate delayed wilting to soil moisture conservation. An infrared thermometer (model OS562, Omega Engineering Inc., Stamford, CT) was used to measure canopy temperature by averaging ten random measurements of each plot between R2 and R5 growth stages (Fehr et al., 1971). Canopy temperature depression was calculated using the built in thermocouple to measure ambient air temperature and then subtracting that from the canopy temperature (Idso, 1982). The readings were taken in July and August between 1200 and 1400, when air temperature was greater than 30°C, skies were clear, with minimal wind. Results show that multiple mechanisms could be involved in soil water conservation by these diverse genotypes as genotypic differences were found in water use efficiency measurements but not by canopy temperature.

In a study utilizing a global representation of 300 grain sorghum genotypes, Mutava et al. (2011) measured canopy temperature with a handheld infrared gun on clear sunny days between 1200 and 1500 h and revealed significant differences between genotypes. The large variation in this study suggests potential for selection in breeding programs.

Applications of canopy temperature measurements for demonstrating an advancement of physiological changes in genetic gain studies related to seed yield has been revealed in several studies on wheat (Lopes et al., 2012; Xiao et al., 2012). Lopes et al. (2012) found genetic yield gains of spring wheat to be 0.7% per year in Mexico and a strong relationship with cooler canopy temperatures at grain filling by measurement with a portable infrared thermometer. An analysis showed no significant quadratic or cubic functions for canopy temperature during grain fill which shows there is still room for improvement in that trait (Lopes et al., 2012). Xiao et al. (2012) found an increase of 62 kg per hectare per year of genetic gains in grain yield of winter wheat in China with improvements in physiological traits including canopy temperature

depression measured by an infrared thermometer and simultaneously measuring air temperature with a digital thermometer. Both of these studies showed genetic differences among entries and related improvements in grain yield to improved physiological traits.

Chlorophyll Fluorescence

As light energy is absorbed by a chlorophyll molecule in a leaf, the energy is either utilized by photochemical or by non-photochemical processes. The photochemical process is known as photosynthesis, while the non-photochemical processes include energy dissipating as infrared radiation (heat) and as red/far-red radiation, which is chlorophyll fluorescence. Of all the light absorbed, only about 1 or 2% is re-emitted as chlorophyll fluorescence. This light is best measured with a chlorophyll fluorometer, which will provide information about photosystem II (PSII) and the extent to which PSII is using the energy absorbed by chlorophyll and the extent to which it is being damaged by excess light (Maxwell and Johnson, 2000). Within the photosynthetic apparatus, PSII is considered the most vulnerable to light-induced damage and will show the first sign of stress in a leaf. Being able to detect plant tolerance to environmental stresses and the amount of damage to the photosynthetic apparatus from these stresses are the key points for chlorophyll fluorescence measurements. With the use of hand held chlorophyll fluorometers the ability to quickly measure plants in the field has increased.

The fluorometer measurements are typically taken on a dark-adapted leaf that receives controlled amounts of light energy from the instrument. At the exposure to light, leaf fluorescence rises to the minimal level of fluorescence (F_o), which is the fluorescence level obtained when the PSII reaction centers are in the ‘open’ state (capable of photochemistry). As a saturating light pulse continues, the PSII reaction centers reach a ‘closed’ state, and results in the

maximal level of fluorescence (F_m) (Barbagallo et al., 2003). The variable fluorescence (Fv) is the difference between F_m and F_o.

The potential use of chlorophyll fluorescence for crop improvement is increasing as described by Baker and Rosenqvist (2004). Genetic differences have been found in measuring chilling tolerance by chlorophyll fluorescence in rice (Oryza sativa L.) genotypes (Sthapit et al., 1995) and responses to high temperatures in wheat (Moffatt et al., 1990). These studies have compared the photochemical activities by using ratios of fluorescence values to compare changes between different leaf samples. The ratio of variable fluorescence to maximum fluorescence (F_v/F_m) is an estimate of maximum quantum efficiency of PSII photochemistry and is a common ratio used to show stress and damage to the PSII reaction centers.

Advancements in fluorescence have led to the use of charge coupled device (CCD) cameras for fluorescence imaging. Peltier-cooled CCD cameras have been used to decrease dark-noise from thermal events within the CCD (Oxborough, 2004). These imaging systems allow for PSII measurements on a spatial level with the ability to view several leaves or plants for plant performance and selection (Baker and Rosenqvist, 2004). The use of chlorophyll fluorescence for a fast and non-invasive measurement has been demonstrated multiple times with improvement of the instruments allowing for more efficient applications in crop improvement programs.

Djanaguiraman et al. (2011) evaluated the effects of high temperature (HT) versus optimum temperature (OT) on one genotype of soybean through the use of controlled environment chambers. Physiological measurements were made with a pulse-modulated fluorometer after 30 minutes of dark adaption of the third trifoliate leaf from the main stem apex between 11:00 and 13:00 hours at R2 growth stage. This measurement allowed for the

determination of the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) and the ratio of minimum fluorescence to maximum fluorescence (Fo/Fm). Results were significant at the $P<0.001$ value and showed a 33% increase in thylakoid membrane damage (Fo/Fm) in the HT plants over the OT plants. This study characterized the response of one genotype and suggested further evaluations of additional genotypes to determine potential genetic variability of HT stress tolerance. In another study, Mutava et al. (2011) utilized a global representation of 300 grain sorghum genotypes and evaluated field measurements on chlorophyll fluorescence. A pulse-modulated fluorometer was used after 30 minutes of dark adaption on the top most fully expanded leaf between 1030 and 1530 h. and revealed significant differences between genotypes (Mutava et al., 2011). The large variation in this study suggests potential for selection in breeding programs. Multiple studies have demonstrated the use of chlorophyll fluorescence for detecting genetic differences in field grown plants of several crop species.

Chlorophyll Content

Chlorophyll concentrations have been measured nondestructively by absorbance methods. A commonly used absorbance chlorophyll meter is the Minolta SPAD-502 meter (Konica Minolta Optics, Inc., Tokyo, Japan). Monje and Bugbee (1992) conducted an experiment in which the dual-wave SPAD-502 chlorophyll meter was tested against a custom-built single-wave meter and then destructive colorimetric measurement was performed to find in vitro chlorophyll concentration to compare the absorbance measurements. A total of six readings were averaged per leaf for comparison. There were no significant differences found between the single-wavelength and SPAD-502 meters, even though results showed that the SPAD-502 meter produced a correlation of 93% with the destructive measurement slightly higher than the single-wavelength meter of 90%. Measurements had a smaller coefficient of variation with the SPAD-

502 meter and were more repeatable than those of the single-wavelength meter. There was a correlation of 98% between the readings of the two meters and both meters overestimated chlorophyll concentrations below 600 mg m^{-2} and above 100 mg m^{-2} causing a curvilinear line of measurements between in vivo and in vitro samples. Averaging multiple readings proved to be more time efficient than in vitro destructive measurements. Monje and Bugbee mentioned that the commercial SPAD-502 meter had better options than the custom-built meter by being able to average and edit stored values even though there were no significant differences between the measurements (Monje and Bugbee, 1992).

Richardson et al. (2002) conducted a study to compare two commercial chlorophyll absorbance meters with several reflectance indices for leaf chlorophyll content by measuring leaves from paper birch (*Betula papyrifera* Marsh.). Hand-held chlorophyll absorbance meters consisted of the CCM-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA) which measures absorbance at 660 and 940 nm compared with the Minolta SPAD-502 meter (Konica Minolta Optics, Inc., Tokyo, Japan), which measures absorbance at 650 and 940 nm. Five measurements per leaf were averaged for each absorbance meter. The reflectance was measured with a UniSpec Spectral Analysis System (PP Systems, Haverhill, Massachusetts, USA) at wavelengths from 306 to 1138 nm and averaged from six passes per each leaf scan. Several indices were used with the reflectance measurements. The best estimate of chlorophyll content was measured with the reflectance indices, which had a higher correlation than either of the absorbance meters. The absorbance meters appeared to be less accurate at higher chlorophyll content levels, just as Monje and Bugbee (1992) found in their study. Between the absorbance meters, the SPAD-502 had a higher correlation with chlorophyll content than the CCM-200 did. Richardson suggests from the results that all of these noninvasive methods worked well since they are fast, easy to

use, and produce reliable estimates of chlorophyll content. Although, use of these instruments between species may prove to be difficult and for accurate estimates of chlorophyll content specific calibration equations should be derived for each species (Richardson et al., 2002). Chlorophyll content measurements have been proven accurate by noninvasive methods by both reflectance indices and absorbance meters. There have been several studies since that apply this to field measurements.

Saitoh et al. (2004) compared several parameters of leaf photosynthesis between wild and cultivated genotypes of soybeans. One of their measurements included SPAD readings with a Minolta SPAD-502 meter (Konica Minolta Optics, Inc., Tokyo, Japan). Genotypes used included two wild types and two cultivated types grown in a greenhouse. SPAD readings were taken on the fully expanded terminal leaflet at 10-day intervals between 32 and 85 days after planting. The chlorophyll concentrations from SPAD measurements were higher in the cultivated genotypes than the wild types throughout all of the testing dates. The highest readings were at R5 stages and decreased in all genotypes during seed fill. The results suggested that during the domestication process, selections were made for superior plants that resulted in an overall increase in photosynthetic rates.

In the previously mentioned study by Djanaguiraman et al. (2011), chlorophyll content was also measured with a self-calibrating Minolta SPAD-502 chlorophyll meter (Konica Minolta Optics, Inc., Tokyo, Japan) on the third trifoliate leaf from the main stem apex between 11:00 and 13:00 hours at R2 growth stage. Results showed that HT stress decreased the chlorophyll content compared with the OT at a significance value of $P<0.001$. In the OT, leaf chlorophyll content increased through the 10^{th} day and remained the same through the 14^{th} day while plants in the HT treatment showed a decrease in leaf chlorophyll content from the 2^{nd} day through the

14th day. As mentioned before, this study was solely on one genotype and further research is needed with multiple genotypes to discover possible genetic variation of chlorophyll content and tolerance to HT.

In a much larger field study, a global representation of 300 grain sorghum genotypes were utilized by Mutava et al. (2011) to measure chlorophyll content. A self-calibrating Minolta SPAD-502 chlorophyll meter (Konica Minolta Optics, Inc., Tokyo, Japan) was used on the top most fully expanded leaf three times throughout the season from booting stage through maturity. No significant differences between genotypes or relationships with seed yield were found in this study.

Chlorophyll content measurements in a study on a genetic gain study in wheat, revealed genotypic differences and a strong relationship with seed yield (Xiao et al., 2012). Xiao et al. (2012) found an increase of 62 kg per hectare per year of genetic gains in grain yield of winter wheat in China with improvements in physiological traits including chlorophyll content measured with a SPAD meter. These results show support for analysis of physiological traits in other species as well.

Leaf Antioxidants

Reactive oxygen species (ROS) are highly active and are produced by normal operation of photosynthesis and electron transport as well as abiotic stresses, but are balanced by scavenging antioxidants and enzymes (Taiz and Zeiger, 2010). High temperature stress, which causes increased ROS, has been shown to increase membrane damage, measured by electrolyte leakage, in grain sorghum (Djanaguiraman et al., 2010b) and soybeans (Djanaguiraman et al., 2010a). Membrane damage was also identified in wheat with decreased membrane stability under both moisture and temperature stress (Sairam et al., 1997). Differences of membrane

stability to high temperature tolerance have been shown between two cultivars of creeping bentgrass that were identified with electrolyte leakage (Liu and Huang, 2000). Measurements of antioxidant enzyme activity have shown decreases under high temperature (Djanaguiraman et al., 2010a; Djanaguiraman et al., 2010b). Emmons and Peterson (2001) found differences between oat cultivars for antioxidant activities based on high-performance liquid chromatography (HPLC). Differences have been found among total antioxidant capacity in *Piper betle* L. leaf extracts by in vitro method on three varieties (Dasgupta and De, 2004), expressed as the equivalent to ascorbic acid and gallic acid. Scavenging of ROS is through a combination of antioxidants and antioxidant enzymes and methodologies have been identified for identifying both. Genetic differences have been revealed in a few studies.

Objectives

There have been several studies revealing genetic differences in various physiological parameters ranging from pollen germination (Kakani et al., 2002; Koti et al., 2004; Kakani et al., 2005; Salem et al., 2007), canopy temperature (Blum et al., 1982; Harris et al., 1984; McKinney et al., 1989b; Kashiwagi et al., 2008; Mutava et al., 2011; Lopes et al., 2012; Xiao et al., 2012), chlorophyll fluorescence (Sthapit et al., 1995; Moffatt et al., 1990; Mutava et al., 2011), chlorophyll content (Saitoh et al., 2004; Xiao et al., 2012), and leaf antioxidants (Emmons and Peterson, 2001; Dasgupta and De, 2004). These results show support for further analysis of physiological traits in other species and the relation that these traits might have to the improvement in seed yield. Strong correlation between traits and seed yield show the possibility that improvements have occurred physiologically while selections were made for seed yield. Several studies looking at the genetic gain of crops has already related physiological traits to the improvement of seed yield (Lopes et al., 2012; Xiao et al., 2012) and that these traits may have

changed with the improvement of seed yield. The objectives of this study were to characterize pollen germination, canopy temperature, leaf chlorophyll content, leaf antioxidants, and leaf fluorescence in soybean cultivars released from the 1920's through 2010 and determine if changes in these traits have occurred with improvement of seed yield.

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CHAPTER 2 - Field Study - Physiological Parameters

Introduction

Food production and security of food for the global population is among the top challenges that face the agriculture industry. The United Nations expects the world population to increase to about 10 billion by year 2050 (United Nations, 2011). The increasing population places more pressure on resources for food production and a dependence on agricultural research to increase the efficiency and output of production agriculture. Plant breeding will be relied upon to make important contributions to the increased production of food and fiber. Advancements in plant breeding are dependent upon the quality and quantity of genetic variation present in the germplasm under evaluation, including differences in physiological traits.

Physiological measurements have detected differences among genotypes for a variety of traits. A simple in vitro pollen germination method originated for soybeans by Gwata et al. (2003) has proven successful in detecting genotypic differences among genotypes. This in vitro method was used by Gwata et al. (2003) to show pollen germination differences between nodulating and nonnodulating soybean genotypes. The same procedure was used in a study by Koti et al. (2004) that showed the response of enhanced ultraviolet-B radiation on soybean pollen with germination ranging from 72 to 92% among treatments. Salem et al. (2007) observed significant variation among 44 genotypes with pollen germination ranging from 70% up to 93% in a study designed to screen soybean pollen for high temperature tolerance. Kakani et al. (2002) examined pollen germination in 21 diverse genotypes of groundnut with genotypic differences in pollen germination ranging from 35% to 73%. Applications of in vitro pollen germination to

screen for potential selection of high temperature tolerance in soybeans have had some success, although there have been limited field based studies.

There have been several studies revealing genetic differences in canopy temperature measurements. Blum et al. (1982) evaluated barley strains and wheat cultivars, showing significant variations in leaf temperatures and a significant correlation across strains between leaf temperature and leaf water potential. Significant differences in canopy temperature were found between 20 soybean genotypes by measuring leaf canopy temperatures with a hand-held infrared thermometer (Harris et al., 1984). In a study utilizing a global representation of 300 grain sorghum genotypes, Mutava et al. (2011) measured canopy temperature with a handheld infrared gun and observed significant differences between grain sorghum genotypes.

Genetic differences have been found in several other traits as well. Chlorophyll fluorescence measurements of chilling tolerance in rice (Oryza sativa L.) genotypes (Sthapit et al., 1995) and responses to high temperatures in wheat (Moffatt et al., 1990) have both revealed significant differences. Chlorophyll content measurements between wild and cultivated genotypes of soybeans, obtained by using a SPAD meter, showed greater concentration of chlorophyll in the cultivated genotypes over the wild types (Saitoh et al., 2004). Emmons and Peterson (2001) found differences between oat cultivars for antioxidant activities based on high-performance liquid chromatography. Additionally, an in vitro method showed significant differences among total antioxidant capacity from three varieties of *Piper betle* L. leaf extracts (Dasgupta and De, 2004).

Several studies have linked genetic differences in physiological traits to improvements in seed yield. Morrison et al. (1999) found an increase in seed yield positively related to photosynthesis, stomatal conductance, and chlorophyll concentration. Jin et al. (2010) found a

positive correlation between soybean seed yield and year of release with an average increase of 0.58% annually and a 0.33% increase per year in photosynthetic rate among cultivars. Lopes et al. (2012) found genetic yield gains of spring wheat to be 0.7% per year as well as a strong relationship with physiological traits including cooler canopy temperatures at grain fill, increased stay-green, earlier heading, and increased thousand kernel weight. Xiao et al. (2012) found an increase of 62 kg per hectare per year in grain yield of winter wheat in China with simultaneous improvements in physiological traits such as chlorophyll content, canopy temperature depression, and photosynthetic rate.

To assess the potential changes in physiological traits over time, the objectives of this study were to characterize pollen germination, canopy temperature, leaf chlorophyll content, leaf antioxidants, and leaf fluorescence in soybean cultivars released from the 1920's through 2010 and determine if changes in these traits have occurred along with improvement of seed yield.

Materials and Methods

Field trials were conducted in Manhattan, KS in 2010 and 2011 in both dryland and irrigated environments. The genotypes evaluated were part of a nationwide genetic gain study, organized by Dr. Brain Diers, University of Illinois. The study consisted of 60 maturity group III (Table A.1) and 54 maturity group IV (Table A.2) cultivars, released from the 1920's through 2010, with both public and private releases as well as genotypes with glyphosate-resistance and non-resistance.

Genotypes were planted in plots arranged in a randomized complete block design with four replications. Plots consisted of four-rows, 3.4 m (11.2 feet) long, spaced 76.2 cm (30 inches) apart, seeded at a planting rate of 24 seeds per meter using a four-row ALMACO cone planter with Kinze row units (ALMACO, Nevada, IA). In 2010, plots were planted on May 27th on a coarse-silty, mixed, superactive, nonacid, mesic Typic Udifluvents (Belvue silt loam) soil and in 2011, plots were planted on May 23rd on a coarse-silty, mixed, superactive, mesic Fluventic Hapludolls (Eudora silt loam) soil. Weeds were controlled with a burn down application before planting and by hand during the growing season. Flood irrigation, on the irrigated experiment, was used as necessary throughout the growing season.

In vitro pollen germination (PG) was determined from both maturity groups evaluated in the irrigated field on a selected group of genotypes. This selected group consisted of twenty genotypes from the maturity group III and twenty genotypes from the maturity group IV tests, which were selected by choosing genotypes from both public and private sectors across all decades in the study to allow for the parameters that are very time and labor intensive to be completed. Twenty to 30 unopened flowers were sampled from each plot beginning at late R1 (Fehr et al., 1971) through the end of flowering. Flowers were picked in the field between 0800 h

and 0900 h, stored in a Petri dish, and arrived at the lab by 0930 h inside a cooler to maintain constant temperature. The Petri dishes were then stacked on the countertop to prepare for pollen extraction. Flowers were dissected with tweezers by removing the sepals and opening the keel petals with the tweezers and jointly removing the pistil and stamen in one motion. Then the tweezers were rotated to point the stamen downward and the apparatus was tapped against the bench top to allow the pollen to fall onto the media slides. Several flowers were used per slide and media slides with the pollen were then incubated at either 28 or 34° C for 30 minutes in incubators (RevSci Incufridge, Revolutionary Science, Shafer, MN) preheated to the assigned temperature. The medium used consisted of 15 g sucrose ($C_{12}H_{22}O_{11}$), 0.03 g calcium nitrate [$Ca(NO_3)_2$ $4H_2O$], and 0.01 g boric acid (H_3BO_3) dissolved in 100 mL of deionized water (Gwata et al., 2003). To the medium 0.5 g of agar was added then slowly heated to dissolve (Salem et al., 2007). Media was prepared the night before and poured onto glass microscope slides, which were placed in Petri dishes to allow for labeling. After incubation, the media slides were then photographed with an Olympus DP70 digital camera on an Olympus BX51 microscope at 10x magnification and pollen grains were counted at a later date. A pollen grain was considered germinated when its tube length equaled the grain diameter. (Luza et al., 1987)

Canopy temperature (CT) measurements were captured between 1000 h and 1400 h, on sunny cloudless days, on all genotypes in all environments from the center of each plot's canopy surface. Measurements consisted of one image per plot by using an infrared camera (FLIR BCAM, FLIR Systems, Wilsonville, OR) several times from R1 through R6. The captured radiometric images were stored and analyzed with FLIR Systems QuickReport Software to obtain the average CT of the entire field of view of the infrared camera.

Leaf chlorophyll content (Chlor) was measured on all genotypes in all environments, using a self-calibrating Minolta SPAD-502 chlorophyll meter (Konica Minolta Optics, Inc., Tokyo, Japan) several times from R1 through R6. Four measurements were averaged from the upper canopy of each plot to obtain the chlorophyll content reading.

Leaf fluorescence was measured between 1000 h and 1400 h on sunny cloudless days, by taking one reading per plot. All genotypes in both environments of the 2010 season were measured from R1 through R6 with a pulse-modulated fluorometer (OS 30, OptiScience, Hudson, NH) after about 30 min of dark adaptation of the leaves with plastic clips. Tags were placed on a branch to signify the leaf that was being used in order to maintain consistent measurements over the season.

Leaf antioxidants were measured by collecting from the top 3rd to 5th trifoliate leaves at R4 and R6. Leaves were stored in 50 mL centrifuge tubes and frozen at the field in a Styrofoam cooler filled with liquid nitrogen. The tubes were then transported in large bags and stored in an -80° C freezer until analyzing. Total antioxidant capacity (TAC) is determined based on reduction of Mo(VI) to Mo(V) by the extract and the formation of a green phosphate/Mo(V) complex at low pH (Prieto et al., 1999). TAC of the soybean leaves was determined by macerating 0.1 gram of leaf sample in 10 ml of ethanol with a mortar and pestle and placing into another 50 mL centrifuge tube. The contents were centrifuged at 3500 rpm for 10 minutes. TAC was measured by combining 0.3 mL of supernatant, 0.3 mL of ascorbic acid (100 mg/mL), and 3.0 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) (Dasgupta and De, 2004). Tubes were then capped and placed in a boiling water bath for 90 minutes and cooled. The color intensity was read at 695 nm and expressed as change in optical density per minute per gram of leaf. The blank was 0.3 ml of ethanol, 3ml of reagent, and 0.3 ml

of ascorbic acid. Electrolyte leakage (EL) of the membranes were measured by weighing 1 gram of leaf sample and placing it in a 50 mL centrifuge tube with 5 ml of 0.4 M mannitol salt solution. After 3 hours of incubation at room temperature, electrical conductivity (C_1) was measured without touching the leaf tissue. The tubes were capped and placed in a boiling water bath for 10 minutes to kill the tissue completely. Electrical conductivity (C_2) was then measured as before. The membrane injury was calculated as C_1/C_2*100 and expressed as a percent.

Prior to harvest, the center rows were used to measure height, lodging, maturity, sudden death syndrome (SDS), shattering, and seed yield. Plant height is a measure of the average height in centimeters from the soil line to the top of the main stem. Lodging scores were assigned on a scale of 1 to 5, with 1 equaling all plants erect and 5 equaling all plants prostrate. Maturity was recorded as the date when 95% of the pods reached mature color. Plants were rated on SDS severity on all environments in 2010 and the irrigated environments in 2011 with disease severity ratings from 1 to 5, with 1 equaling no disease and 5 equaling all plants showing severe symptoms. The center two rows were harvested with a plot combine. Seed yield was recorded as kilograms per hectare, adjusted to 13% moisture. Yield components were analyzed from a hand harvested section of one of the border rows, 0.33 m in length, on all environments of the selected genotypes for both maturity groups in the 2010 growing season and only from the maturity group IV environments in 2011. Total plants, nodes, and pods were counted. Total seeds were determined by seed weight, which was found by weighing a counted sample of 200 seeds.

All of the data were analyzed with the Glimmix procedure in SAS 9.2 (SAS Institute, 2008). Analysis of variance was determined with environment and genotype as fixed effects and replication nested within environment as a random effect. Environment represented year and irrigation or dryland. Means were subjected to Tukey-Kramer's pairwise comparison test at the

$p<0.05$ level. Relationships between parameters were determined by the Correlation procedure in SAS 9.2 (SAS Institute, 2008).

Results

Weather varied between growing seasons with the 2010 season overall slightly cooler and much wetter than the 2011 season (Table A.3). The 2011 season started off near normal precipitation, but then became much drier and warmer as the plants reached reproductive stage through seed fill. Temperatures in 2010 tended to range from 0.5°C cooler in May and July to 2.0°C warmer in June and August through October, compared to the 30 year average. Temperatures in 2011 tended to be near the 30 year average in May and September, but ranging up to 3.5°C warmer in June through August and in October. Precipitation for the 2010 season was short 95 mm and for the 2011 season short 189 mm, compared to the 30 year average.

Analyses of variance for both maturity groups across all four environments showed that seed yield, plant height, lodging, maturity, and SDS all varied significantly among environments and genotypes as well as significant interactions between genotypes and environments (Table 2.1). Canopy temperature varied significantly among environments for the maturity group III and maturity group IV entries (Table 2.1). Genotypes varied significantly ($p<0.01$) in both maturity groups, and no significant interactions between genotypes and environments were observed. Leaf chlorophyll content (Chlor.) varied significantly ($p<0.01$) among genotypes for both maturity groups and environments of the maturity group III (Table 2.1). There were no significant differences between environments of the maturity group IV and no significant interactions between genotypes and environments of either maturity group for leaf chlorophyll content measurements.

Yield components showed significant differences between genotypes for each component measured for the maturity group IV genotypes and all parameters except total pods for the maturity group III (Table A.4). Leaf chlorophyll fluorescence showed significant differences

between genotypes for the maturity group IV and no significant differences between environments or genotype by environment interaction while the maturity group III had no significant differences between genotypes but a significant difference between environments with no genotype by environment interaction (Table A.5). Leaf antioxidant levels revealed no significant differences for genotypes on either TAC or EL for both maturity groups (Table A.6). Environments were significantly different for both TAC and EL for maturity group IV and the EL measurement for maturity group III. In vitro pollen germination results showed significant differences for environments, genotypes, and genotype by environment interactions for both maturity groups (Table 2.2). There was significant genotype by environment interactions for all of the maturity group III parameters (Figure A.2) and the 34°C incubation parameter of the maturity group IV (Figure A.3). The analysis of variance across both temperatures and two environments showed no significant genotype by temperature or genotype by temperature by environment interaction (Table 2.2). In summary, there were no genotypic differences observed in leaf chlorophyll fluorescence for maturity group III or leaf antioxidant measurements of TAC and EL. Genotypic differences were revealed in seed yield, yield components, leaf chlorophyll fluorescence for maturity group IV, pollen germination, leaf chlorophyll content, and canopy temperature.

Year of release had a significant relationship with seed yield, plant height, lodging, maturity, SDS, canopy temperature, and chlorophyll content for both maturity groups (Table 2.3). In addition, MG IV showed a strong, significant relationship between year of release and chlorophyll fluorescence (Table 2.3). Seed yield increased at a rate of about 23.1 kg ha^{-1} yr^{-1} and 26.2 kg ha^{-1} yr^{-1} for the MG III and MG IV, respectively (Figure A.4a and Figure A.5a). Seed yield had a positive relationship with year of release for all environments of MG III and MG IV

entries (Figure 2.1a and Figure 2.2a). The highest yielding genotypes produced about 2600 kg ha^{-1} more than the lowest yielding genotypes. Plant height showed a negative relationship with year of release for MG III (r = -0.38**)(Figure A.6a) and MG IV (r = -0.37**) (Figure A.7.a) entries. The tallest indeterminate genotypes were about 60 cm taller than the shortest genotypes. There was a single determinate genotype in the study. Plant lodging showed a negative relationship with year of release for the MG III (r = -0.78**) (Figure A.6.b) and MG IV (r = -0.61**) (Figure A.7.b) entries. Genotypes with the highest lodging scores, a prostrate architecture, were earlier releases dating from the 1920's through most of the 1940's. Maturity showed a positive relationship with year of release for the MG III (r = 0.51**) (Figure A.6.c) and MG IV (r = 0.60**) (Figure A.7.c) entries. The later maturing genotypes consisted of releases from the 1980's to present day releases. Sudden death syndrome (SDS) showed a negative relationship with year of release for the MG III (r = -0.60**) (Figure A.6.d) and MG IV (r = -0.39**) (Figure A.7.d) entries. Canopy temperature showed a negative relationship with year of release for all environments of MG II and MG IV entries (Fig. 2.1b and Fig. 2.2b). Genotypes with the coolest canopy temperature were about 2°C cooler than the warmest genotypes in the study. Chlorophyll content showed a positive relationship with year of release for all environments of MG III and MG IV entries (Fig. 2.1c and Fig. 2.2c). Genotypes with the highest chlorophyll content were about 7 SPAD units higher than the genotypes with lowest chlorophyll content. Chlorophyll fluorescence showed a positive relationship with year of release for both environments of the MG IV entries (Fig. 2.2d) while the MG III entries showed no significant relationship (Fig. 2.1d). The MG IV genotypes with the highest chlorophyll fluorescence were higher in Fv/Fm ratio by about 0.099 than the genotypes with the lowest chlorophyll fluorescence. Canopy temperature, chlorophyll content, and chlorophyll fluorescence all showed

a significant relationship among each other as well as with the other measured parameters such as lodging, maturity, and SDS (Table 2.3).

Total antioxidant capacity showed a significant positive relationship with year of release (r = 0.49*) for MG IV, but not for MG III (Table 2.4). Electrolyte leakage (EL) showed no significant correlations with year of release or seed yield nor did any of the pollen germination parameters (Figure 2.3), although a significant positive relationship was observed between EL and PG at the 34°C parameter (Table 2.4).

Yield components show strong significant relationships with year of release and, as expected, with seed yield. Both maturity groups showed a positive relationship between year of release and total plants, MG III (r = 0.82**) and MG IV (r = 0.78**), as well as with total nodes (r = 0.56*), total pods (r = 0.58**), and total seeds (r = 0.67**) in the MG IV entries (Table. 2.4). Total seeds were also highly correlated with other yield component parameters, including a positive relationship with total plants and total pods in both maturity groups as well a positive relationship between total pods and total nodes in both maturity groups (Table 2.4). Other significant relationships in MG III consisted of total nodes with total pods per nodes, and a relationship of total plants with total seeds per pods and total seeds (Table 2.4). There are also multiple correlations between the MG III yield components and EL and PG parameters, but none of those are found in the MG IV (Table 2.4). Other significant positive relationships in MG IV consisted of total plants with total pods and total nodes, and a relationship of total seeds with total nodes and total pods per nodes (Table 2.4).

Seed yield has a strong significant relationship with year of release and also shows strong relationships with all of the traits that are correlated with year of release. The strong positive relationship between maturity and seed yield raises concerns that increased maturity results in

increased seed yield due to the strong relationship. Maturity has a slope of 0.06 days year^{-1} for the MG III and 0.14 days year^{-1} for the MG IV, showing an increase of 0.5 to 1.5 days per decade before genotypes are reaching maturity. This increased growing period could allow for more physiological growth and create potential differences between early and later maturing genotypes within the same maturity group and possible differences between physiological measurements taken. But by splitting up the genotypes into three groups (early, middle, and late maturing) within each maturity group, differences among these groups are found for MG III entries (Figure A.8) and MG IV entries (Figure A.9). The later maturing genotypes tended to yield higher than the earlier maturing genotypes and tended to be comprised of more recent releases. While the earlier maturing genotypes tended to yield less, the early and middle maturing groups were comprised of releases from throughout the entire range of years of release which contains the lower yielding genotypes. When looking at the slope of the lines for seed yield plotted against year of release with each maturity 'third' group identified, there is a positive slope for seed yield found within each maturity group for MG III entries (Figure A.8c) and MG IV entries (Figure A.9c). There is a range of maturities found within the highest yielding group of genotypes. This shows that there has been increasing seed yield for all relative maturity groups, not a trend of later maturing genotypes. Of all the physiological measurements in this study, canopy temperature showed the strongest relationship with year of release followed by chlorophyll content.

Discussion

The results of this study did show significant improvement in seed yield among entries over year of release and a strong, significant relationship between year of release with seed yield, plant height, lodging, maturity, yield components, SDS, CT, and chlorophyll content for both maturity groups along with chlorophyll fluorescence for maturity group IV. Several other studies have related increased seed yield from several decades of entry releases with the improvement of physiological parameters (Lopes et al., 2012; Xiao et al., 2012; Jin et al., 2010; Morrison et al., 1999). Here, physiological improvements were found and related to seed yield on several parameters as well. Genetic differences were found in canopy temperature with decreasing canopy temperature for higher yielding, more recent releases; which is similar to the results of a study by Lopes et al. (2012) on wheat. Another study on wheat (Xiao et al., 2012), found significant differences between genotypes with increasing chlorophyll content over year of release, similar to the results found in this study. Xiao et al. (2012) did not find significant improvement in canopy temperature depression measurements on their study in wheat, which is not consistent with the results in this study for CT. Photosynthetic rates have been found to increase as seed yield has improved in soybeans in a couple of studies (Jin et al., 2010; Morrison et al., 1999), indicating the potential for other physiological improvements in soybeans.

Pollen germination showed significant differences among genotypes but did not have a significant correlation with year of release or seed yield. Average PG among genotypes ranged from 85.2 to 94.2% and 78.9 to 93.1% for MG IV (Table A.2) and MG III (Table A.1), respectively. The range of pollen germination in this study is similar to the study by Salem et al. (2007) although pollen germination in this study is higher than found by Walker (2012). Neither study, by Salem et al. (2007) nor Walker (2012), found significant correlations with seed yield

either. One possible explanation for the lack of significant correlations with seed yield is the genotype by environment interactions found in pollen germination. There was much more variability in pollen germination in 2010 than was found in 2011. The method used for pollen germination is very time consuming and labor intensive and as a result allows for human error with the large number of technicians working on the project, possibly an explanation for the variability between years. Another possibility could be that the self-pollinated flowers of soybeans contain plenty of viable pollen to fertilize the flower and that the lack of developed pods or seeds is related more to abortion by the plant due to genetic and or environmental constraints rather than damaged pollen.

Canopy temperature showed significant differences among genotypes and significant correlations with year of release and seed yield. Previous studies have found a significant negative correlations between canopy temperature and seed yield (Kashiwagi et al., 2008; Lopes et al., 2012) identical to the relationship in this study for both MG III (Figure A.10) and MG IV (Figure A.11) genotypes (Table 2.3). Xiao et al. (2012) did not find significant improvement in canopy temperature depression measurements on their study in wheat, which does not agree with the results in this study for CT. Other studies in soybeans have found significant differences among entries for canopy temperature depression (Harris et al., 1984; McKinney et al., 1989b). On canopy temperature, the decrease found over time shows that more recent releases are yielding higher and have cooler canopy temperatures, due to increased transpiration. The increased transpiration provides more water to the leaves to keep the canopies cooler and to be used in photosynthesis. The continued photosynthesis, during increased atmosphere temperatures, results in more carbon synthesized into carbohydrates and apparently provides increased seed yield. Canopy temperature measurements are revealing the status of transpiration

within the plant; i.e., whether the genotype is transpiring lots of water quickly (cooler canopy) or the genotype is not transpiring as much water (warmer canopy).

Chlorophyll content showed significant differences among genotypes and significant correlation with year of release and seed yield. Chlorophyll content has been found to have a significant positive correlation with seed yield in winter wheat (Xiao et al., 2012) similar to the relationship observed in this study for both MGIII and MGIV soybeans (Table 2.3).

Yield components showed significant differences between genotypes (Table A.4) and had significant correlations with year of release (Table 2.4). Correlations with other parameters (Pollen germination) were not found in the two year data of the MG IV entries, although there were correlations in the single year collection of data of the MG III entries. A possible explanation is the effect of abortion by the plant due to genetic and or environmental constraints.

Genotypic differences were found in leaf chlorophyll fluorescence and a significant correlation with year of release for the MG IV entries, but not in the MG III entries. Other studies have also found significant differences among genotypes with chlorophyll fluorescence (Sthapit et al., 1995; Moffatt et al., 1990; Mutava et al., 2011). There are several possible reasons why genetic differences were not consistently found in both maturity groups of this study. Possibly, not enough measurements per plot were taken as the instrument read only a small area of one leaflet per plot. Improvements could be made to the method by decreasing the total number of genotypes analyzed and increasing the number of repetitions per plot. Another possibility is that chlorophyll fluorescence isn't as adaptable to dicots as it is on monocots; other successful studies revealing genotypic differences have been on rice (Sthapit et al., 1995), wheat (Moffatt et al., 1990), and grain sorghum (Mutava et al., 2011).

There were no genotypic differences found in leaf antioxidant measurements of TAC and EL, although there was significant positive correlation with year of release and seed yield for TAC of the MG IV entries. With no genotypic differences but a significant trend with year of release and seed yield, there is evidence of some relationship. Possibly a more precise measurement (high performance liquid chromatography) may be needed to detect any possible differences as this study may not have been exact enough. Significant differences among genotypes were found in another study (Dasgupta and De, 2004), but they were not consistent with the results found here.

Conclusions

This study characterized pollen germination, canopy temperature, leaf chlorophyll content, leaf antioxidants, and chlorophyll fluorescence in soybean cultivars released from the 1920's through 2010 and showed changes in leaf chlorophyll content and canopy temperature, which have occurred with the improvement of seed yield.

Pollen germination shows potential in revealing genotypic differences but fails to exhibit a relationship with year of release and seed yield. Leaf antioxidants and chlorophyll fluorescence for the MG III entries showed no genotypic differences in this study. The method for in vitro pollen germination and leaf antioxidant are both time consuming and labor intensive which reduces the total number of genotypes capable of being measured. Chlorophyll fluorescence shows potential in being a quick, in-the-field measurement that with increased measurements could possibly provide genotypic differences on soybeans while measuring a large number of genotypes. Leaf chlorophyll content, canopy temperature, and leaf chlorophyll fluorescence on the MG IV entries all revealed genotypic differences and strong correlations with year of release and seed yield. These measurements provide a rapid, in-field analysis that produced robust results in a minimal amount of time. Canopy temperature showed the strongest relationship with seed yield and allows for the opportunity of field-based high-throughput phenotyping, which is currently being explored with successful findings of implementing canopy temperature with spectral radiometer data.

The use of canopy temperature as a high-throughput field-based phenotyping tool is being implemented and the efficiency of collecting large amounts of data has been increasing. Canopy temperature measurements fit well with high-throughput field-based phenotyping systems for measurements of transpiration and differences in stomata (Jones et al., 2009;

Furbank and Tester, 2011) as White et al. (2012) describes with their experience in using a high-clearance tractor equipped with infrared imagery instruments, along with many other instruments on board. Increased technology will aid in the ability of researchers to cover larger populations of genotypes in an effort to decrease the cost of field-based phenotyping.

This study shows evidence of improvements in physiological parameters that have occurred with the improvement of seed yield and reveals opportunities to implement these parameters into crop improvement and plant breeding programs.

Figures and Tables

Table 2.1 Analysis of variance and F-values for several agronomic and physiological traits of Maturity Group III and IV soybean across four environments.

Source	d.f.	Yield	Height	Lodging	Maturity	SDS[†]	CT	Chlor
					Maturity Group III			
Environment (Env)	3	101.21**	8.82**	73.76**	122.85**	9.8*	6.24*	25.62**
Genotype (Gen)	59	47.97**	36.88**	26.59**	31.45**	2.70**	2.04**	7.00**
Gen X Env	177	3.57**	1.58**	2.89**	3.99**	2.0**	0.3 NS[§]	0.61 NS
					Maturity Group IV			
Env	3	258.04**	10.97**	257.01**	166.02**	1041.94**	28.27**	1.76 NS
Gen	53	60.78**	23.59**	19.63**	63.85**	7.98**	2.86**	9.64**
Gen X Env	159	4.02**	1.39**	3.91**	4.58**	3.54**	0.59 NS	0.93 NS

[*] Significant at the 0.05 probability level.
[**] Significant at the 0.01 probability level.
[§] NS, not significant.
[†] SDS, sudden death syndrome; CT, canopy temperature; Chlor, chlorophyll content by SPAD (soil plant analysis development).

Table 2.2 Analysis of variance and F-values for pollen germination across two environments and two temperature parameters for Maturity Group III and IV soybean.

Source	d.f.	PG[†]
	Maturity Group III	
Environment (Env)	1	40.33**
Genotype (Gen)	19	6.19**
Temperature (Temp)	1	0.03 NS[§]
Gen X Env	19	5.55**
Gen X Temp	19	0.54 NS
Temp X Env	1	6.36*
Gen X Temp X Env	19	0.37 NS
	Maturity Group IV	
Env	1	197.23**
Gen	19	3.06**
Temp	1	8.65**
Gen X Env	19	1.67*
Gen X Temp	19	0.61 NS
Temp X Env	1	3.76 NS
Gen X Temp X Env	19	0.86 NS

[*] Significant at the 0.05 probability level.
[**] Significant at the 0.01 probability level.
[§] NS, not significant.
[†] PG, pollen germination.

Table 2.3 Pearson's correlation coefficients among agronomic, physiological traits and year of release in Maturity Groups III (n=60) and IV (n=54) soybean.

Traits	Year	Yield	Height	Lodging	Maturity	SDS†	CT	Chlor
	Maturity Group III							
Yield	0.89**							
Height	-0.38**	-0.30*						
Lodging	-0.78**	-0.60**	0.48**					
Maturity	0.51**	0.62**	0.13	-0.22				
SDS	-0.60**	-0.53**	0.27*	0.59**	-0.39**			
CT	-0.87**	-0.87**	0.19	0.70**	-0.59**	0.55**		
Chlor	0.65**	0.68**	-0.32*	-0.36**	0.43**	-0.30*	-0.53**	
Flr	0.20	0.31*	-0.01	-0.10	0.11	-0.22	-0.26*	0.21
	Maturity Group IV							
Yield	0.87**							
Height	-0.37**	-0.33*						
Lodging	-0.61**	-0.46**	0.21					
Maturity	0.60**	0.58**	0.01	-0.21				
SDS	-0.39**	-0.39**	0.01	0.62**	0.01			
CT	-0.79**	-0.79**	0.23	0.50**	-0.57**	0.24		
Chlor	0.78**	0.67**	-0.44**	-0.61**	0.30*	-0.49**	-0.54**	
Flr	0.67**	0.62**	-0.06	-0.38**	0.46**	-0.37**	-0.56**	0.68**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† SDS, sudden death syndrome; CT, canopy temperature; Chlor, chlorophyll content by SPAD (soil plant analysis development); Flr, chlorophyll fluorescence.

Table 2.4 Pearson's correlation coefficients among pollen germination, leaf antioxidant capacity and electrolyte leakage, yield and yield components, and year of release in Maturity Groups III and IV soybean (n=20)

	Year	Yield	TAC†	EL	PG28	PG34	PGavg	ttlPLTS	SW	ttlNod	ttlPOD	ttlPod/N	ttlSeeds
Maturity Group III													
Yield	0.89**												
TAC	0.28	0.20											
EL	0.20	0.07	-0.14										
PG28	-0.20	-0.04	-0.05	0.19									
PG34	0.30	0.31	0.19	0.61**	0.59**								
PGavg	-0.04	0.05	0.06	0.38	0.93**	0.82**							
ttlPLTS	0.82**	0.76**	0.41	-0.03	-0.43	0.03	-0.30						
SW	0.36	0.25	0.29	0.51*	0.24	0.58**	0.45*	0.08					
ttlNod	-0.04	0.24	-0.05	-0.56*	0.18	-0.22	-0.01	0.16	-0.41				
ttlPOD	0.00	0.19	0.15	-0.66**	-0.31	-0.49*	-0.45*	0.31	-	0.54*			
ttlPod/N	0.10	-0.19	0.17	0.31	-0.56*	-0.08	-0.36	0.11	0.12	-0.86**	-0.14		
ttlSeeds	0.18	0.28	0.21	-0.48*	-0.40	-0.46*	-0.49*	0.55*	-	0.41	0.79**	-0.01	
ttlS/Pod	0.26	0.21	0.19	-0.07	-0.27	-0.20	-0.26	0.51*	-0.18	0.07	0.16	0.13	0.73**
Maturity Group IV													
Yield	0.87**												
TAC	0.49*	0.47*											
EL	-0.25	-0.28	-0.25										
PG28	0.32	0.29	0.04	-0.31									
PG34	0.34	0.35	0.25	-0.28	0.69**								
PGavg	0.32	0.31	0.15	-0.35	0.96**	0.79**							
ttlPLTS	0.78**	0.87**	0.26	-0.20	0.37	0.34	0.35						
SW	0.34	0.24	0.28	-0.18	0.21	0.30	0.32	0.05					
ttlNod	0.56*	0.75**	0.39	-0.23	0.11	0.15	0.15	0.64**	0.17				
ttlPOD	0.58**	0.77**	0.38	-0.12	0.08	0.09	0.11	0.71**	-0.10	0.85**			
ttlPod/N	0.34	0.41	0.25	0.06	0.08	0.02	0.06	0.41	-0.36	0.15	0.63**		
ttlSeeds	0.67**	0.85**	0.41	-0.16	0.08	0.18	0.12	0.72**	-0.09	0.77**	0.93**	0.62**	
ttlS/Pod	0.34	0.34	0.20	-0.08	-0.08	0.18	-0.02	0.14	0.03	-0.02	0.09	0.20	0.42

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† TAC, total antioxidant capacity; EL, electrolyte leakage; PG28, pollen germination at 28°C; PG34, pollen germination at 34°C; PGavg, average pollen germination of 28°C and 34°C; ttlPLTS, total plants; SW, seed weight; ttlNod, total nodes; ttlPOD, total pods; ttlPod/N, total pods per nodes; ttlSeeds, total seeds; ttlS/Pod, total seeds per pods.

Figure 2.1 Relationships between year of release and (a) seed yield (b) canopy temperature (c) chlorophyll content and (d.) chlorophyll fluorescence in MG III soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=60).

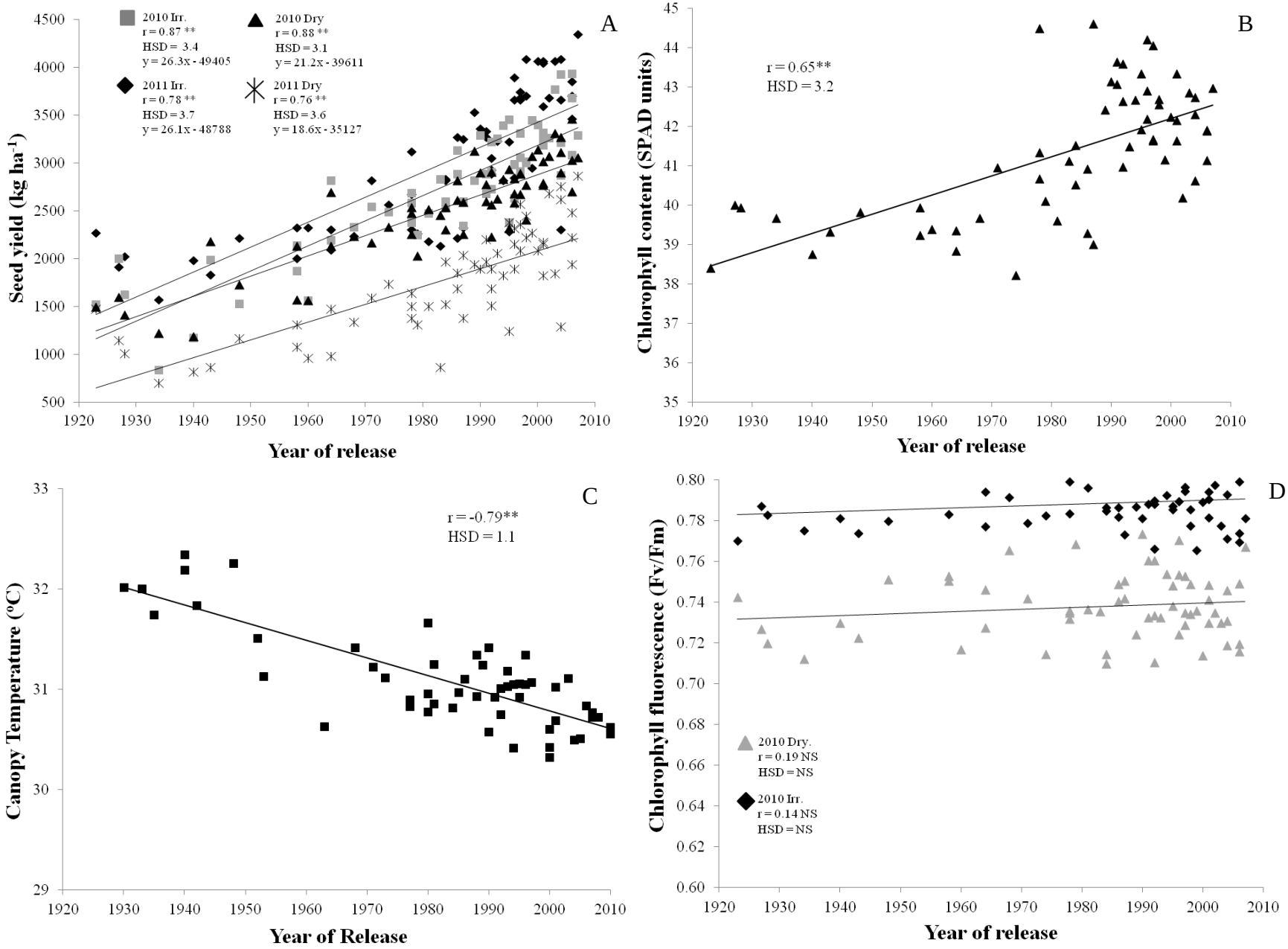


Figure 2.2 Relationships between year of release and (a) seed yield (b) canopy temperature (c) chlorophyll content and (d.) chlorophyll fluorescence in MG IV soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=54).

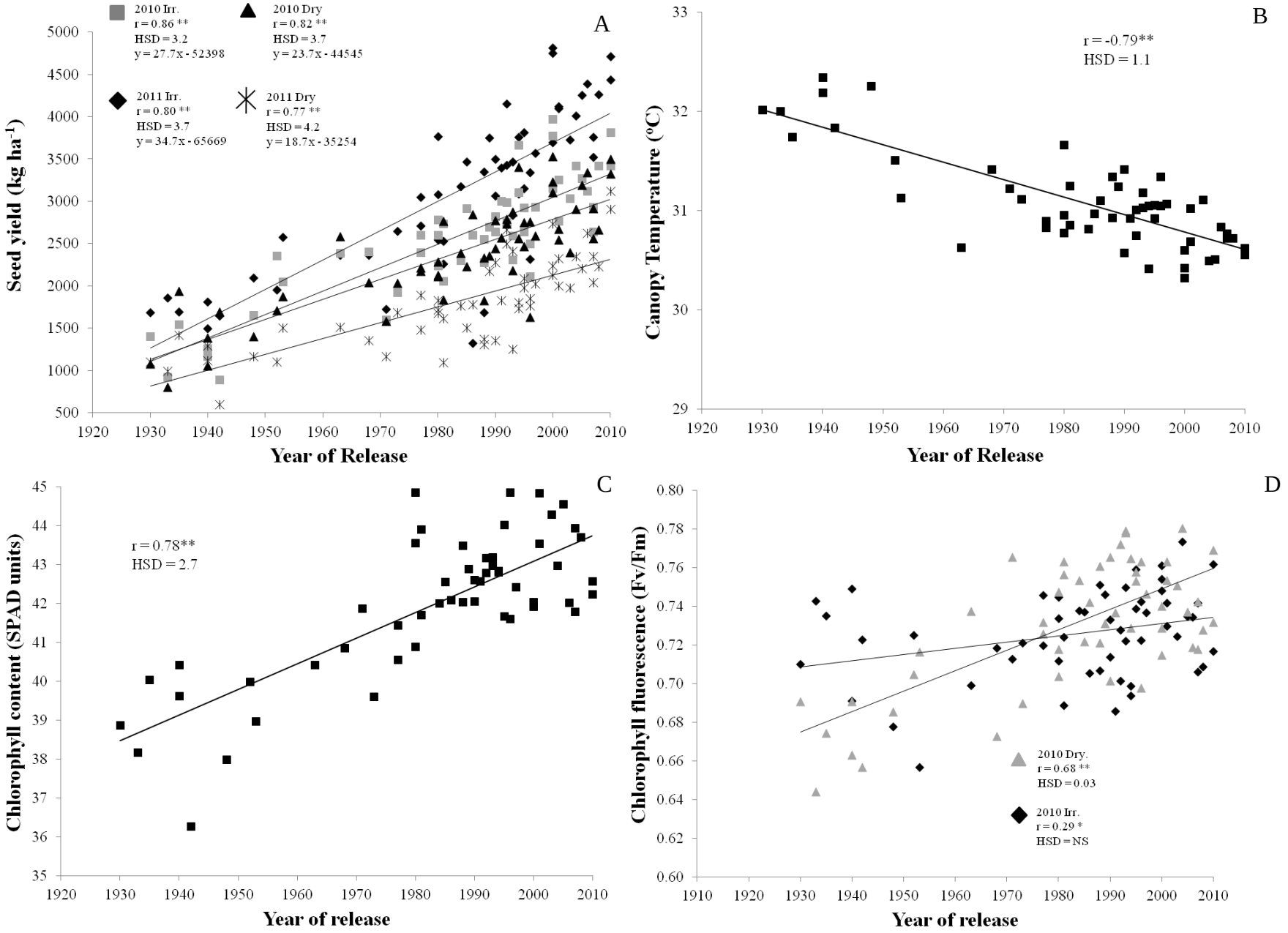
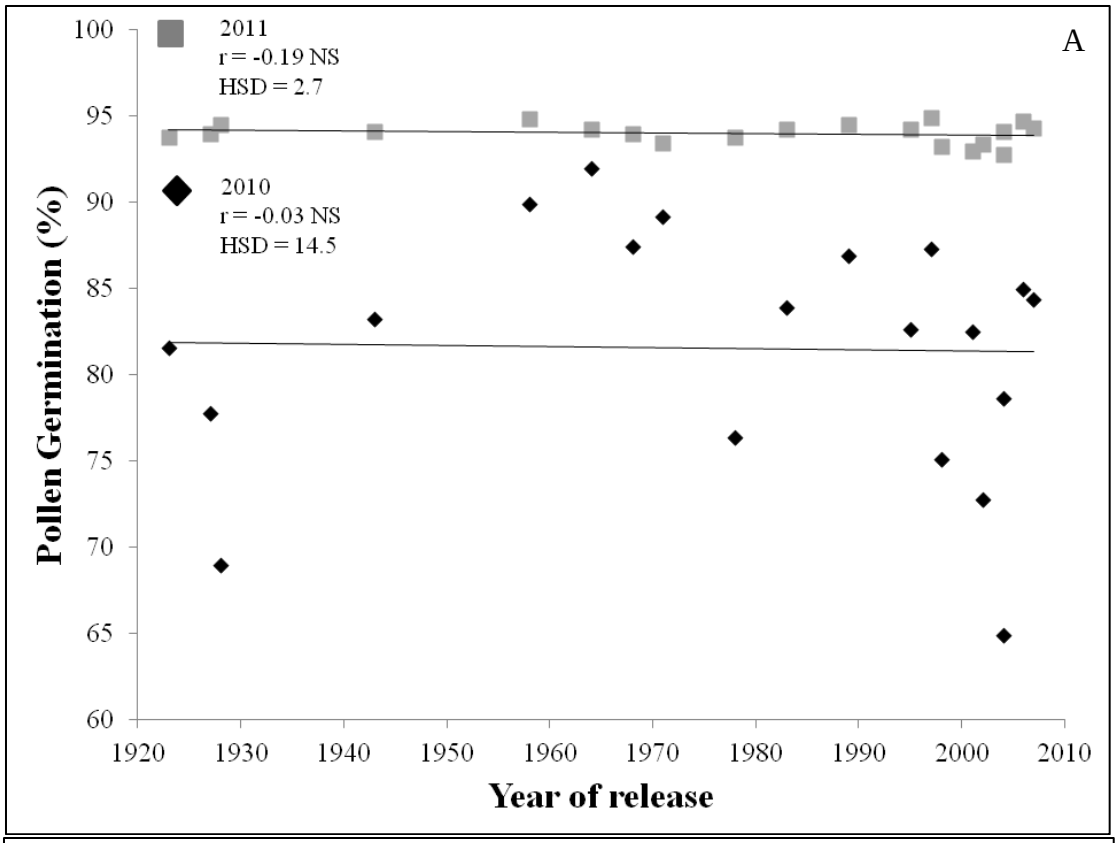
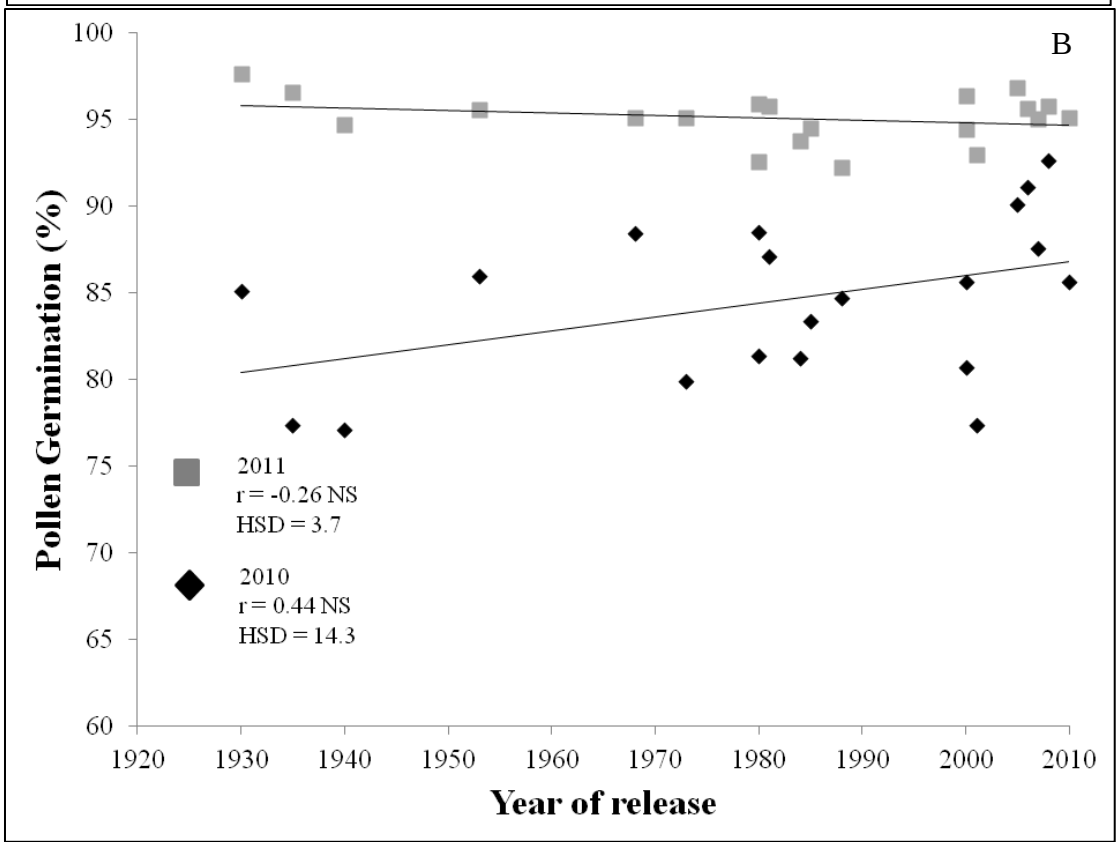


Figure 2.3 Relationships between year of release and in vitro pollen germination in (a) MG III and (b) MG IV soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=20).





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Appendix A - Supplementary Data

Figures and Tables Within Appendices

Figure A.1 Average U.S. soybean yields from 1924 - 2011 from USDA.

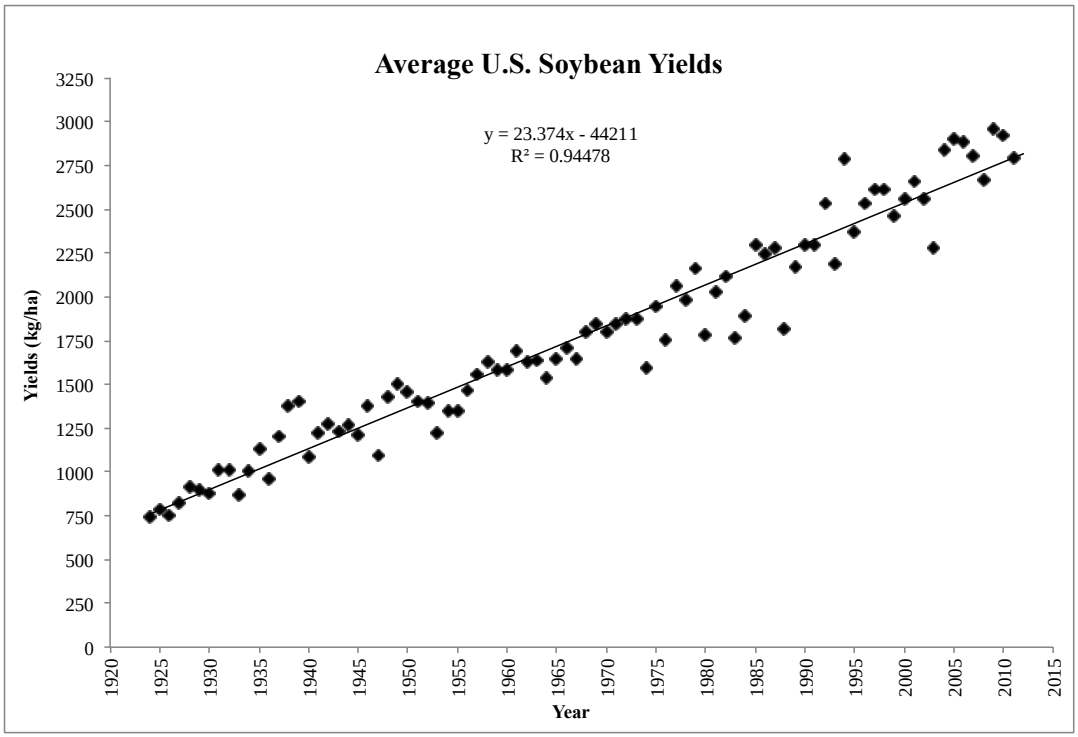


Table A.1 MG III soybean genotypes information, agronomic, and physiological parameter means grown in Manhattan, KS from both 2010 and 2011 (n=60).

Year of Release	Name	Seed Yield	Height	Lod	Mat	SDS	CT	SPAD	Flr	PG28	PG34	PGavg	TAC	EL
		kg ha^{-1}	cm				°C		Fv/Fm	——— % ———				%
1923	Dunfield	1688	101	3.8	18	2.3	32.0	38.4	0.77	88.4	88.3	87.7	33.0	75.9
1927	Illini	1666	134	3.3	23	2.3	31.4	40.0	0.77	89.1	82.5	85.9	36.1	68.6
1928	AK (Harrow)	1515	122	3.8	20	2.5	31.9	39.9	0.76	86.0	78.9	81.7	35.0	70.6
1934	Mandell	1084	110	3.6	17	2.0	32.3	39.7	0.76	-	-	-	-	-
1940	Mingo	1293	134	3.7	21	2.3	32.3	38.8	0.74	-	-	-	-	-
1943	Lincoln	1715	112	2.3	19	2.3	31.6	39.3	0.75	90.6	87.5	88.7	34.8	75.8
1948	Adams	1661	108	3.3	21	2.4	31.9	39.8	0.76	-	-	-	-	-
1958	Ford	1629	115	2.4	18	1.8	31.4	39.9	0.77	-	-	-	-	-
1958	Shelby	1975	118	2.5	18	2.1	31.1	39.2	0.77	93.4	91.9	92.4	33.3	75.6
1960	Ross	1601	116	2.8	19	2.4	31.4	39.4	0.76	-	-	-	-	-
1964	Adelphia	1850	108	2.1	20	2.3	31.1	39.4	0.74	-	-	-	-	-
1964	Wayne	2322	120	2.4	24	2.0	30.7	38.8	0.75	93.2	93.4	93.1	35.2	79.0
1968	Calland	2033	113	2.1	19	2.2	31.0	39.7	0.76	92.5	90.2	90.7	32.5	77.3
1971	Williams	2279	124	2.2	24	1.9	30.3	41.0	0.76	92.8	91.0	91.3	42.3	75.5
1974	Woodworth	2277	117	2.4	21	1.9	30.8	38.2	0.76	-	-	-	-	-
1978	Cumberland	2333	116	2.5	20	1.8	30.6	41.3	0.77	-	-	-	-	-
1978	Oakland	2080	113	1.6	21	2.0	30.6	40.7	0.75	-	-	-	-	-
1978	Private 3- 1	2469	95	2.0	22	1.9	30.8	44.5	0.77	84.4	89.8	85.1	38.3	74.7
1979	Pella	1961	110	1.8	20	2.0	30.7	40.1	0.77	-	-	-	-	-
1981	Williams 82	2167	125	2.3	25	2.1	30.8	39.6	0.74	-	-	-	-	-
1983	Private 3-15	2070	98	2.3	20	1.8	30.6	41.1	0.75	90.4	88.9	89.1	41.7	73.6
1984	Harper	2461	110	1.8	23	1.9	30.4	41.5	0.76	-	-	-	-	-
1984	Zane	2309	106	1.8	20	2.0	30.8	40.5	0.76	-	-	-	-	-
1986	Chamberlain	2410	120	2.1	22	2.0	30.2	39.3	0.75	-	-	-	-	-
1986	Private 3- 2	2705	116	2.4	23	1.9	30.5	40.9	0.76	-	-	-	-	-
1987	Pella 86	2076	106	1.9	21	1.8	31.0	39.0	0.78	-	-	-	-	-
1987	Resnik	2619	94	2.2	21	2.0	30.7	44.6	0.77	-	-	-	-	-
1989	Private 3- 9	2854	101	2.0	22	2.0	30.4	42.4	0.78	91.0	90.4	90.7	38.7	74.8
1990	Private 3-10	2861	105	1.8	25	2.0	30.5	43.1	0.77	-	-	-	-	-
1991	Private 3- 3	2629	104	2.1	24	1.8	30.7	43.1	0.77	-	-	-	-	-
1991	Private 3-16	2799	122	2.1	27	1.9	30.7	43.6	0.76	-	-	-	-	-
1992	Dunbar	2468	107	2.3	20	2.3	30.5	42.6	0.77	-	-	-	-	-
1992	Private 3-17	2474	103	1.9	24	1.8	30.5	41.0	0.76	-	-	-	-	-
1992	Thorne	2684	101	2.2	21	2.3	30.6	43.6	0.77	-	-	-	-	-
1993	Private 3-18	2790	102	1.8	18	2.0	31.0	41.5	0.77	-	-	-	-	-
1994	Private 3-19	2716	111	2.1	25	2.0	30.7	42.7	0.75	-	-	-	-	-
1995	IA 3004	2476	110	1.9	20	2.1	30.2	41.9	0.77	-	-	-	-	-
1995	Macon	2578	106	2.4	26	2.0	30.6	43.3	0.77	89.4	88.6	88.4	33.8	75.4
1996	Maverick	2885	125	2.6	25	2.0	30.6	44.2	0.76	-	-	-	-	-
1996	Private 3- 4	2638	102	2.1	25	2.0	30.5	42.2	0.75	-	-	-	-	-
1996	Private 3-11	2839	98	2.3	22	2.0	30.5	42.9	0.77	-	-	-	-	-
1997	Pana	2872	129	2.3	23	1.8	30.6	44.1	0.76	-	-	-	-	-
1997	Private 3- 5	3106	109	2.6	26	1.9	30.2	41.7	0.77	-	-	-	-	-
1997	Private 3-12	2975	106	2.3	27	1.9	30.0	41.6	0.77	92.3	90.1	91.1	36.3	78.4
1998	IA 3010	2987	91	1.8	22	1.9	30.5	42.6	0.75	87.3	85.5	84.2	31.4	78.0
1998	Private 3- 6	2925	112	2.1	26	1.8	30.2	42.7	0.76	-	-	-	-	-
1999	Private 3- 7	2935	110	1.9	23	1.9	30.0	41.2	0.78	-	-	-	-	-
2000	Private 3-20	3172	99	1.8	24	1.8	30.3	42.2	0.77	-	-	-	-	-
2001	IA 3014	2959	114	2.4	21	2.3	30.5	43.3	0.75	-	-	-	-	-
2001	Private 3-21	3128	102	1.6	21	1.8	30.4	41.6	0.77	89.1	88.4	87.7	31.2	72.9
2001	U98-311442	2971	104	1.9	24	1.8	30.4	42.2	0.77	-	-	-	-	-
2002	Private 3- 8	3173	113	1.8	27	1.7	30.0	40.2	0.79	83.0	84.4	83.1	34.7	71.0
2003	IA 3023	3247	106	2.0	23	1.8	30.4	42.9	0.78	-	-	-	-	-
2004	IA 3024	3405	104	2.3	23	1.8	29.8	42.8	0.76	-	-	-	-	-
2004	NE3001	2339	55	1.2	18	1.8	31.0	40.6	0.75	75.0	84.6	78.9	39.3	77.0
2004	Private 3-13	3258	103	2.0	23	1.9	30.2	42.3	0.77	86.9	88.5	86.4	39.1	72.6
2006	KS3406RR	3098	108	2.0	26	1.8	30.6	41.9	0.76	-	-	-	-	-
2006	Private 3-22	2896	105	2.1	20	2.1	30.2	41.1	0.79	-	-	-	-	-
2006	Private 3-23	3393	102	2.1	26	2.1	29.8	41.9	0.78	90.3	92.3	89.8	39.5	75.8
2007	Private 3-14	3391	108	1.9	23	2.0	30.1	43.0	0.78	91.7	89.1	89.3	41.4	72.7
	HSD‡ (0.05)	234	6	0.3	1	0.3	1.7	3.2	NS	11.4	8.9	10.4	NS	NS

‡HSD, honestly significant difference.

Table A.2 MG IV soybean genotypes information, agronomic, and physiological parameter means grown in Manhattan, KS from both 2010 and 2011 (n=54).

Year of Release	Name	Seed Yield	Height	Lod	Mat	SDS	CT	SPAD	Flr	PG28	PG34	PGavg	TAC	EL
		kg ha^{-1}	cm				°C		Fv/Fm	———	%	———		%
1930	Macoupin	1321	128	2.8	23	2.1	32.0	38.9	0.70	90.9	91.4	91.3	22.2	79.1
1933	Scioto	1145	105	3.7	21	2.2	32.0	38.2	0.69	-	-	-	-	-
1935	Boone	1650	113	3.1	28	2.2	31.7	40.0	0.70	85.3	87.5	86.9	27.3	78.7
1940	Chief	1276	145	2.5	26	1.5	32.3	39.6	0.72	84.9	87.6	85.9	26.4	76.5
1940	Patoka	1374	100	2.8	22	2.3	32.2	40.4	0.68	-	-	-	-	-
1942	Gibson	1211	107	3.3	22	2.4	31.8	36.3	0.69	-	-	-	-	-
1948	Wabash	1581	118	2.4	21	1.8	32.3	38.0	0.68	-	-	-	-	-
1952	Perry	1783	109	2.6	26	2.3	31.5	40.0	0.71	-	-	-	-	-
1953	Clark	2002	113	2.1	25	1.6	31.1	39.0	0.69	90.0	95.0	90.8	25.9	77.8
1963	Clark 63	2215	120	2.2	25	1.9	30.6	40.4	0.72	-	-	-	-	-
1968	Cutler	2040	107	1.6	24	1.6	31.4	40.9	0.70	91.5	94.5	91.8	24.1	78.7
1971	Bonus	1519	126	2.1	20	1.7	31.2	41.9	0.74	-	-	-	-	-
1973	Private 4-12	2073	116	2.3	28	2.1	31.1	39.6	0.71	87.2	90.7	87.5	27.7	76.9
1977	Franklin	2287	127	2.1	29	1.8	30.9	40.6	0.74	-	-	-	-	-
1977	Union	2345	120	2.4	25	1.7	30.8	41.4	0.73	-	-	-	-	-
1980	Douglas	2148	119	2.7	35	2.3	30.8	40.9	0.73	93.4	91.4	92.2	25.8	77.9
1980	Private 4- 6	2667	101	2.1	24	1.5	31.7	43.6	0.73	87.1	87.2	87.0	25.7	79.7
1980	Private 4- 7	2381	98	1.9	25	1.6	31.0	44.9	0.72	-	-	-	-	-
1981	Lawrence	1813	115	2.1	28	1.7	30.9	41.7	0.73	-	-	-	-	-
1981	Sparks	2412	115	2.2	25	1.6	31.2	43.9	0.74	92.0	91.3	91.4	27.7	73.1
1984	Private 4-13	2410	103	2.6	24	1.9	30.8	42.0	0.75	86.0	89.5	87.5	26.5	74.1
1985	Private 4- 1	2531	113	2.4	25	1.8	31.0	42.6	0.73	87.3	92.0	88.9	29.1	78.2
1986	Morgan	2144	120	2.3	32	1.9	31.1	42.1	0.72	-	-	-	-	-
1988	Flyer	2402	101	1.9	24	1.9	31.3	43.5	0.76	89.5	86.5	88.5	24.8	78.1
1988	Spencer	1777	115	1.9	30	1.9	30.9	42.0	0.71	-	-	-	-	-
1989	Private 4- 2	2744	118	2.5	28	2.0	31.2	42.9	0.74	-	-	-	-	-
1990	Private 4- 8	2503	108	2.3	23	1.7	31.4	42.1	0.71	-	-	-	-	-
1990	Private 4- 9	2717	112	1.8	26	1.6	30.6	42.6	0.75	-	-	-	-	-
1991	Corsica	2701	98	2.0	27	1.5	30.9	42.6	0.71	-	-	-	-	-
1992	Private 4- 3	2866	108	1.6	34	1.6	31.0	43.2	0.76	-	-	-	-	-
1992	Private 4-14	3136	120	2.4	34	1.6	30.8	42.8	0.74	-	-	-	-	-
1993	KS4694	2548	108	2.2	35	1.8	31.0	43.2	0.75	-	-	-	-	-
1993	Private 4-15	2436	118	2.6	34	2.0	31.2	43.0	0.76	-	-	-	-	-
1994	Private 4-16	3159	103	2.1	33	1.9	30.4	42.8	0.71	-	-	-	-	-
1994	Stressland	2622	108	2.0	26	1.6	31.0	42.8	0.73	-	-	-	-	-
1995	Cisne	2754	99	2.4	29	1.7	31.1	44.0	0.75	-	-	-	-	-
1995	Mustang	2705	119	2.0	30	1.6	30.9	41.7	0.76	-	-	-	-	-
1996	Omaha	2615	98	2.1	28	1.8	31.0	44.9	0.75	-	-	-	-	-
1996	Private 4-17	1956	109	2.0	32	2.2	31.3	41.6	0.71	-	-	-	-	-
1997	Private 4-18	2779	108	2.2	31	1.6	31.1	42.4	0.74	-	-	-	-	-
2000	Private 4-10	3638	105	2.3	32	1.8	30.3	42.0	0.73	-	-	-	-	-
2000	Private 4-11	3624	115	2.1	34	1.9	30.6	42.0	0.75	90.7	93.5	91.0	27.9	73.3
2000	Private 4-23	3022	113	2.1	34	1.8	30.4	41.9	0.74	88.3	90.8	87.6	25.3	77.1
2001	LS93-0375	2859	97	1.9	27	1.5	31.0	44.8	0.75	-	-	-	-	-
2001	Private 4- 4	3088	114	2.0	34	1.8	30.7	43.5	0.75	84.6	90.0	85.2	27.2	81.7
2003	LN97-15076	2786	105	1.8	29	1.6	31.1	44.3	0.74	-	-	-	-	-
2004	Private 4- 5	3170	101	2.1	35	1.9	30.5	43.0	0.78	-	-	-	-	-
2005	LD00-3309	3232	105	1.9	24	2.0	30.5	44.6	0.74	92.7	95.3	93.5	26.5	74.3
2006	Private 4-19	3370	111	2.3	36	1.9	30.8	42.0	0.73	92.6	94.4	93.4	28.8	74.9
2007	KS4607	2694	96	1.9	35	1.9	30.7	41.8	0.71	89.7	93.1	91.3	27.5	77.0
2007	Private 4-21	2990	102	2.3	27	1.8	30.8	43.9	0.74	-	-	-	-	-
2008	Private 4-20	3143	98	1.9	26	1.8	30.7	43.7	0.72	93.8	94.6	94.2	30.4	78.6
2010	Private 4-22	3734	110	2.3	29	1.5	30.6	42.6	0.77	89.5	90.5	90.4	26.6	76.7
2010	Private 4-24	3576	117	2.6	36	1.9	30.6	42.2	0.72	-	-	-	-	-
	HSD‡ (0.05)	250	6	0.3	2	0.2	1.1	2.7	0.04	11.7	8.9	10.6	NS	NS

‡HSD, honestly significant difference.

Table A.3 Summary of Manhattan, KS weather for 2010 and 2011 growing season and 30 year average.

Year	Month	Average Air Temperature Maximum	Average Air Temperature Minimum	Total Precipitation
		°C		mm
2010	May	22.8	11.9	92.2
	June	31.2	19.3	168.2
	July	32.4	21.8	106.4
	Aug.	34.0	19.9	81.3
	Sept.	28.8	14.9	76.2
	Oct.	23.7	7.4	27.2
	Total			551.4
2011	May	24.3	11.7	131.1
	June	31.5	18.8	121.2
	July	36.5	23.2	52.8
	Aug.	34.4	20.2	59.2
	Sept.	26.7	11.6	37.1
	Oct.	22.9	7.1	55.9
	Total			457.2
30 year average	May	24.8	12.0	129.3
	June	30.1	17.2	144.8
	July	33.1	20.2	112.3
	Aug.	32.3	18.8	104.6
	Sept.	27.7	13.2	87.1
	Oct.	20.9	6.2	68.3
	Total			646.4

Table A.4 Analysis of variance and F-values for selected 20 genotypes across two environments for the MG III and four environments for the MG IV.

Source	d.f.	Ttl. Plants	Ttl. Nodes	Ttl. Pods	Pod/Nod	Ttl. Seeds	Seed/Pod	SW
				Maturity Group III				
Environment (Env)	1	0.01 NS[§]	2.77 NS	8.90 NS	40.41**	14.74*	9.33 NS	2.56 NS
Genotype (Gen)	19	3.70**	5.45**	0.89 NS	9.42**	2.03*	4.10**	17.80**
Gen X Env	19	0.64 NS	1.04 NS	1.05 NS	1.55 NS	1.80*	3.20**	1.16 NS
				Maturity Group IV				
Env	3	3.63 NS	0.32 NS	7.18**	20.13**	11.17**	9.68**	31.77**
Gen	19	4.22**	4.95**	7.09**	6.03**	8.59**	9.31**	10.31**
Gen X Env	57	1.12 NS	0.77 NS	1.05 NS	1.65**	1.54*	2.39**	1.20 NS

[*] Significant at the 0.05 probability level.
[**] Significant at the 0.01 probability level.
[§]NS, not significant.

Table A.5 Analysis of variance and F-values for all genotypes across two environments.

Source	d.f	Fluorometer
——— Maturity Group III ———		
Environment (Env)	1	37.64**
Genotype (Gen)	59	0.29 NS[§]
Gen X Env	59	0.22 NS
——— Maturity Group IV ———		
Env	1	0.55 NS
Gen	53	1.97**
Gen X Env	53	1.31 NS

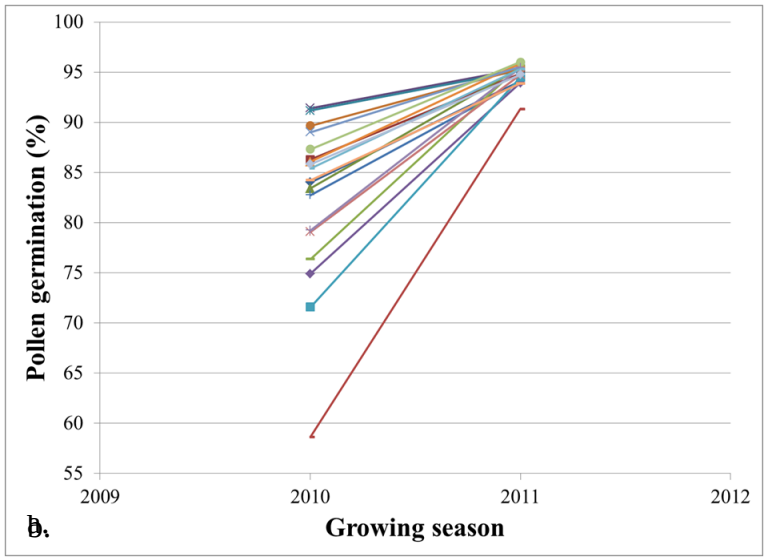
[*] Significant at the 0.05 probability level.
[**]Significant at the 0.01 probability level.
[§]NS, not significant.

Table A.6 Analysis of variance and F-values for selected 20 genotypes across four environments.

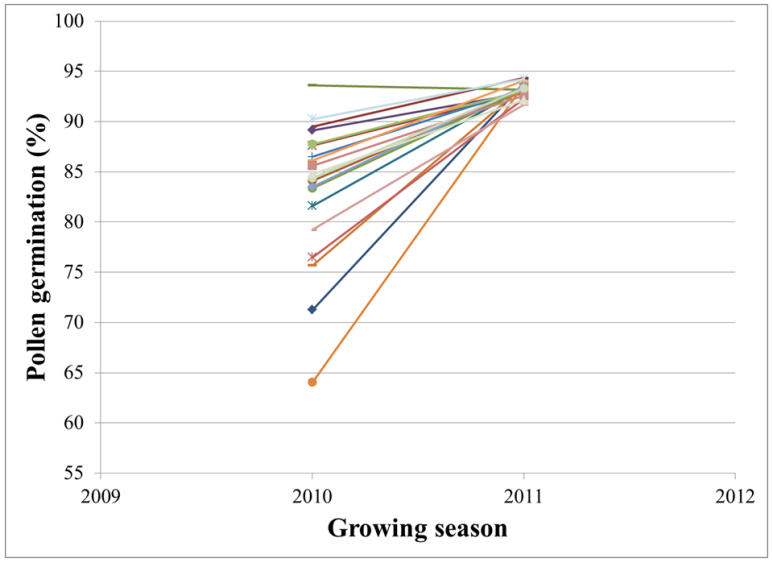
Source	d.f.	TAC	EL
———— Maturity Group III ————			
Environment (Env)	3	0.49 NS[§]	25.20**
Genotype (Gen)	19	0.73 NS	0.98 NS
Gen X Env	57	0.88 NS	0.64 NS
———— Maturity Group IV ————			
Env	3	138.56**	47.22**
Gen	19	0.4 NS	0.90 NS
Gen X Env	57	0.68 NS	0.52 NS

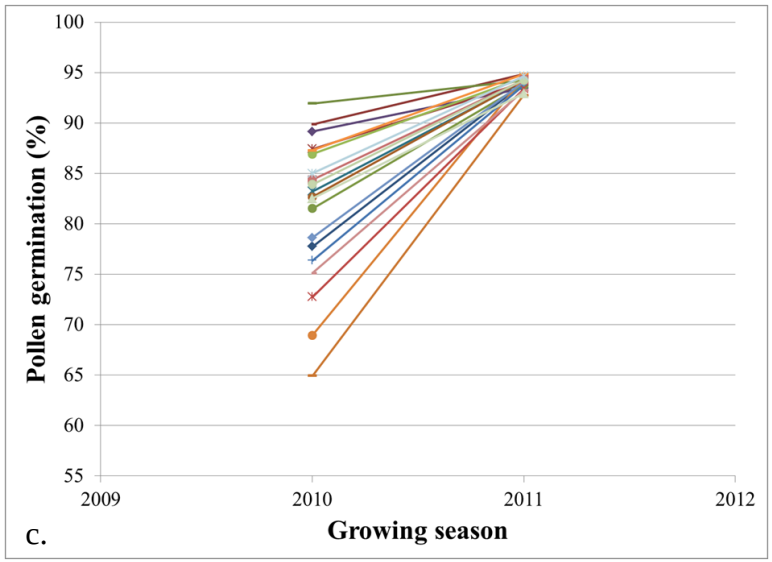
[*] Significant at the 0.05 probability level.
[**]Significant at the 0.01 probability level.
[§]NS, not significant.

Figure A.2 Plot of MGIII soybean genotypes, grown in Manhattan, KS in 2010 and 2011, by pollen germination percent for 28° C (A.), 34° C (B.), and the average of 28° and 34° C (C), showing the GxE result (n=60).



b.





c.

Figure A.3 Plot of MGIV soybean genotypes, grown in Manhattan, KS in 2010 and 2011, by pollen germination percent for 34° C, showing the GxE result (n=54).

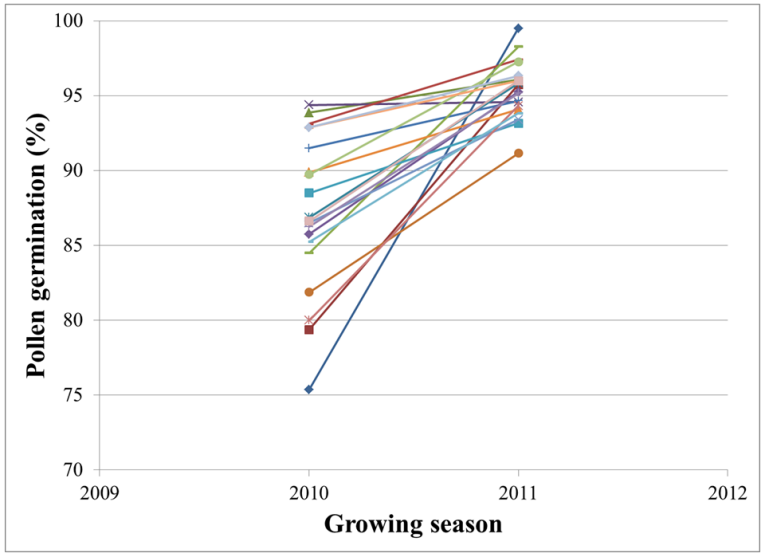


Figure A.4 Relationships between year of release and (a) seed yield (b) canopy temperature (c) chlorophyll content and (d.) chlorophyll fluorescence in MG III soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=60).

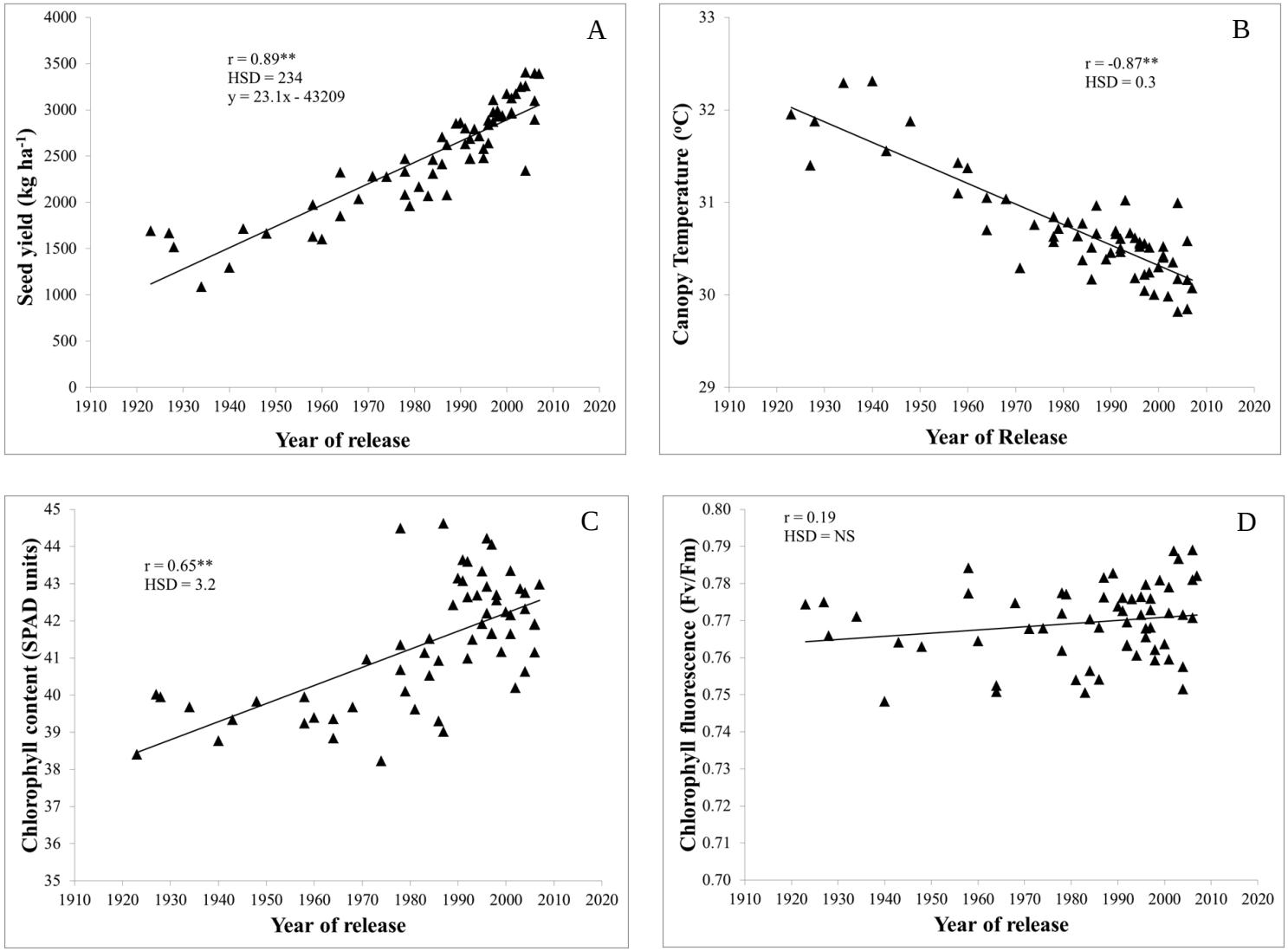


Figure A.5 Relationships between year of release and (a) seed yield (b) canopy temperature (c) chlorophyll content and (d.) chlorophyll fluorescence in MG IV soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=54).

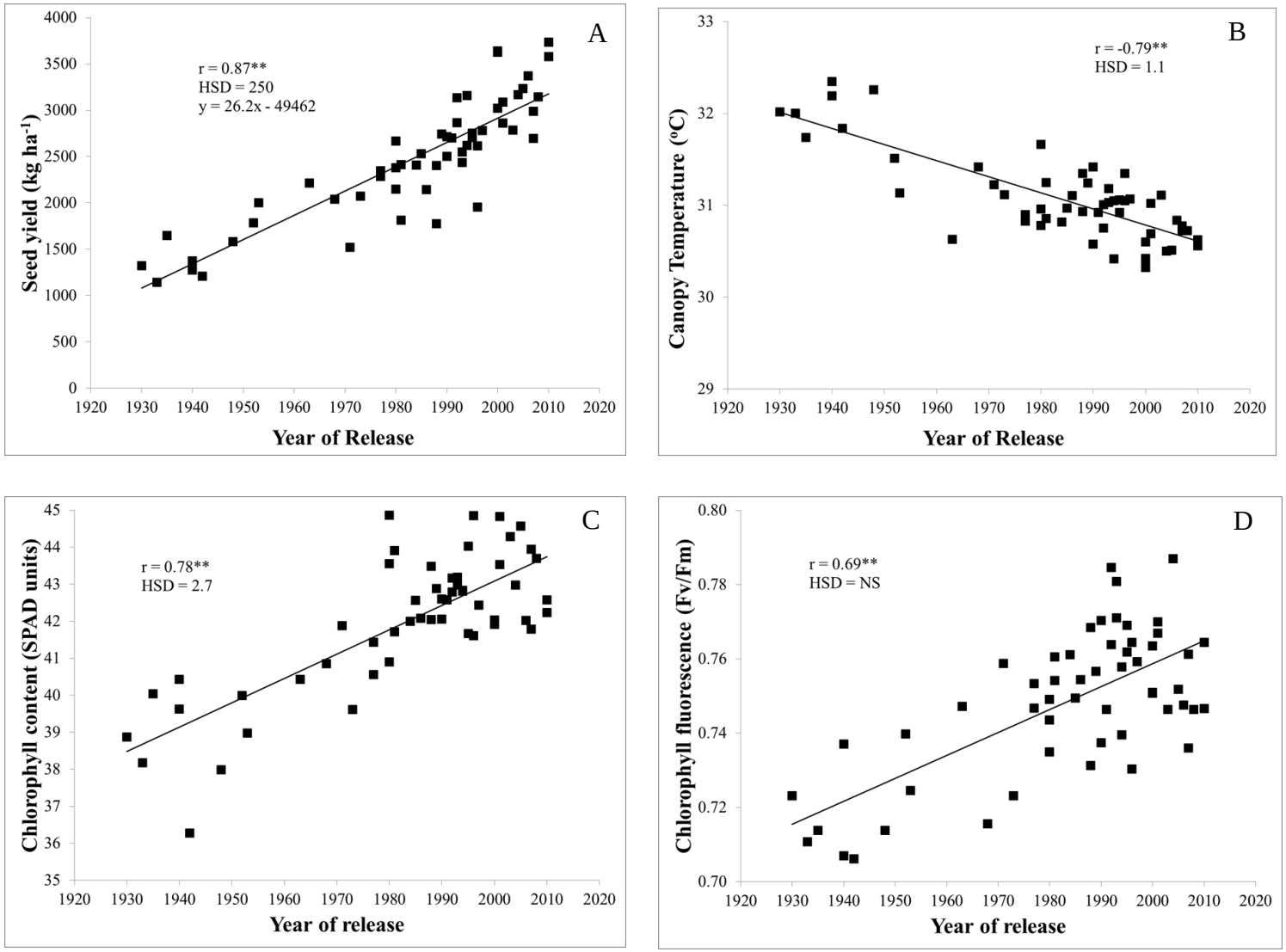


Figure A.6 Relationships between year of release and (a) height (b) lodging (c) maturity and (d.) SDS in MG III soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=60).

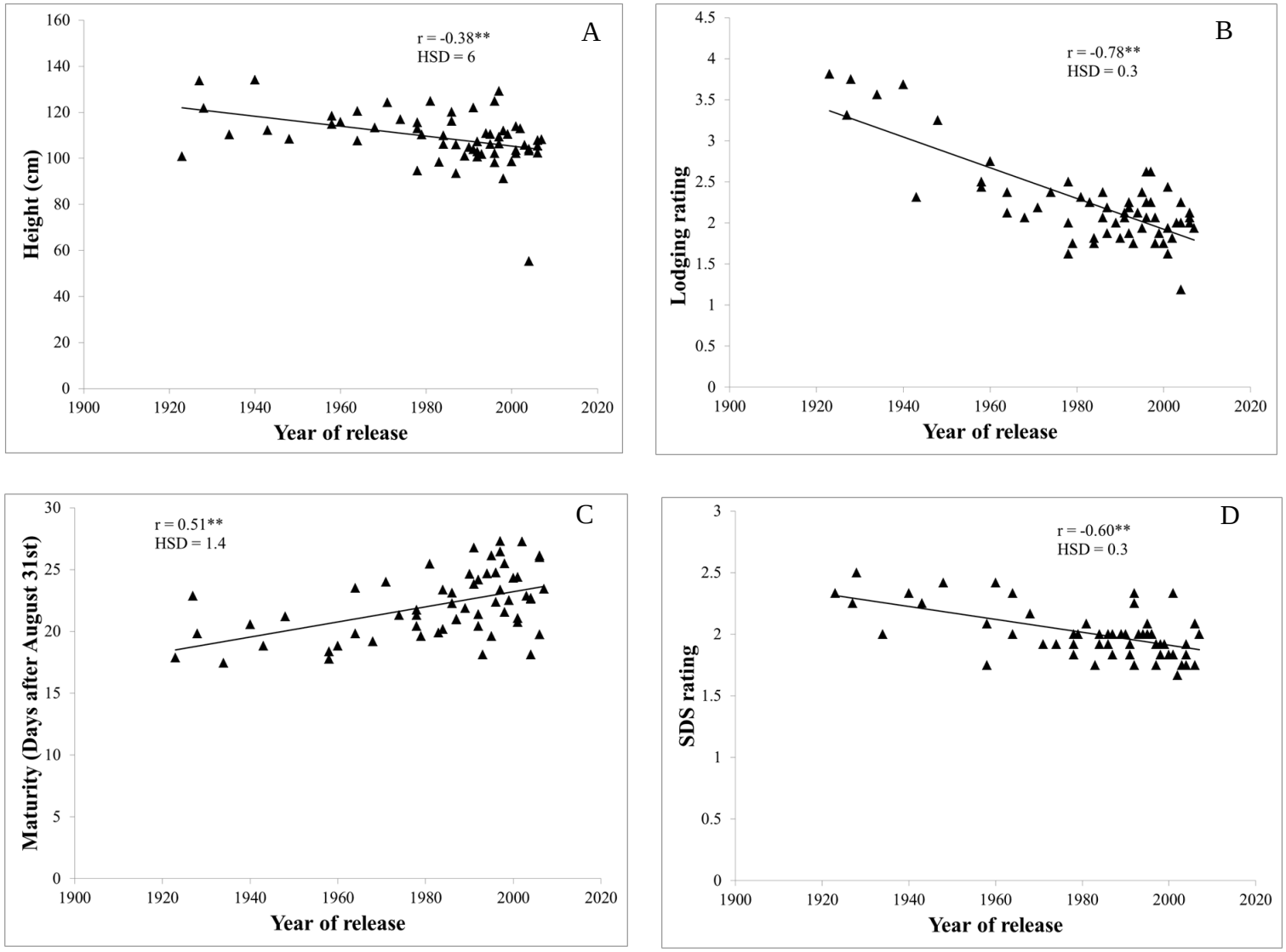


Figure A.7 Relationships between year of release and (a) height (b) lodging (c) maturity and (d.) SDS in MG IV soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=54).

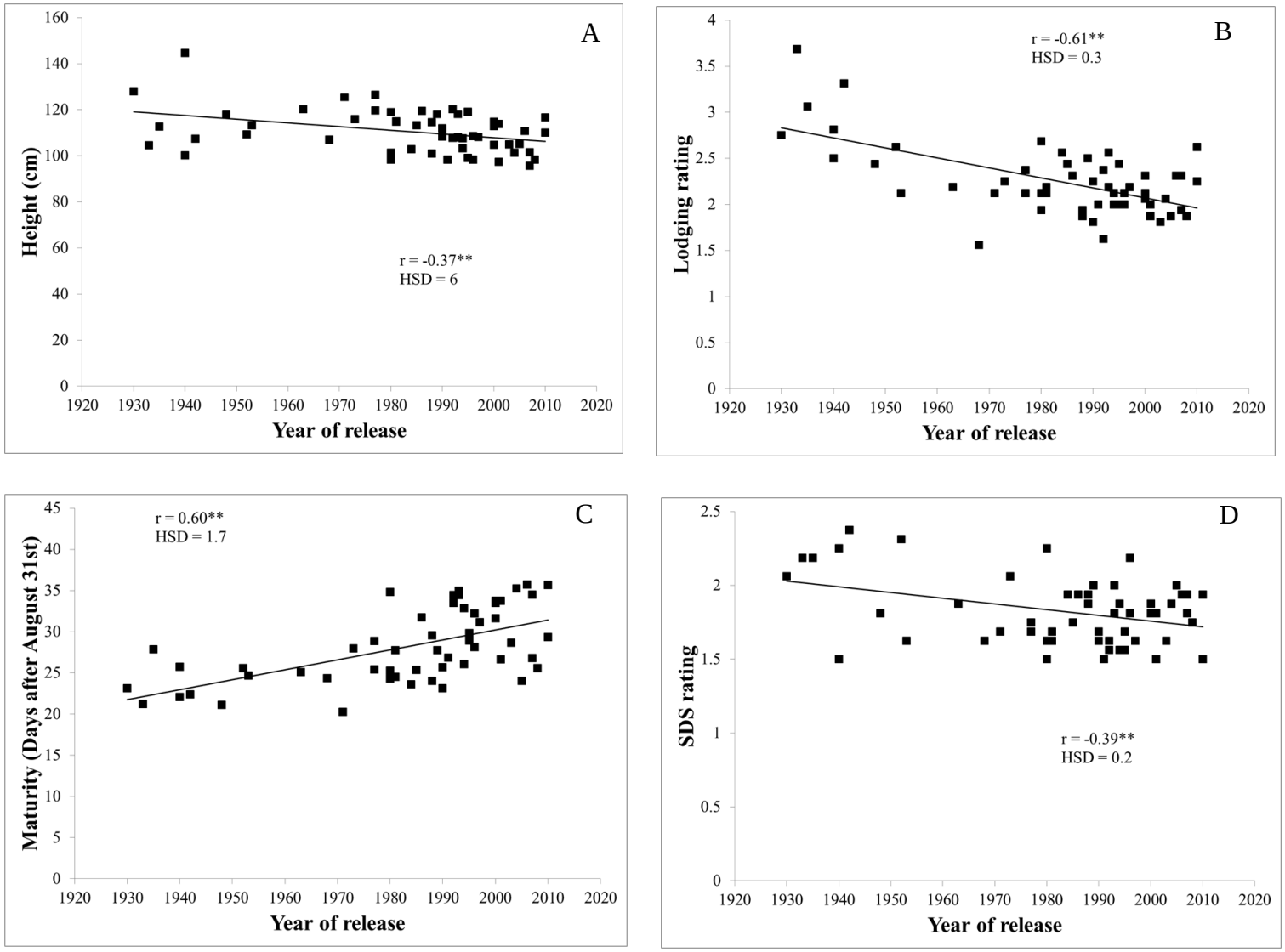
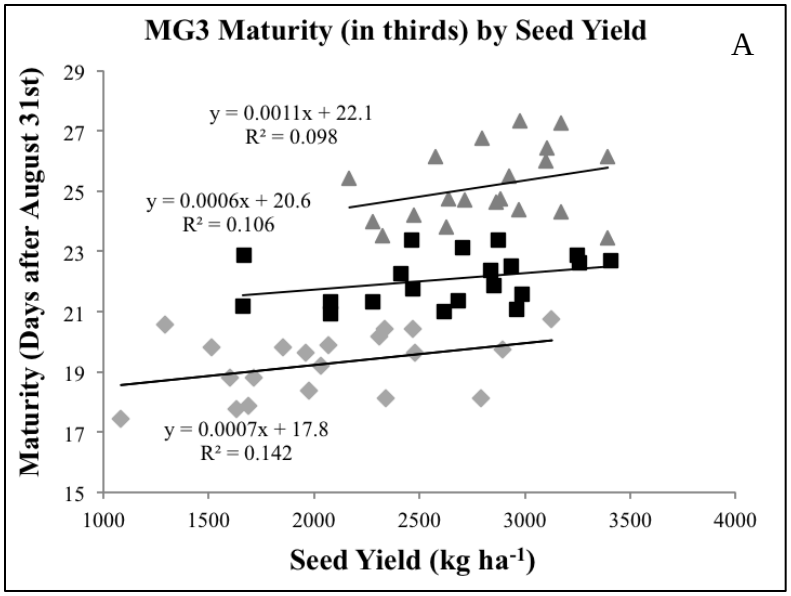
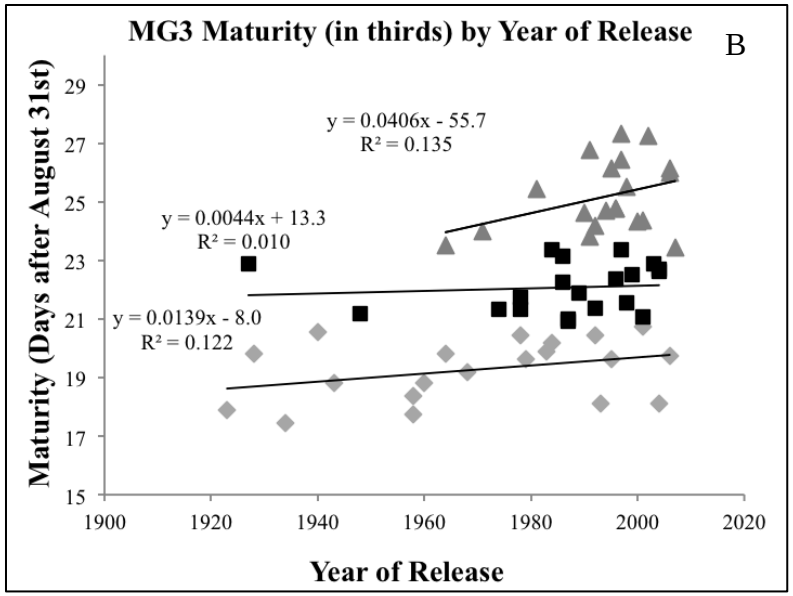


Figure A.8 Relationships between maturity, segmented into thirds, and (a) seed yield (b) year of release and (c) the relationship of seed yield and year of release in MG III soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=60).





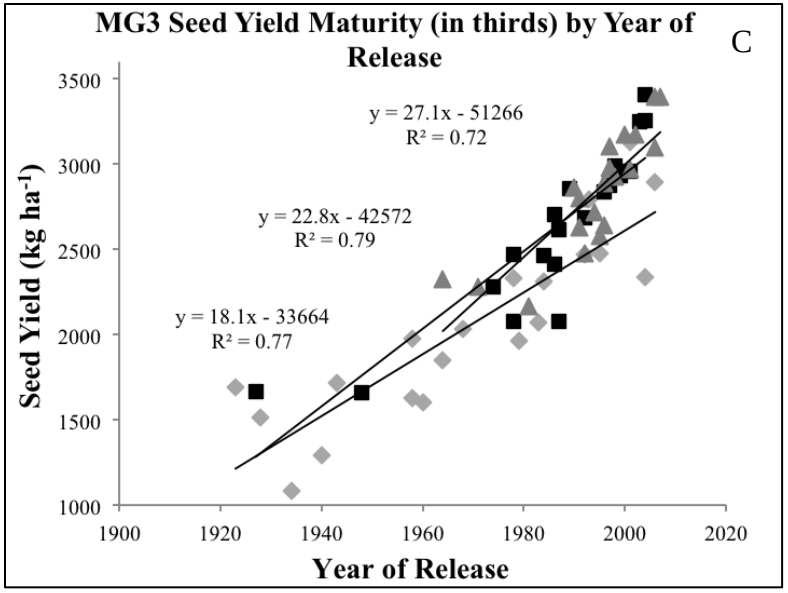


Figure A.9 Relationships between maturity, segmented into thirds, and (a) seed yield (b) year of release and (c) the relationship of seed yield and year of release in MG IV soybean genotypes grown in Manhattan, KS in 2010 and 2011 (n=54)

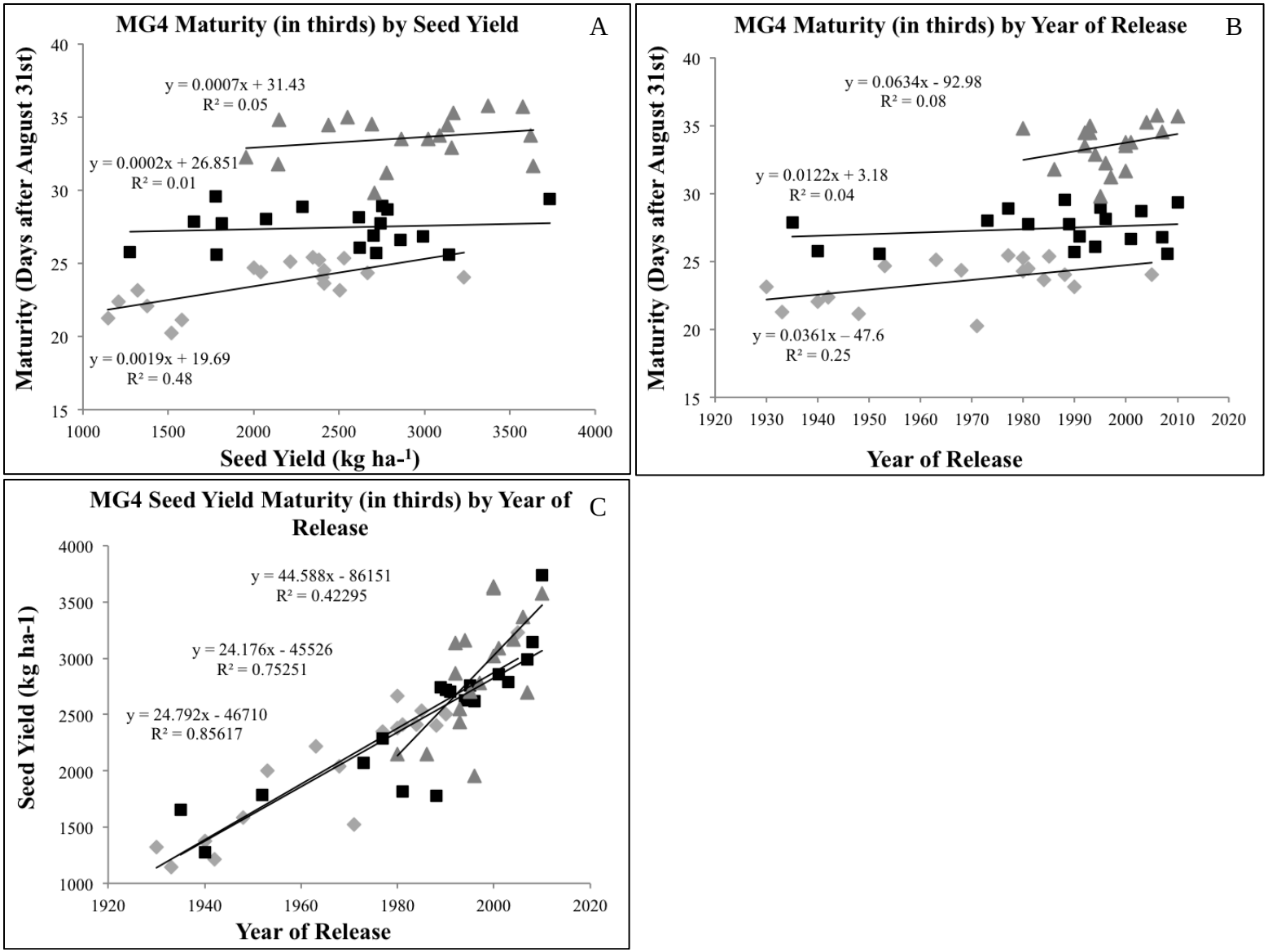


Figure A.10 Correlation of MGIV soybean genotypes, grown in Manhattan, KS from both 2010 and 2011, by seed yield with canopy temperature (n=54).

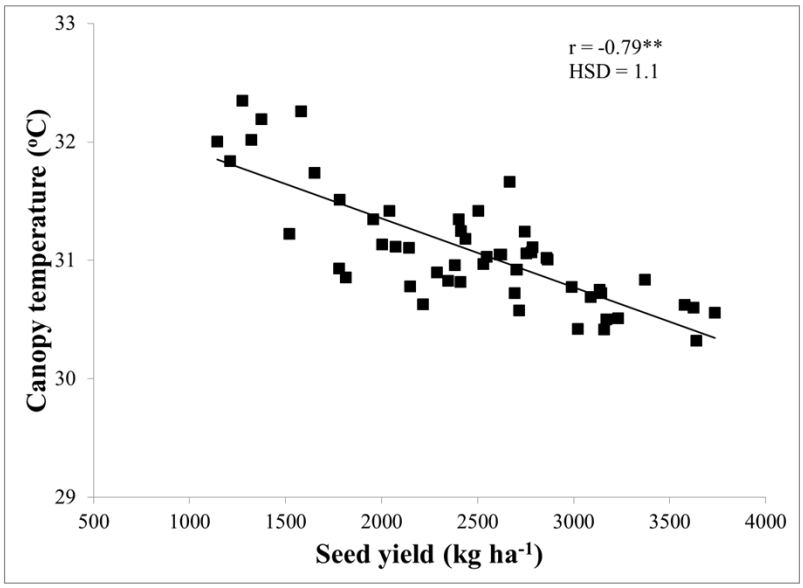


Figure A.11 Correlation of MGIII soybean genotypes, grown in Manhattan, KS from both 2010 and 2011, by seed yield with canopy temperature (n=60).