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Physiological Characterization of the SoyNAM Parental Lines under Field Conditions

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Physiological Characterization of the SoyNAM Parental Lines under Field Conditions

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ABSTRACT

The narrow genetic pool of soybean (*Glycine max* L. Merr.) in North America can limit its future yield gains. Among the worldwide germplasm collection of 45,000 unique Asian landraces, only 80 contribute 99% to the collective parentage of North American soybean cultivars. Among these 80 landraces, just 17 contribute to 86% of the collective parentage of the modern cultivars. The Soybean Nested Association Mapping population (SoyNAM) was therefore developed with the objective of diversifying the soybean gene pool in North America. Forty diverse soybean genotypes from maturity groups (MG) 1 through 5 were crossed with a common MG 3 parent to develop 40 recombinant inbred populations. Each of these populations has 140 recombinant inbred lines (RILs) and have been genotyped with molecular markers and characterized for few important traits. This experiment was conducted during three consecutive summers, in Fayetteville, Arkansas with the objective to phenotype the SoyNAM parental lines for yield and drought-related traits. And, then identify the extreme genotypes among these parental genotypes, which have either not been mapped previously or if mapped have not been mapped very extensively.

Canopy coverage was estimated through aerial digital images taken 3 to 4 times until canopy closure. After canopy closure, during late vegetative or early R1 stage, shoot samples were taken that were used to determine N_2 derived from the atmosphere (NDFA), shoot nitrogen and ureide concentrations, and carbon isotope ratio ($\delta^{13}C$, an indirect measure of water use efficiency). Two harvests were made at mid-R5 and two weeks later, to calculate seed growth rate and effective filling period. Wilting measurements were taken towards the end of irrigation cycles when drought symptoms started appearing. Yield and harvest index (HI) were determined from a bordered section of each plot at maturity. Statistical analysis indicated that several parents

differed statistically from the hub parent. Some genotypes were also identified as common extreme parents for more than one trait. Identification of such divergent parental lines will aid in selecting recombinant inbred populations for future quantitative trait loci mapping (QTL) studies.

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DEDICATION

This thesis is dedicated to the farmers of India who are absolute heroes and became my source of inspiration in choosing this field of crop science. My husband Kaushik for believing in me, my choices and standing my side with all his love and unwavering support. My mother who inspired me to work hard for building my life, to fight all the odds and stay focused to carry out my work passionately and at the same time enjoy each and every step of it.

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1.1 Introduction

The US is the world's largest producer of soybean *(Glycine max* L. Merr.), contributing 34% to the world's soybean production, and soybean is the second most important crop of the US. However, the soybean gene pool in North America is quite narrow; only 17 accessions contribute 86% of the parentage to modern cultivars (Carter et al., 2004; Gizlice et al., 1994). This narrow genetic base can limit future yield gains. The Soybean Nested Association Mapping population (SoyNAM) was therefore developed with the objective of diversifying the soybean gene pool. By crossing 40 diverse soybean genotypes from maturity groups (MG) 1 through 5 with a common MG 3 parent, 40 recombinant inbred populations were developed. These 40 recombinant inbred populations were genotyped with molecular markers and characterized for maturity, nematode rating, and a few other important traits.

Although several years of research on physiological and biochemical aspects of the crop has provided considerable insight into traits that influence plant growth and crop yield, none of this research has made a significant contribution to cultivar improvement, as it has failed in aiding in problem identification and germplasm selection (Sinclair et al., 2004). The reason for not using physiological traits is the difficulty involved in making many of these measurements on a large number of genotypes. The SoyNAM populations, which were developed with the motive to diversify the soybean gene pool and to identify and map traits of interest, can play a major role in solving this problem and are a tremendous resource that can be utilized to develop a new 'toolbox' for breeders to use. However, the very first step in developing this toolbox is to characterize the parents of the SoyNAM populations.

The current research focuses on identification of extreme parental genotypes of the SoyNAM population and characterization of the SoyNAM parental genotypes for yield and

drought-related physiological traits, that have either not been mapped previously, or if mapped, have not been mapped very extensively. By phenotyping the parental genotypes, it will allow identification of specific mapping populations that will likely have the most segregation for traits of interest. Identification of the most divergent parental lines for these traits will aid in selecting recombinant inbred populations for future quantitative trait loci (QTL) mapping studies.

1.2 Literature Review

1.2.1 Soybean Morphology, Origin, and History

Soybean (2n = 40, diploid number of chromosomes) belongs to the leguminosae family and produces seeds containing ~20% oil and ~40% protein. The growth habit of this plants is categorized into determinate and indeterminate. Wild soybean (*Glycine soja*) is indeterminate (Tian et al., 2010). Indeterminate soybean varieties begin to flower when plants are around half of their final height, whereas determinate varieties bloom relatively uniformly in the top and bottom positions of the plant (Fehr and Caviness, 1977). Also, indeterminate plants are relatively tall and have smaller leaves on the top than on the lower portion of the plant, while determinate varieties possess similar sized leaves at both the positions of the plant (Fehr and Caviness, 1977). Determinate varieties have a terminal raceme with a cluster of flowers along a central stem, indeterminate plants do not possess a raceme and instead have a zigzag-pattern in the upper nodes (Fehr and Caviness, 1977). Determinate varieties have typically been grown in the Southern U.S., whereas indeterminate ones were mostly grown in the Northern U.S. (McWilliams et al., 1999). In the recent years, indeterminate varieties have become more prominent in the Southern U.S. (Purcell et al., 2014).

Soybean initiates flowering under short photoperiods, hence is a short-day plant (Kumudini, 2000). These plants are sensitive to the length of photoperiod and are adapted to

different latitudes. Based on the adaptation for specific latitudes, soybean cultivars have been classified into different maturity groups (MGs) ranging from 000 in Canada to 9 in the tropics (McWilliams et al., 1999). Maturity groups typically grown in Arkansas are 3, 4 and 5.

Soybean originated in Southeast Asia, specifically China (Qiu and Chang, 2010) and was first domesticated in China around 1100 BC (NCSPA, 2014) from its closest wild relative *Glycine soja* (Guo et al., 2010; Joshi et al., 2013). During the first century CE, Japan and many other neighboring countries grew soybean (NCSPA, 2014). The main difference between *G. max* and *G. soja* is that of seed color, seed size, seed oil and protein concentration, grain yield, growth habit (upright vs. prostate), and stress tolerance (Joshi et al., 2013).

A colonist, who planted it at Savannah, Georgia in 1765, introduced soybean to North America. Benjamin Franklin also sent some soybean seeds to a friend to plant in his garden in 1770. In 1851, soybean seeds were distributed to American farmers in Illinois and other corn-belt states (these seeds were a gift from a crew-member rescued from a Japanese fishing boat in the Pacific Ocean in 1850). During the 1870s soybean gained popularity among farmers, and they began planting soybean as forage for livestock (NCSPA, 2014). Later, soybean plants flourished in the North Carolina because of its characteristic and suitable hot and humid summers. In 1904, after the identification of soybean as a useful protein and oil source, and as a means to preserve soil nitrogen quality (by George Washington Carver, the American chemist), soybean was adopted as a rotational crop by cotton (*Gossypium hirsutum*) growers (NCSPA, 2014). It began to be grown as a food crop during the early 20th century and emerged as a major crop of the U.S. over the past three decades (USSEC, 2017).

1.2.2 Soybean Present Status

Soybean is one of the most important and the most cultivated oilseed crops worldwide. It is produced in about 50 countries with the United States, Brazil, Argentina, and China being the major producers. The United States (U.S.) has been the world's leading producer for the past half-century and accounts for one-third of the global production (USSEC, 2017). Soybean is the second highest hectarage field crop in the U.S. after corn (*Zea mays* L. Merr) (USDA, ERS, 2017); and represents 34% of total world soybean production (Soystats, 2016). About 90% of the oilseed production within the United States is from soybean (USDA, ERS, 2017), and the country exports over 60% of soybean as grain, cake (meal), and oil with the largest annual exports in 2008-10 of 48 MT (Fischer et al., 2014). A booming world market for vegetable oils and animal feed is fast emerging, driving a need to increase soybean production. Arkansas currently ranks 10th in soybean production in the nation, producing about 150 million bushels that values more than $ 1.5 billion, and exporting 37% of its produce (Arkansas farm Bureau, 2016). Arkansas is also the edamame capital of U.S. In 2014, soybean and soybean products were the largest agricultural export in Arkansas, worth $1.2 billion out of $3.72 billion worth of total agriculture exports (Arkansas Farm Bureau, 2016).

1.2.3 Narrow Genetic Base

There are several reasons for the narrow genetic base of soybean. Soybean is an autogamous species and has also undergone several genetic bottlenecks resulting in a small gene pool. The three major genetic bottlenecks are: 1) domestication in Asia to produce Asian landraces, 2) the introduction of Asian landraces to North America, and 3) selective breeding over 75 years (Hyten et al., 2006). Other than this, the wild perennial Glycine species have not been exploited for genetic improvement and broadening the genetic base of soybean (Chung and

Singh 2008). Therefore, soybean cultivars being grown worldwide have a narrow genetic base and diversity. Despite this apparent limitation of the narrow genetic base for production, soybean breeding has continued to make significant progress to date (Singh, 2017). However, future genetic gains in productivity can be adversely affected due to this reduction in genetic diversity. Also, reduced genetic diversity can also lead to susceptibility to emerging diseases (Hyten et al., 2006).

Among a collection of about 45,000 unique Asian landraces maintained in the *G. max* germplasm collection worldwide, there is vast genetic diversity; however, just 80 (0.02%) of these landraces contribute to 99% of collective parentage of North American soybean cultivars (Carter et al., 2004). Among these 80, just 17 contribute to 86% of the collective parentage of modern cultivars. In the remaining 63, each contributes to less than 1% (Gizlice et al., 1994). Thus, the soybean gene pool in North America is quite narrow.

Nucleotide sequence variation was evaluated in 120 soybean genotypes that included representative members of four distinct populations, namely: 25 elite North American soybean cultivars, 17 Asian landraces that were founders of North American elite cultivars, 52 Asian landraces (other than founders of American elite cultivars), and 26 diverse accessions of wild-type soybean (*Glycine soja*) (Hyten et al., 2006). This variation study revealed that the effects of domestication and introduction combined with subsequent intensive selection resulted in sequence diversity losses in elite cultivars versus *G. soja* as measured by Θ, ¶, and haplotype diversity. The two common measures of nucleotide diversity are Θ and ¶; Θ is the number of polymorphic sites in a genotypic sample corrected for sample size (Watterson, 1975; Hyten et al., 2006), and ¶ is the expected heterozygosity per nucleotide (Tajima, 1983; Hyten et al., 2006). No allelic diversity was detected among elite cultivars for 40% of genes analyzed. The

domestication bottleneck caused 50% reduction in diversity, 81% elimination of rare alleles, and significant change in allele frequency in 60% of genes analyzed (Hyten et al., 2006). Overall, it seems that the domestication bottleneck was more severe in soybean than in other crops. The data indicated that modern breeding has minimally affected allelic structure compared to historical bottlenecks. Therefore, low nucleotide diversity in the modern elite North-American cultivars seems both due to an unusually low level of genetic variability in the wild *progenitor G. soja* (¶ = 0.00217, Θ = 0.00235), as well as due to the reduction in diversity that occurred during domestication and introduction. The introduction of favorable alleles from diverse soybean backgrounds is considered an important means of soybean improvement (Gizlice et al., 1994; Carter et al., 2004).

1.2.4 SoyNAM

The Soybean Nested Association Mapping population (SoyNAM) was developed by crossing 40 diverse genotypes with a single reference parent (IA3023, an elite high yielding cultivar developed at Iowa State University) and advancing progenies to the F6 generation to create 40 recombinant inbred populations, each consisting of 140 recombinant inbred lines (RILS) (SoyBase, 2018). The mating design, molecular techniques, and statistical models provide a potential means of mapping various agronomic traits in this population (SoyBase, 2018).

The 40 diverse genotypes selected for the SoyNAM population are shown in Table 1.1. All of the 40 mapping populations have been genotyped with molecular markers using 6K bead chip, and characterized for yield, disease and nematode ratings, maturity, plant height, development stages, and a few other important traits.

1.2.4.1 Characteristics of Parents

Phenotypic characteristics of the parental genotypes for maturity group, stem termination (determinate or indeterminate), flower color (purple, white), pubescence color (gray, tawny), pod color (tan, black, brown, and others), seed coat luster (dull, shiny and others), seed coat color (yellow, green, brown, black, mottled and others), and hilum color (black, imperfect black, brown, buff, yellow) are summarized in Table A.1.

Phenotyping the parental genotypes will allow identification of specific mapping populations that will likely have the most segregation for traits of interest. This research will focus on phenotyping physiological traits that are important for crop growth, yield, and drought tolerance that have not been mapped extensively or, in some cases, not at all. These traits include canopy coverage, radiation use efficiency, seed growth rate, effective filling period, seed weight, N_2 fixation, ureide concentration, canopy temperature, wilting, water use efficiency (measured through carbon isotopic ratios), harvest index and seed yield.

1.3 Physiological Traits

1.3.1 Crop Growth and Yield-associated Traits

1.3.1.1 Radiation Interception and Canopy Coverage

Canopy coverage is an indirect estimate of radiation interception, and radiation interception by a plant is the preliminary necessity for plant growth; hence an important trait with respect to yield. Monteith (1977) discovered that the dry matter accumulation rate varied in direct proportion to the amount of intercepted radiation. Gifford et al. (1984) found that canopy's light interception capacity determines yield. It would, therefore, be valuable to further understand the relationship between radiation interception (RI) and crop growth.

Measurement of photosynthetically active radiation (PAR), above and beneath a canopy, near solar noon when there is no obstruction of light by the clouds (Board et al., 1992; Egli,

1994a; Flenet et al., 1996; Purcell, 2000) is the most familiar method for the measurement of the fraction of radiation intercepted (FRI); where

$$FRI = (1- (PAR\ beneath\ canopy) \times (PAR\ above\ canopy)^{-1}) \quad [1]$$

These measurements are made using light quantum sensors that integrate PAR along a meter length. To measure RI, these quantum sensors are either placed perpendicular to a row (Egli, 1994a), or placed parallel to a row beneath the canopy, and multiple measurements are taken between the rows and averaged (Board et al., 1992).

Purcell (2000) demonstrated that FRI could be determined based on canopy coverage values, obtained from the analysis of digital images made from above a crop. Purcell found that canopy coverage obtained through this process, and FRI was strongly correlated. The method assumes that soil background is distinguishable from leaves, leaves have a smaller leaf transmission than absorption, and the angle between camera and horizon approximates solar angle. If these assumptions are correct, then canopy coverage (i.e., the fraction of ground area covered by leaves) is approximately equivalent to FRI obtained in the unobstructed light. The method allows canopy coverage measurements, irrespective of solar radiation and solar angle restriction, therefore is of great significance. Additionally, the digital-image analysis method is an inexpensive, fast and efficient alternative to the methods of directly estimating radiation interception (Fiorani et al., 2012). In digital imaging, the software identifies green pixels from the hue, saturation, and brightness. Canopy coverage is determined as the fraction of green pixels divided by the total number of pixels per frame (Purcell, 2000). This analysis method has now become a widely accepted high-throughput method for canopy coverage or FRI determination (Gasper and Conley, 2015; Xavier et al., 2017).

In soybean, canopy coverage and cumulative intercepted photosynthetically active radiation (CIPAR) have been found to relate well with grain yield (Edwards et al., 2005; De Bruin and Pedersen, 2009; Gaspar and Conley, 2015). Canopy coverage has also shown a strong association with individual seed weight (Place et al., 2011). Edward et al. (2005) and Gasper and Conley (2015) reported that soybean canopies that captured 400 to 600 MJ-m^{-2} CIPAR by R6 maximized yield; so the final plant population must be high enough to attain the required CIPAR. Faster canopy coverage also enhances water use efficiency, through increased transpiration (Purcell and Specht, 2004).

Kaler et al. (2018) identified 11 QTLs associated with canopy coverage, for two different dates of measurement, on a GWAS panel of 373 genotypes.

1.3.1.2 Radiation Use Efficiency

The amount of dry matter produced is proportional to the intercepted photosynthetically active radiation (IPAR). Thus, radiation use efficiency (RUE) is defined as the amount of biomass produced for each unit of intercepted solar radiation and can be considered an integrated, long-term measure of photosynthesis (Monteith, 1977; Kiniry et al., 1989). Crop biomass (BM) is hence, the product of IPAR and RUE as proposed by Monteith (1977).

RUE is estimated by collecting sequential plant mass samples from a defined area during a growing season, and regressing the dried BM (g m^{-2}) against IPAR (MJ m^{-2}). The slope of this regression defines RUE (g MJ^{-1}, Sinclair and Muchow, 1999; Monteith, 1977). The typical RUE values (BM over PAR basis) for a soybean crop are ≈ 1.3 to 2.5 g MJ^{-1} (Sinclair and Muchow, 1999).

Yield stagnates or begins to decrease as the population density reaches a critical threshold (Wiggans, 1939; Weber et al., 1966; Purcell et al., 2002). This is because there is a

decrease in RUE with an increase in plant population density (Purcell et al., 2002), and dry matter accumulation is a function of RUE. The reason for this decrease in RUE at high population density is still unknown. The self- thinning rule could be a reason for this decrease in yield, but that signifies a decrease in plant density as the plant biomass increases (Yoda et al., 1963; Harper, 1977; Li et al., 2000).

The decrease in specific leaf nitrogen (N) concentration at high population densities also decreases RUE, as the amount of N that can be obtained from soil is fixed (Sinclair and Horie, 1989). Accordingly, at high population densities, N is distributed across a large leaf area, which decreases specific leaf N concentration (SPLN). This SPLN drop was observed in soybean by a linear relationship obtained between RUE and SPLN, with maximum RUE at 1.2 gN m^{-2} SPLN (Sinclair and Horie, 1989, Pengelly et al., 1999). Deficiency of water or other nutrients can also result in decreased SPLN and hence a decrease in RUE.

1.3.1.3 Seed Growth Rate, Effective Filling Period, and Seed Yield

Egli (1994b) defined yield as the weight of seeds produced from a unit area, however, if we look closely yield is actually produced by a rate expressed over an interval of time. Thus, the time aspect or the duration of seed growth known as the seed fill duration (SFD) is also important. Seed fill duration (SFD) refers to the duration from growth stage R5 to R7 (Egli, 1994b) and is a heritable trait (Salado- Navarro et al., 1985). It is difficult to measure the duration of seed fill because of the difficulty to accurately determine when the seed begins and terminates accumulating dry weight. Effective filling period (EFP) calculated as a quotient of final seed mass and seed growth rate (SGR, Daynard et al., 1971; Egli et al., 1978) is therefore frequently used as an estimation of the seed fill duration. Studies have reported that the EFP was found significantly correlated with the whole plant measure of SFD. Final weight per seed in

soybean is thus a product of seed growth rate (SGR, g $seed^{-1}$ day^{-1}) and effective filling period (EFP, days) (Van Roekel et al., 2015).

To measure SGR, multiple plant samples are taken during the linear phase of seed growth, between mid-R5 and maturity. The pods are removed from the plants immediately after getting the plants from the field (mid-R5) and then dried (Egli, 1975). The dried pods are shelled, and the seed weight for each sample is measured and seeds are counted. Average seed mass is obtained by dividing seed weight by seed number. The increase in average seed weight is obtained by taking the difference of the average seed weight of the two harvests or by regressing if there are more than two samples. This increase is divided by the number of days between sampling dates to finally get the SGR (Egli, 1975; Swank et al., 1987), assuming that both harvest dates are during the linear phase of seed growth.

Although final weight per seed in soybean is a function of SGR and EFP, EFP is more frequently related to seed yield (Dunphy et al., 1979; Smith and Nelson, 1986) than is SGR. Daynard et al. (1971) reported that there was less than 16% variation in grain yield among corn hybrids due to differences in SGR, but 71 to 80% yield variation with respect to EFP. Long EFP is associated with high seed yield (Boerma and Ashley, 1988). Similarly, Hanway and Weber (1971) concluded that variation in the duration of the filling period was the main cause of yield differences among the eight soybean cultivars that they studied, as the cultivars had same dry matter accumulation rate. Egli and Leggett (1973) reported a similar association between yield and the effective filling period for other soybean cultivars. Sinclair and De Wit (1976) also stated that the strategies for lengthening EFP were critical for soybean yield increase. Several other studies, Dunphy et al. (1979), Boote (1981), Nelson (1986), and Gay et al. (1980) confirmed a relation between EFP and seed yield.

The factors controlling SGR are quite well known, but much less is known about the factors controlling the duration of seed fill, but it is known that the filling period is regulated by both plant and seed mechanisms (Egli, 1994b). Egli (1990) reported that cessation of seed dry matter accumulation (seed growth) which determines the EFP, is controlled by the physiological responses of the seed to the environment and is not a pre-determined characteristic of the seed. The cessation of dry matter accumulation in the seed is controlled by the ability of the seed to continue water uptake and cell expansion; when net water uptake stops, continued dry matter accumulation causes desiccation and eventual cessation of dry matter accumulation (Egli, 1990). Cell water uptake is a function of the osmotic gradient across the cell wall (Lockhart, 1965). Assimilate availability also regulates net water uptake and cell expansion. Furthermore, assimilate availability and physical characteristics of the pod and/or seed may interact to control cell expansion, thus impacting EFP (Egli, 1990). Seed moisture is also closely related to the stage of seed development. Seed water uptake increases in the initial phase of seed development reach infinity by the time 80-90% of the seed dry mass is accumulated, and declines sharply thereafter (Fraser et al., 1982; Swank et al., 1987).

1.3.1.4 Seed Weight and Harvest Index

Harvest index (HI) is the ratio of total grain weight to mature plant weight and is an important trait associated with the noticeable increases in crop yield that occurred in the twentieth century. The ratio of grain weight to total plant weight was first noted by Beaven (1914) and termed as “migration coefficient” (Donald and Hamblin, 1976). Modern crop plants mostly have shorter and stiffer stems than previous crops, which is a trait related directly to increased HI (Sinclair, 1998). Improvements in HI highlight the importance of C allocation in grain production and reflect the progress in the partitioning of assimilated photosynthate to the

harvestable product (Sinclair, 1998). Photosynthate is, however, only one resource involved in the change in HI. Another critical component of grain is nitrogen (N), and partitioning of N to seed could have a crucial influence on HI. N accumulation and HI have a direct relationship because the grain and straw are not of equal N concentration; therefore, any major shifts in the relative fraction of grain and straw require substantial changes in N accumulation by the plant (Sinclair, 1998). However, selection of germplasm solely based on increased HI has failed to generate plant material that gave higher yields in soybean (Buzzell and Buttery, 1977). For further increases in yield, it is, therefore, necessary to select for germplasm that responds to nitrogen applications and have high HI as well.

Spaeth et al. (1984) suggested that the HI within soybean cultivars was relatively stable and that regardless of individual plant size the proportion of seed mass (i.e., HI) was relatively constant. Constant HI within cultivars would be a useful tool in the prediction of seed yield from total biomass (Donald and Hamblin, 1976; Spaeth et al., 1984). Harvest index is, however, not always independent of environment. Schapaugh and Wilcox (1980) found in soybean that HI of late maturing cultivars dropped significantly in a weak year. Edwards and Purcell (2005) indicated that there was a linear decrease in HI as CIPAR (cumulative intercepted photosynthetically active radiation) increased. Edwards and Purcell (2005) also indicated that with increased plant population, HI generally increased slightly in early maturity groups MGs (00,0), decreased slightly in MG 5 and 6, and showed no response to increasing plant populations for MG 1 through 4.

1.3.2 Traits Associated with Ameliorating Drought Effects

Soybean is an important source of plant protein and oil worldwide (Dhanapal, 2015b). The crop faces several challenges in the form of different abiotic stresses like drought, salinity,

and extreme temperature that obstruct plant growth at different developmental stages. Among these challenges, drought is the most daunting challenge (Tuberosa, 2013). Drought is the most prevalent, controlled by multiple genes, and is greatly affected by the environmental factors (Blum, 2005; Pinto et al., 2010).

From the agronomic perspective, drought stress refers to decreased soil water content because of reduced rainfall or irrigation, resulting in abnormal plant development and or yield loss in the field (Purcell and Specht, 2004). Drought stress differs from water- deficit stress, which usually refers to treatments inside a growth chamber or a greenhouse (Purcell and Specht, 2004).

1.3.2.1 Nitrogen Fixation Rate, Nitrogen Concentration, and Nitrogen Derived from Atmosphere

Nitrogen (N) is the most limiting nutrients for most crops. Physiological and biochemical studies have demonstrated that maintenance of an appropriate carbon (C) and N balance is essential, and that, when plants are deficient in N, photosynthesis declines (Coruzzi and Zhou, 2001). An increase in yield has been reported in legumes when the amount of N fixed for a unit amount of C invested is enhanced (Denison, 2015). Likewise, when photosynthesis is decreased due to drought, N_2 fixation in soybean drops (King and Purcell, 2006). With legumes, although there is the advantage that they fix atmospheric N_2, N_2 fixation is affected by several environmental conditions.

Muchow and Sinclair (1986) concluded that N accumulation could be an important constraint on final seed yield for soybean. Sinclair (1998) also pointed out that N is a critical component of grain and its partitioning to seed could have a crucial influence on HI and yield. While analyzing the photosynthate and N requirements of seeds, Sinclair and De Wit (1976) found soybean to be unique among crop species they studied. Having both a high protein and

lipid content, soybean required the highest rate of N supply to the seed, yet produced biomass at one of the lowest rates. Therefore, soybean needs to translocate large amounts of N from vegetative tissues during seed-fill to sustain seed growth. The loss of N from the vegetative plant parts causes a loss of physiological activity that leads to senescence. Sinclair and De Wit (1976) proposed that this N loss could limit EFP and thereby limit total seed production and yield. However, the effects of this characteristic would be dependent on the rate of photosynthate supplied to the seed, rate of external N supply, and the rate of N translocation within the plant.

Nitrogen requirement of soybean is met by a combination of uptake and assimilation of inorganic soil N and biological N_2 (atmospheric nitrogen) fixation. If inorganic soil N is abundant, N_2 fixation decreases and the proportion of N in the crop derived from N_2 fixation declines. In contrast, if inorganic N is very low, N_2 fixation contributes to the majority of the crop's N needs (Harper, 1987). Most estimates show that 25-60% of total N in the mature plants is obtained from symbiotic N_2 fixation, with the remaining portion being soil derived (Deibert et al., 1979; Zapata et al., 1987). However, for soils low in organic matter, N_2 fixation contributes to the majority (85 to 90 %) of N accumulation. In an experiment conducted in soil with less than 1% organic matter, Mastrodomenico and Purcell (2012) found that N_2 fixation contributed 90% of seed N content at maturity. Mastrodomenico and Purcell (2012) also demonstrated that in water sufficient conditions, N_2 fixation and N accumulation continue at a high rate until the end of seed fill, which is in agreement with the previous research (Leffel et al., 1992; Spaeth and Sinclair, 1983).

There are differences in the sensitivity of N_2 fixation to drought among soybean genotypes. This sensitivity of N_2 fixation to drought has been evaluated as a change in shoot tissue N concentration in response to drought (King and Purcell, 2006; King et al., 2014). The

assumption here being that the decrease in N concentration during drought reflects a decrease in N_2 fixation relative to C assimilation (i.e., photosynthesis) and that N_2 fixation is the only contributor to shoot N, ignoring mineral N uptake and assimilation. Genotypes with high shoot N concentration under well-watered conditions, when exposed to drought had a reduction in shoot N concentration; conversely, genotypes possessing low shoot N concentration under well-watered conditions had little or no change in N when exposed to drought (King and Purcell, 2006). In later experiments, King et al. (2014) assessed 175 maturity group (MG) 4 soybean accessions for low shoot N and identified two relatively high yielding accessions that were able to maintain high rates of nitrogenase activity at considerably lower soil moisture content than commercial cultivars or other accessions with high shoot N.

Shearer et al. (1980) introduced an alternate method to evaluate differences among cultivars for N_2 fixation based on the natural abundance of the ^{15}N isotope. The basis of the method is that the concentration of ^{15}N is naturally greater in the soil than in the atmosphere. Therefore, there will be a dilution of ^{15}N in the plant tissue of a plant actively fixing atmospheric N_2 compared to a plant that derives N exclusively from the soil. The inclusion of a non-N_2–fixing reference crop (non-nodulating genotype) serves to account for N obtained from the soil. Based on the ratio of ^{15}N to ^{14}N in the air ($R_{air\ N2}$) and the ratio of ^{15}N to ^{14}N in the sample (R_{sample}) as represented by $\delta^{15}N$, nitrogen derived from the atmosphere (NDFA) is expressed as follows (Kohl and Shearer, 1981):

$$NDFA = ((\delta^{15}N_{ref} - \delta^{15}N_{samp}) / (\delta^{15}N_{ref} - \delta^{15}N_0)) \times 100 \quad [2]$$

Here, $\delta^{15}N_{ref}$ refers to the composition of a plant completely dependent on soil N, $\delta^{15}N_{samp}$ signifies the composition of the individual samples, and $\delta^{15}N_0$ refers to the composition of a sample, totally dependent on N_2 fixation. $\delta^{15}N_{ref}$ is determined by measuring the $\delta^{15}N$ of a non-

nodulating reference crop, and $\delta^{15}N$ (data from the stable isotope facility is obtained as this) is determined based on eq. [3]:

$$\delta^{15}N = 1000\ (R_{sample} - R_{air\ N2}) / R_{air\ N2} \quad [3]$$

$\delta^{15}N_0$ represents an average value of shoot $\delta^{15}N$ of three cultivars which were completely dependent on N fixed from atmospheric N_2 and is a constant (-1.30) as determined for soybean by Bergersen et al. (1989).

1.3.2.2 Ureide Concentration

The vulnerability of N_2 fixation to soil drying can largely impact soybean yield (Purcell and King, 1996). Therefore, improving drought tolerance to N_2 fixation is a key step for improving soybean's performance during drought (Sinclair et al., 2010). However, the physiological basis of N_2 fixation inhibition by water deficits in legume nodules is not clearly known. The final products of N_2 fixation in soybean reported from nodules are ureides (allantoin and allantoate; Ohyama and Kumazawa, 1978). The sensitivity of N_2 fixation in soybean appears to be associated with high ureide accumulation in the shoots (Serraj et al., 1999b).

In response to soil water deficit, the phloem flow to nodules decreases, resulting in a reduction of water available to export N_2 fixation products along the xylem; commonly known as feedback inhibition (Sinclair and Serraj, 1995; Serraj and Sinclair, 1996; Pate et al., 1969; Serraj et al., 1999b, 2001). This is proposed to cause accumulation of N_2 fixation products like ureides in leaves and nodules, causing a decrease in nitrogenase activity. While studying common beans (*Phaseolus vulgaris* L.), Coleto et al. (2014) realized that even though ureide accumulation was a general stress-associated response; not the cause or signal of N_2 fixation repression. Ureide in shoots of drought-sensitive genotypes was at a greater concentration than the drought-tolerant

ones, which is similar to results from King and Purcell (2005) and King et al. (2014) for soybean. A possible regulatory action for allantoin, in which it influences the production of abscisic acid, thereby influencing stress tolerance was proposed by Watanabe et al. (2014).

Although the relationship between the sensitivity of N_2 fixation to drought and ureide accumulation in the shoots is not completely understood, it is evident that there are genotypic differences in the extent to which ureide accumulation occurs during drought. Genotypes with drought sensitive N_2 fixation accumulate large amounts of ureides in the shoot, whereas the genotypes with drought-tolerant N_2 fixation accumulate significantly less ureides under water deficit (King and Purcell, 2005; King et al., 2014; Purcell et al., 1998; Serraj et al., 1999b). Hence, ureide concentration can be used to identify genotypes that have the ability to continue N_2 fixation even in relatively low soil moisture (Sinclair et al., 2000; King et al., 2014).

Ureide concentration has been mapped by using a biparental mapping population of 97 recombinant inbred lines (RILs) developed from a cross between 'KS4895' and 'Jackson' (Hwang et al., 2013). KS4895 has drought sensitive N_2 fixation and 'Jackson' has drought tolerant N_2 fixation (King and Purcell, 2005, 2006; Purcell et al., 2000). Hwang et al. (2013) identified five quantitative trait loci (QTLs) for ureide concentration under irrigated conditions, using composite interval mapping (CIM). Under drought conditions, two QTLs for ureide concentration were recognized using CIM; one of these identified on Gm19 was at the same position as a QTL under irrigated conditions (Hwang et al., 2013). Ray et al. (2015) used GWAS to identify 53 putative loci on 18 chromosomes that were associated with ureide concentration. Two of these putative loci were detected near previously reported QTLs associated with ureide concentration, and 30 loci were found near genes associated with ureide metabolism.

1.3.2.3 Canopy Wilting

The most common abiotic stress that causes yield decline in soybean is the water deficit stress (Purcell and Specht, 2004). Irrigation could be a solution but is often not a viable option due to unavailability of a water source or the associated exorbitant costs. A trait that appears promising for improving drought tolerance in soybean is slow canopy wilting under water deficit.

Canopy wilting appears as the first visible symptom of soil water deficit in soybean (King et al., 2009). Sloane et al. (1990) first reported that the soybean genotypes differ in the commencement and severity of canopy wilting experienced under drought. Later, King et al. (2009) found significantly large differences among soybean genotypes for how rapidly the wilting symptoms appear during the onset of drought. Yield advantages have also been observed for slow wilting genotypes under drought conditions with no significant penalty in the absence of drought, as evident from a modeling study (Sinclair et al., 2010) and yield data of a recently released slow wilting cultivar (Carter et al., 2016).

Studies have tried to investigate the possible reasons for drought tolerance in the slow wilting genotypes. The slow-wilting genotype PI 416937 has a highly prolific root system (Pantalone et al., 1996), greater root mass and root surface area (Hudak and Patterson, 1995), and greater lateral root growth compared with fast wilting genotypes (Hudak and Patterson, 1996). There does not, however, appear to be a causal relationship between rooting characteristics of PI 416937 and wilting, as row spacing had no effect on canopy wilting (King et al., 2009). Rather, King et al. (2009) found that soil water conservation was responsible for the slow wilting of soybean genotype PI416937. It is hypothesized that such genotypes conserve soil moisture when it is abundant and utilize it later during drought when the soil moisture supply of other genotypes

gets exhausted. Fletcher et al, (2007) demonstrated that under water-limited conditions, transpiration of slow wilting genotypes plateaus as water-vapor deficit (VPD) begins to exceed ~2kPa, whereas transpiration for fast-wilting genotypes continues to increase linearly with increasing VPD.

Studies have found that the slow wilting genotypes may have low RUE due to decreased transpiration. At the field level, conserving soil moisture by restricting transpiration can result in a decrease in RUE (g MJ^{-1}).

$$RUE = T \times WUE \times \Delta PAR^{-1} \quad [4]$$

where, T is the quantity of water transpired (L) during a portion of the growing cycle,
WUE is the average water use efficiency (g L^{-1}) during the same time period, and
Δ PAR refers to the cumulative amount of photosynthetically active radiation (PAR, MJ m^{-2}) intercepted by the crop during the same time period.

Ries et al. (2012) and King et al. (2009) found that RUE of slow wilting genotypes under well-watered conditions was low compared with fast wilting genotypes, supporting the hypothesis that slow wilting is associated with soil water conservation. Bai and Purcell (2018b) have demonstrated a relationship between slow wilting and increased WUE of genotypes.

QTLs associated with slow wilting have been mapped in biparental populations (Abdel – Haleem et al., 2012; Charlson et al., 2009; Hwang et al., 2015, 2016) as well as in GWAS panel (Kaler et al., 2017a).

1.3.2.4 Thermal Infrared Imaging and Canopy Temperature

Canopy temperature is an important drought- associated trait and a useful tool to evaluate differences in drought tolerance among soybean genotypes. Early work with infrared thermometers proved successful in monitoring evapotranspiration rates in crops (Stone and

Horton, 1974) and the estimation of daily crop temperatures (Blad and Rosenberg, 1976). Stone and Horton (1974) used an infrared thermometer to measure canopy temperature of sorghum (*Sorghum bicolor*), but measurements were inaccurate especially when canopy coverage was incomplete (Blad and Rosenberg, 1976). 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). Leaf and canopy temperatures have become quite easy and fast to measure with infrared thermometers and have been related to plant water stress.

Barley *(Hordeum vulgare*) and wheat (*Triticum aestivum*) cultivars were evaluated for leaf temperatures along with the leaf water potential measurements (Blum et al., 1982). Results from measurements made during vegetative growth stages on three different days as water stress increased, had significant variations in leaf temperatures and a significant correlation across genotypes between leaf temperature and leaf water potential. Thus, indicating 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).

Harris et al. (1984) used a hand-held infrared thermometer to evaluate 20 soybean genotypes for leaf canopy temperatures; there was a significant correlation between air temperature and canopy temperature and a negative correlation between canopy temperatures and seed yield. The study also found that canopy temperatures tend to be more highly correlated with seed yield when evaporative demands are relatively high compared to when evaporative demands are relatively low. Result point that infrared thermometers may be successfully implemented into breeding programs for selection purposes. Similarly, Yousfi et al. (2016) found

that grain yield was positively correlated with decreased canopy temperature during grain filling, in bread and durum wheat (r=0.68 and r=0.82).

Thermal imaging focuses on plant water relations because the evaporative cooling due to transpiration lowers the canopy temperature (Jones et al., 2009). A thermal imaging system was evaluated by Kashiwagi et al. (2008) to examine temperature differences in plant canopies. Thermal images were captured from early flowering until late pod fill stage with an infrared camera between 1400 and 1430 hours on a diverse set of chickpea germplasm. The sequential color gradients were extracted from the images to obtain the numerical thermal data and were analyzed by an image analysis software, 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 had significant differences among genotypes. A significant correlation (r=0.60) was found in the relationship between the relatively cool canopy area and the seed yield. The authors concluded that the cool canopies were due to higher transpiration rates, as root systems supplied more water resulting in increased yields. Canopy temperature measurements in wheat (Lopes et al., 2012) were associated with genetic yield gains of up to 0.7% per year in Mexico and had a strong relationship with cooler canopy temperatures during grain filling. Xiao et al. (2012) found an increase in genetic gain of the yield of wheat of 62 kg per hectare per year with improvements in physiological traits including canopy temperature. Both of these studies found genetic differences among entries and related improvements in grain yield to improved physiological traits.

Aerial infrared (IR) imaging to measure canopy temperature is a useful, high-throughput based phenotyping tool for drought tolerance assessment (Araus and Cairns, 2014; Yousfi et al., 2016). Being non- invasive, the technique does not require any contact with the plants which can

affect stomatal responses (Guilioni et al., 2008). Other than this, the technique is rapid, cost-effective, and very detailed (Araus and Cairns, 2014). It also helps to overcome the bottlenecks for drought tolerance improvement through the expeditious recognition of specific drought tolerance traits. Limitations in the field phenotyping have significantly affected our potential to decode the genetics of quantitative traits, particularly those associated with yield and stress tolerance (Blum, 2011). Over the past decade, the technique has been developed for a wide range of agricultural applications (Chapman et al., 2014), but was initiated in agriculture by Jones et al. (2009) who applied it for the evaluation of stomatal conductance in grapevines.

Aerial IR thermography provides a quantitative measure of drought stress which overcomes several limitations of visual ratings (Bai and Purcell, 2018a). Aerial canopy temperature in soybean is also found positively correlated with wilting and negatively correlated with yield. Thus, canopy temperature seems a promising tool for rapid characterization of drought-associated characteristics to soybean.

Kaler (2017c) has mapped QTLs associated with canopy temperature in soybean GWAS panel of 345 accessions, and these were also coincident with the delayed canopy wilting QTLs.

1.3.2.5 Water Use Efficiency and Carbon Isotope Composition ($\Delta^{13}C$ or $\delta^{13}C$)

Drought is one of the chief factor limiting crop productivity under water-limited conditions (Tuberosa, 2013). However, over-time plants have evolved several morphological and physiological mechanisms to tolerate and escape drought. Water use efficiency (WUE) being one such physiological adaptation against drought (Baum et al., 2007), is an important trait for improving crop productivity under water-deficit conditions (Blum, 2009; Condon et al. 2004; Sinclair, 2012). As per Condon et al. (2004), selection for increased water use efficiency was the key for wheat yield increment under the late season drought conditions. Sinclair (2012)

demonstrated the same for soybean, that soybean genotypes with improved WUE can be used to develop cultivars with higher yield under drought.

In general, WUE is defined as the amount of dry matter accumulated per unit amount of water utilized as transpiration (Gilbert et al., 2011; Passioura, 1977). At leaf scale, WUE is the ratio of net CO_2 assimilated by photosynthesis and the amount of water transpired. Grain yield under water-limited conditions is expressed as a function of the amount of water transpired (T), WUE, and harvest index (HI) (grain yield = T × WUE × HI; Passioura, 1977). However, WUE has not been used as direct selection criteria in the crop breeding programs evaluating a large number of genotypes under field conditions due to the limitations caused by arduous measurement techniques and large environmental effects (Tardieu, 2013).

The 1980s saw the development of a reliable screening method for WUE, utilizing carbon isotope composition of plant tissue (Farquhar et al., 1982 and O'Leary, 1981). This method utilizes the fact that, naturally about 1.1 % of the carbon in the biosphere occurs in the form of the stable ^{13}C isotope and the remaining 98.9 % is ^{12}C (Condon et al., 2002), and that the molar abundance ratio of ^{13}C / ^{12}C is generally higher in atmospheric CO_2 than in plant tissues due to discrimination against heavier ^{13}C at the time of diffusion of CO_2 into plant tissue at gaseous exchange sites (Farquhar et al., 1989). Rubisco also has a higher preference towards ^{12}C over ^{13}C, indicated by the kinetic constants of the two reactions (Farquhar et al., 1989; Farquhar and Richards, 1984). As a result of this discrimination, the carbon isotopic composition varies.

Several studies have confirmed and utilized the relationship between carbon isotope composition of plant and WUE, in different crops such as wheat (Triticum aestivum L.) (Condon et al., 1990), bean (Phaseolus vulgaris L.) (White, 1993), and peanut (Arachis hypogea L.) (Wright et al., 1994). The carbon isotopic composition of plants can either be expressed as $\Delta^{13}C$

(carbon isotope discrimination) or as carbon isotope ratio ($\delta^{13}C$). Carbon isotope discrimination ($\Delta^{13}C$) is a representation of ^{13}C / ^{12}C in plant tissue relative to ^{13}C / ^{12}C in the air (Farquhar and Richards, 1984). Carbon isotope ratio ($\delta^{13}C$) is a ratio of ^{13}C / ^{12}C in plant tissue to ^{13}C / ^{12}C in air. A positive correlation exists between carbon isotope ratio ($\delta^{13}C$) and WUE while a negative correlation exists between carbon isotope discrimination ($\Delta^{13}C$) and WUE (Condon et al., 1990). Farquhar et al. (1982) pointed that the reason for the relation between WUE and carbon isotope composition is the common relationship between the ratio of CO_2 inside and outside of the leaf. Both WUE and carbon isotope composition show an increment, as the ratio of internal to external CO_2 depletes.

Also, different studies have preferred different plant parts for ^{13}C analysis, and all those different plant tissues were found to have characteristic ^{13}C concentrations (Chen et al., 2012). Craig (1953) reported higher ^{13}C concentration in woody stems compared with the leaves. Zhao et al. (2004) identified that grain and root tissues had the highest $\delta^{13}C$ values compared to other tissues in rice (*Oryza sativa* L. Merr). Leaves became the most preferred plant parts for tissue analysis due to simplicity in handling and lab analysis (Farquhar and Richards, 1984; Munjonji et al., 2017). Kaler et al. (2017b) and Bai and Purcell (2018b) used entire shoot samples to assess $\delta^{13}C$ in soybean, which seem to be a more representative sample than specific plant parts.

Due to sufficient genotypic variation, stability across environments and high broad-sense heritability, $\delta^{13}C$ and $\Delta^{13}C$ appear to be promising surrogate measures for WUE that can be used in the breeding programs of legumes as well as cereals; targeting improvement in crop drought tolerance (Specht et al., 2001; Dhanapal et al., 2015). QTLs associated with carbon isotope composition and WUE has been identified by Dhanapal et al. (2015b) and Kaler et al. (2017b).

1.4 Objectives

This proposed research has two objectives described below:

1. To characterize the SoyNAM parental lines for yield, seed growth rate, effective filling period, canopy coverage, radiation use efficiency, nitrogen accumulation, ureide concentration, wilting, and harvest index, when grown under optimum growing conditions.
2. To identify the most extreme genotypes among the 41 SoyNAM genotypes based on the above traits.

In Chapter 2, we will evaluate the distribution of parental genotypes with respect to each yield-related trait and identify extreme genotypes among the 41 parental genotypes of SoyNAM. Also, the relation of each these traits with yield.

Similarly, in chapter 3, we will evaluate the distribution of parental genotypes with respect to drought-related traits and identify extreme genotypes among the parental genotypes of the population, and the relation of each these traits with yield.

Tables and figures

Table 1.1 List of genotypes selected as SoyNAM parents.

High Yielding Lines	Lines With Diverse Ancestry	PIs With High Yields in Drought
4J105-3-4	LG03-2979	PI 398881
5M20-2-5-2	LG03-3191	PI 427136
CL0J095-4-6	LG00-3372	PI 437169B
CL0J173-6-8	LG04-4717	PI 518751
HS6-3976	LG04-6000	PI 561370
LD00-3309	LG05-4292	PI 404188A
LD01-5907	LG05-4317	PI 574486
LD02-4485	LG05-4464	PI 507681B
LD02-90550	LG05-4832	
Magellan	LG90-2550	
Maverick	LG92-1255	
NE3001	LG94-1128	
Prohio	LG94-1906	
S06-13640	LG97-7012	
SKYLLA	LG98-1605	
TN05-3027		
U03-100612		

References

Abdel-Haleem, H., T.E. Carter Jr., L.C. Purcell, C.A. King, L.L. Ries, P. Chen, W. Schapaugh Jr., T.R. Sinclair, and H.R. Boerma. 2012. Mapping of quantitative trait loci for canopy-wilting trait in soybean (Glycine max L. Merr). TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik 125:837–46.

Araus, J.L., and J.E. Cairns. 2014. Field high-throughput phenotyping: The new crop breeding frontier. Trends Plant Sci. 19:52–61. https://doi.org/10.1016/j.tplants.2013.09.008.

Arkansas Farm Bureau, 2016. http://www.arfb.com/pages/arkansas-agriculture/commodity-corner/soybean/

Bai, H., L.C. Purcell. 2018a. Aerial canopy temperature differences between fast- and slow-wilting soybean genotypes. J Agro Crop Sci. 00:1–9. https://doi.org/10.1111/jac.12259.

Bai, H., and L.C. Purcell. 2018b. Response of carbon isotope discrimination and oxygen isotope composition to mild drought in slow-and fast-wilting soybean genotypes. J Crop Improv. 32(2):239-253.

Baum M., M. Von Korff, P. Guo, B. Lakew, A. Hamwieh, S. Lababidi, S.M. Udupa, H. Sayed, W. Choumane, S. Grando, S. Ceccarelli. 2007. Molecular approaches and breeding strategies for drought tolerance in barley. In: Varshney R, Tuberosa R Genomics-Assisted Crop Improvement, Volume 2: Genomics Applications in Crops. Springer, Dordrecht:51–79.

Beaven, E.S. 1914. Rep. Br. Assoc. 83, 660.

Bergersen, F., J. Brockwell, R. Gault, L. Morthorpe, M. Peoples, and G. Turner. 1989. Effects of available soil N and rates of inoculation on nitrogen fixation by irrigated soybeans and evaluation of $\delta^{15}N$ methods for measurement. Aust J. Agric. Res. 40 (4):763-780.

Blad, B.L., and N.J. Rosenberg. 1976. Measurement of crop temperature by leaf thermocouple, infrared thermometry and remotely sensed thermal imagery. Agron. J. 68 (4):635-641.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Aust J Agric. Res. 56(11):1159-1168.

Blum, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Res. 112:119-123.

Blum, A. 2011. Phenotyping and selection. In Plant breeding for water-limited environments. Springer New York: 153-216.

Blum, A., J. Mayer, and G. Gozlan. 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Res. 5:137-146.

Board, J., M. Kamal, and B. Harville. 1992. Temporal importance of greater light interception to increased yield in narrow-row soybean. Agron. J. 84:575-579.

Boerma, H., and D. Ashley. 1988. Canopy photosynthesis and seed-fill duration in recently developed soybean cultivars and selected plant introductions. Crop Sci. 28:137-140.

Boote, K. J. 1981. Response of Soybeans in Different Maturity Groups to March Plantings in Southern USA 1. Agron. J. 73(5): 854-859.

Buzzell, R., and B. Buttery. 1977. Soybean harvest index in hill-plots. Crop Sci. 17:968-970.

Carter, T. E., R. Nelson, C. Sneller, and Z. Cui. 2004. Soybeans: Improvement Production and Uses. Agron. J. 16:303-416.

Carter, T.E., S.M. Todd, and A.M. Gillen. 2016. Registration of 'USDA-N8002'soybean cultivar with high yield and abiotic stress resistance traits. Journal of Plant Registrations. 10(3):238-245.

Chapman, S. C., T. Merz, A. Chan, P. Jackway, S. Hrabar et al. 2014. Pheno-copter: A low-altitude, autonomous remote-sensing robotic helicopter for high-throughput field based phenotyping. Agronomy 4:279–301. https://doi.org/10.3390/agronomy4020279.

Charlson, D.V., S. Bhatnagar, C.A. King, J.D. Ray, C.H. Sneller, T.E. Carter, and L.C. Purcell. 2009. Polygenic inheritance of canopy wilting in soybean [Glycine max (L.) Merr.]. Theoretical and Applied Genetics. 119: 587-594.

Chen, J., S.X. Chang, and A.O. Anyia. 2012. Quantitative trait loci for water-use efficiency in barley (Hordeum vulgare L.) measured by carbon isotope discrimination under rain-fed conditions on the Canadian Prairies. Theor. and applied genet. 125:71-90.

Chung, G., and R. J. Singh. 2008. Broadening the genetic base of soybean: a multidisciplinary approach. Critical Reviews in Plant Sci. 27:295-341.

Coleto I., M. Pineda, A. P. Rodin, A. M. De Ron, and J. M. Alamillo. 2014. Comparison of 495 inhibition of N2 fixation and ureide accumulation under water deficit in four common 496 bean genotypes of contrasting drought tolerance. Annals of Botany 113: 1071-1082.

Condon, A., G. Farquhar, and R. Richards. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. Functional Plant Biol. 17:9-22.

Condon, A.G., R. Richards, G. Rebetzke, and G. Farquhar. 2002. Improving intrinsic water-use efficiency and crop yield. Crop Sci. 42:122-131.

Condon, A.G., R. Richards, G. Rebetzke, and G. Farquhar. 2004. Breeding for high water-use efficiency. Journal of experimental botany 55:2447-2460.

Coruzzi, G.M., and L. Zhou. 2001. Carbon and nitrogen sensing and signaling in plants: emerging 'matrix effects'. Current opinion in plant biology 4:247-253.

Craig, H. 1953. The geochemistry of the stable carbon isotopes. Geochimica et Cosmochimica Acta 3:53-92.

Daynard, T., J. Tanner, and W. Duncan. 1971. Duration of the grain filling period and its relation to grain yield in corn, Zea mays L. Crop Sci. 11:45-48.

De Bruin, J.L., and P. Pedersen. 2009. New and old soybean cultivar responses to plant density and intercepted light. Crop Sci. 49:2225-2232.

Deibert, E., M. Bijeriego, and R. Olson. 1979. Utilization of ^{15}N Fertilizer by Nodulating and Non-Nodulating Soybean Isolines 1. Agron. J. 71:717-723.

Denison, R. F. 2015. A Darwinian perspective on improving nitrogen-fixation efficiency of legume crops and forages. In Crop Physiology (Second Edition). 207-222.

Dhanapal, A.P., J.D. Ray, S.K. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, P.B. Cregan, Q. Song, and F.B. Fritschi. 2015b. Genome-wide association study (GWAS) of carbon isotope ratio ($\delta^{13}C$) in diverse soybean [Glycine max (L.) Merr.] genotypes. Theoretical and Applied Genetics 128:73-91.

Donald, C., and J. Hamblin. 1976. The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Advances in agronomy 28:361-405.

Dunphy, E., J. Hanway, and D. Green. 1979. Soybean yields in relation to days between specific developmental stages. Agron. J. 71:917-920.

Edwards, J.T., and L.C. Purcell. 2005. Soybean yield and biomass responses to increasing plant population among diverse maturity groups: I. Agronomic Characteristics. Crop science 45:1770-1777.

Edwards, J.T., L.C. Purcell, and D.E. Karcher. 2005. Soybean yield and biomass responses to increasing plant population among diverse maturity groups: II. Light Interception. Crop Science 45:1778-1785.

Egli, D. B. 1975. Rate of accumulation of dry weight in seed of soybeans and its relationship to yield. Canadian Journal of Plant Science *55*(1):215-219.

Egli, D.B. 1990. Seed water relations and the regulation of the duration of seed growth in soybean. Journal of Experimental Botany 41:243-248.

Egli, D.B. 1994a. Mechanisms responsible for soybean yield response to equidistant planting patterns. Agron. J. 86:1046-1049.

Egli, D. B. 1994b. Seed growth and development. Physiology and determination of crop yield (No. physiologyandde, pp. 127-148). American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Egli, D. B., and J. E. Leggett.1973. Dry Matter Accumulation Patterns in Determinate and Indeterminate Soybeans 1. Crop Science 13(2): 220-222.

Egli, D. B., J. E. Leggett, and J. M. Wood. 1978. Influence of Soybean Seed Size and Position on the Rate and Duration of Filling1. Agron. J. 70(1):127-130.

Farquhar, G., and R. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Functional Plant Biology 11:539-552.

Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Functional Plant Biology 9:121-137.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annual review of plant biology 40(1):503-537.

Fehr, W. R., and C. E. Caviness. 1977. Stages of soybean development. Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa.

Field Analyzer. 2018. Evaluate regions of aerial images for Coverage, Dark Green Color Index (DGCI), Infrared Intensity, and Normalized Difference Vegetation Index (NDVI). Carlin Purcell. https://www.turfanalyzer.com/field_analyzer.html

Fiorani, F., U. Rascher, S. Jahnke, and U. Schurr .2012. Imaging plants dynamics in heterogenic environments. Current opinion in biotechnology 23(2): 227-235.

Fischer, R. A., D. Byerlee, and G. Edmeades. 2014. Crop yields and global food security. ACIAR: Canberra, ACT.

Fletcher, A. L., T. R. Sinclair, and L. H. Allen Jr. 2007. Transpiration responses to vapor pressure deficit in well-watered ‘slow-wilting’ and commercial soybean. Environmental and Experimental Botany 61:145–51.

Flenet, F., J.R. Kiniry, J.E. Board, M.E. Westgate, and D.C. Reicosky. 1996. Row spacing effects on light extinction coefficients of corn, sorghum, soybean, and sunflower. Agronomy Journal 88:185-190.

Fraser, J., D. Egli, and J. Leggett. 1982. Pod and Seed Development in Soybean Cultivars with Differences in Seed Size 1. Agron. J. 74:81-85.

Gaspar, A.P., and S.P. Conley. 2015. Responses of canopy reflectance, light interception, and soybean seed yield to replanting suboptimal stands. Crop Sci. 55:377-385.

Gay, S., D. B. Egli, and D. A. Reicosky. 1980. Physiological Aspects of Yield Improvement in Soybeans 1. Agronomy Journal 72(2): 387-391.

Gifford, R. M., J. H. Thorne, W. D. Hitz, and R. T. Giaquinta. 1984. Crop productivity and photoassimilate partitioning. Science 225(4664): 801-808.

Gilbert, M. E., M. A. Zwieniecki, and N. M. Holbrook. 2011. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. Journal of Experimental Botany 62(8): 2875-2887.

Gizlice, Z., T. Carter, and J. Burton. 1994. Genetic base for North American public soybean cultivars released between 1947 and 1988. Crop Sci. 34:1143-1151.

Guilioni, L., H. G. Jones, I. Leinonen, and J. P. Lhomme. 2008. On the relationships between stomatal resistance and leaf temperatures in thermography. Agricultural and Forest Meteorology. 148:1908–1912. https://doi.org/10.1016/j.agrformet.2008.07.009.

Guo, J., Y. Wang, C. Song, J. Zhou, L. Qiu, H. Huang, and Y. Wang. 2010. A single origin and moderate bottleneck during domestication of soybean (Glycine max L. Merr): implications from microsatellites and nucleotide sequences. Annals of Botany 106:505-514.

Hanway, J., and C. Weber. 1971. Dry matter accumulation in eight soybean (Glycine max (L.) Merrill) varieties. Agron. J. 63:227-230.

Harper, J.L., 1977. Population Biology of Plants. Academic Press, London.

Harper, J.E. 1987. Nitrogen metabolism. H.R. Boerma et al. (ed.) Soybean: Improvement, production, and uses.2nd ed. Agron. Monogr. 16. ASA, CSSA, SSSA, Madison, WL. 497-533.

Harris, D., W. Schapaugh, and E. Kanemasu. 1984. Genetic diversity in soybeans for leaf canopy temperature and the association of leaf canopy temperature and yield. Crop Sci. 24:839-842.

Hatfield, J., R. Reginato, and S. Idso. 1984. Evaluation of canopy temperature-evapotranspiration models over various crops. Agricultural and forest meteorology 32:41-53.

Hudak, C.M., and R.P. Patterson. 1995. Vegetative growth analysis of a drought-resistant soybean plant introduction. Crop Sci. 35:464–471.

Hudak, C.M., and R.P. Patterson. 1996. Root distribution and soil moisture depletion pattern of a drought-resistant soybean plant introduction. Agron. J. 88:478-485.

Hwang, S., C.A. King, M.K. Davies, J.D. Ray, P.B. Cregan, and L.C. Purcell. 2013. QTL analysis of shoot ureide and nitrogen concentrations in soybean [Glycine max (L.) Merr.]. Crop Sci. 53:2421-2433.

Hwang, S., C.A. King, J.D. Ray, P.B. Cregan, P. Chen, T.E. Carter, Z. Li, H. Abdel-Haleem, K.W. Matson, W. Schapaugh, and L.C. Purcell. 2015. Confirmation of delayed canopy wilting QTLs from multiple soybean mapping populations. Theoretical and applied genetics. 128(10):2047-2065.

Hwang, S., C.A. King, P. Chen, J.D. Ray, P.B. Cregan, T.E. Carter, Z. Li, H. Abdel-Haleem, K.W. Matson, W. Schapaugh, and L.C. Purcell. 2016. Meta-analysis to refine map position and reduce confidence intervals for delayed-canopy-wilting QTLs in soybean. Molecular breeding: 36(7): 91.

Hyten, D.L., Q. Song, Y. Zhu, I.Y. Choi, R.L. Nelson, J.M. Costa, J.E. Specht, R.C. Shoemaker, and P.B. Cregan. 2006. Impacts of genetic bottlenecks on soybean genome diversity. Proceedings of the National Academy of Sciences 103:16666-16671.

Jones, H. G., R. Serraj, B. R. Loveys, L. Z. Xiong, A. Wheaton, and A. H. Price. 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Functional Plant Biology. 36:978–989. https://doi.org/10.1071/FP09123.

Joshi, T., B. Valliyodan, J. H. Wu, S. H. Lee, D. Xu, and H.T. Nguyen. 2013. Genomic differences between cultivated soybean, G. max and its wild relative G. soja. BMC genomics 14:S5.

Kaler, A.S., J.D. Ray, W.T. Schapaugh, C.A. King, and L.C. Purcell. 2017a. Genome-wide association mapping of canopy wilting in diverse soybean genotypes. Theoretical and applied genetics 130:2203-2217.

Kaler, A. S., A. P Dhanapal, J. D. Ray, C. A. King, F. B. Fritschi, and L. C. Purcell. 2017b. Genome-wide association mapping of carbon isotope and oxygen isotope ratios in diverse soybean genotypes. Crop Science 57(6):3085-3100.

Kaler, A. S. 2017c. Genomic and Physiological Approaches to Improve Drought Tolerance in Soybean. Ph.D. dissertation. University of Arkansas, Fayetteville, AR.

Kaler, A.S., J.D. Ray, W.T. Schapaugh, M.K. Davies, C.A. King, and L.C. Purcell. 2018. Association mapping identifies loci for canopy coverage in diverse soybean genotypes. Molecular Breeding 38(5):50.

Kashiwagi, J., L. Krishnamurthy, H. Upadhyaya, and P. Gaur. 2008. Rapid screening technique for canopy temperature status and its relevance to drought tolerance improvement in chickpea. Journal of SAT Agricultural Research 6:105-4.

King, C.A., and L.C. Purcell. 2005. Inhibition of N_2 fixation in soybean is associated with elevated ureides and amino acids. Plant Physiology 137:1389-1396.

King, C.A., and L.C. Purcell. 2006. Genotypic variation for shoot N concentration and response to water deficits in soybean. Crop science 46:2396-2402.

King, C.A., L.C. Purcell, and K.R. Brye. 2009. Differential wilting among soybean genotypes in response to water deficit. Crop Science 49:290-298.

King, C.A., L.C. Purcell, A. Bolton, and J.E. Specht. 2014. A possible relationship between shoot N concentration and the sensitivity of N_2 fixation to drought in soybean. Crop Sci. 54:746-756.

Kiniry, J., C. Jones, J. O'toole, R. Blanchet, M. Cabelguenne, and D. Spanel. 1989. Radiation-use efficiency in biomass accumulation prior to grain-filling for five grain-crop species. Field Crops Research 20:51-64.

Kohl, D.H., and G.B. Shearer. 1981. The use of soils lightly enriched in ^{15}N to screen for N_2-fixing activity. Plant and Soil 60:487-489.

Kumudini, S. 2000. Soybean phenology. University of Guelph. https://www. uoguelph.ca/plant /research/homepages/ttollena/research/soy.html (accessed 02 Oct. 2018).

Leffel, R., P. Cregan, A. Bolgiano, and D. Thibeau. 1992. Nitrogen metabolism of normal and high-seed-protein soybean. Crop Sci. 32:747-750.

Li, B. L., H. I. Wu, and G. Zou. 2000. Self-thinning rule: a causal interpretation from ecological field theory. Ecological Modelling 132(1-2):167-173.

Lockhart, J.A. 1965. An analysis of irreversible plant cell elongation. Journal of theoretical biology 8:264-275.

Lopes, M.S., M. Reynolds, Y. Manes, R. Singh, J. Crossa, and H. Braun. 2012. Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a "historic" set representing 30 years of breeding. Crop Sci. 52:1123-1131.

Mastrodomenico, A.T., and L.C. Purcell. 2012. Soybean nitrogen fixation and nitrogen remobilization during reproductive development. Crop science 52:1281-1289.

McWilliams, D.A., D.R. Berglund, and G.J. Endres. 1999. Soybean growth and management quick guide-A1174. North Dakota State University Extension Service.

Monteith, J.L., 1977. Climate and the efficiency of crop production in Britain. Philosophical Transactions of the Royal Society of London B: Biological Sciences 281(980):277-294.

Muchow, R., and T. Sinclair. 1986. Water and nitrogen limitations in soybean grain production II. Field and model analyses. Field Crops Res. 15:143-156.

Munjonji, L., K.K. Ayisi, P. Boeckx, and G. Haesaert. 2017. Stomatal Behavior of Cowpea Genotypes Grown Under Varying Moisture Levels. Sustainability 10:12.

NCSPA. 2014. History of soybeans. North Carolina Soybean Producers Association, INC. http://ncsoy.org/media-resources/history-of-soybeans/ (accessed Sept. 29, 2018).

Nelson, R. L. 1986. Defining the Seed-filling Period in Soybeans to Predict Yield 1. Crop Sci. 26(1):132-135.

Ohyama, T., and K. Kumazawa. 1978. Incorporation of ^{15}N into various nitrogenous compounds in intact soybean nodules after exposure to $^{15}N_2$ gas. Soil Science and Plant Nutrition 24:525-533.

O'Leary, M. H. 1981. Carbon isotope fractionation in plants. Phytochemistry 20(4):553-567.

Pantalone, V., G. Rebetzke, J. Burton, and T. Carter. 1996. Phenotypic evaluation of root traits in soybean and applicability to plant breeding. Crop Sci. 36:456-459.

Passioura, J. 1977. Grain yield, harvest index, and water use of wheat. J. Aust. Inst. Agric. Sci. 43:117-120.

Pate, J., B. Gunning, and L. Briarty. 1969. Ultrastructure and functioning of the transport system of the leguminous root nodule. Planta 85:11-34.

Pengelly, B., F. Blamey, and R. Muchow. 1999. Radiation interception and the accumulation of biomass and nitrogen by soybean and three tropical annual forage legumes. Field Crops Research 63:99-112.

Pinto, R.S., M.P. Reynolds, K.L. Mathews, C.L. McIntyre, J.-J. Olivares-Villegas, and S.C. Chapman. 2010. Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and applied genetics 121:1001-1021.

Place, G. T., S. C. Reberg-Horton, T. E. Carter, and A. N. Smith. 2011. Effects of soybean seed size on weed competition. Agronomy journal 103(1):175-181.

Purcell, L.C., and C.A. King. 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. Journal of Plant Nutrition 19:969-993.

Purcell, L.C., R. Serraj, M. de Silva, T.R. Sinclair, and S. Bona. 1998. Ureide concentration of field-grown soybean in response to drought and the relationship to nitrogen fixation. Journal of Plant Nutrition 21:949-966.

Purcell, L.C. 2000. Soybean canopy coverage and light interception measurements using digital imagery. Crop Science 40:834-837.

Purcell, L.C., C.A. King, and R.A. Ball. 2000. Soybean cultivar differences in ureides and the relationship to drought tolerant nitrogen fixation and manganese nutrition. This paper is published with the approval of the director of the Arkansas Agric. Exp. Stn.(manuscript number 99081). Research supported in part by the United Soybean Board, project 8206. Crop Sci. 40:1062-1070.

Purcell, L.C., R.A. Ball, J. Reaper, and E.D. Vories. 2002. Radiation use efficiency and biomass production in soybean at different plant population densities. Crop Sci. 42:172-177.

Purcell, L.C., and J.E. Specht. 2004. Physiological traits for ameliorating drought stress. p. 569-620. In: (eds. J.E. Specht and H.R. Boerma) Soybeans: Improvement, production, and uses. Agronomy Monographs, 3rd edition. 16 ASA CSSA SSSA, Madison, WI. Purcell. 2009. Polygenic inheritance of canopy wilting in soybean [Glycine max (L.) Merr.].TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik 119:587–94.

Purcell, L.C., M. Salmeron, and L. Ashlock. 2014. Soybean growth and development. In: Arkansas soybean production handbook, MP197. University of Arkansas Division of Agriculture Research & Extension.

Qiu, L.J., and R.Z. Chang. 2010. The origin and history of soybean, *In* G. Singh, ed. The soybean: botany, production and uses. CABI.

Ray, J.D., A.P. Dhanapal, S.K. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, D. Boykin, P.B. Cregan, and Q. Song. 2015. Genome-wide association study of ureide concentration in diverse maturity group IV soybean [Glycine max (L.) Merr.] accessions. G3: Genes, Genomes, Genetics:g3. 115.021774.

Ries, L.L., L.C. Purcell, T.E. Carter, J.T. Edwards, and C.A. King. 2012. Physiological traits contributing to differential canopy wilting in soybean under drought. Crop Sci. 52:272-281.

Salado-Navarro, L.R., T.R. Sinclair, and K. Hinson. 1985. Comparisons Among Effective Filling Period, Reproductive Period Duration, and R5 to R7 in Determinate Soybeans 1. Crop science 25(6):1050-1054.

Schapaugh, W., and J. Wilcox. 1980. Relationship between harvest indices and other plant characteristics in soybean. Crop Sci. 20:529-533.

Serraj, R., and T. R. Sinclair. 1996. Processes contributing to N_2-fixation insensitivity to drought in the soybean cultivar Jackson. Crop Sci. 36:961–968.

Serraj, R., V. Vadez, R.F. Denison, and T.R. Sinclair. 1999b. Involvement of ureides in nitrogen fixation inhibition in soybean. Plant physiology 119:289-296.

Serraj, R., V. Vadez, and T. R. Sinclair. 2001. Feedback regulation of symbiotic N_2 fixation under drought stress. Agronomie 21: 621–626.

Shearer, G., D.H. Kohl, and J.E. Harper. 1980. Distribution of ^{15}N among plant parts of nodulating and non-nodulating isolines of soybeans. Plant Physiology 66:57-60.

Sinclair, T., and C. T. De Wit. 1976. Analysis of the carbon and nitrogen limitations to soybean yield. Agron. J. 68:319-324.

Sinclair, T., and T. Horie. 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. Crop Sci. 29:90-98.

Sinclair, T.R. 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38:638-643.

Sinclair, T.R., and R.C. Muchow. 1999. Radiation use efficiency. Advances in agronomy 65:215-265.

Sinclair, T. R., and R. Serraj. 1995. Legume nitrogen fixation and drought. Nature 378: 344.

Sinclair, T., L. Purcell, V. Vadez, R. Serraj, C.A. King, and R. Nelson. 2000. Identification of soybean genotypes with N_2 fixation tolerance to water deficits. Crop Sci. 40:1803-1809.

Sinclair, T.R., L.C. Purcell, and C.H. Sneller. 2004. Crop transformation and the challenge to increase yield potential. Trends in plant science 9:70-75.

Sinclair, T.R., C.D. Messina, A. Beatty, and M. Samples. 2010. Assessment across the United States of the benefits of altered soybean drought traits. Agron. J. 102:475-482.

Sinclair, T.R. 2012. Is transpiration efficiency a viable plant trait in breeding for crop improvement? Functional Plant Biology 39:359-365.

Singh, R. 2017. Botany and Cytogenetics of Soybean:11-40. The Soybean Genome. Springer.

Sloane, R.J., R.P. Patterson, and T.E. Carter. 1990. Field drought tolerance of a soybean plant introduction. Crop Sci. 30:118-123.

Smith, J.R., and R.L. Nelson. 1986. Relationship between seed-filling period and yield among soybean breeding lines. Crop Sci. 26:469-472.

Soystats. 2016. The American Soybean Association. http://soystats.com/international-world-soybean-production/. (Accessed Sept. 19, 2018).

SoyBase. 2018. Integrating Genetics and Genomics to Advance Soybean Research. USDA. https://www.soybase.org/SoyNAM/soynamdetails.php. (Accessed Sept. 19, 2018).

Spaeth, S., and T. Sinclair. 1983. Variation in nitrogen accumulation and distribution among soybean cultivars. Field Crops Res. 7:1-12.

Spaeth, S.C., H. Randall, T. Sinclair, and J. Vendeland. 1984. Stability of soybean harvest index. Agron. J. 76:482-486.

Specht, J., K. Chase, M. Macrander, G. Graef, J. Chung, J. Markwell, M. Germann, J. Orf, and K. Lark. 2001. Soybean response to water. Crop Sci. 41:493-509.

Stone, L., and M. Horton. 1974. Estimating evapotranspiration using canopy temperatures: field evaluation. Agronomy Journal 66:450-454.

Swank, J., D. Egli, and T. Pfeiffer. 1987. Seed growth characteristics of soybean genotypes differing in duration of seed fill. Crop Sci. 27:85-89.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437-460.

Tardieu, F.O. 2013. Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Frontiers in physiology 4:17.

Tian, Z.X., X.B. Wang, R. Lee, Y.H. Li, J.E. Specht, R.L. Nelson, P.E. Mcclean, L.J. Qiu, and J.X. Ma. 2010. Artificial selection for determinate growth habit in soybean. Proceedings of the National Academy of Sciences of the United States of America. 107:8563-8568.

Tuberosa, R. 2013. Phenotyping for drought tolerance of crops in the genomics era. Frontiers in physiology 3:347.

USDA, ERS. 2017. United States Department of Agriculture, Economic Research Services. https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/background/ (accessed Oct 2, 2018) (Last updated May 01, 2017).

USSEC. 2017. United States soybean export council. https://ussec.org/

Van Roekel, R.J., L.C. Purcell, and M. Salmerón. 2015. Physiological and management factors contributing to soybean potential yield. Field Crops Research 182:86-97.

Watterson, G. 1975. On the number of segregating sites in genetical models without recombination. Theoretical population biology 7:256-276.

Watanabe S., M. Matsumoto, Y. Hakomori, H. Takagi, H. Shimada, and A. Sakamoto. 2014. The 584 purine metabolite allantoin enhances abiotic stress tolerance through synergistic 585 activation of abscisic acid metabolism. Plant, Cell and Environment 37: 1022-1036.

Weber, C., R. Shibles, and D. Byth. 1966. Effect of plant population and row spacing on soybean development and production. Agronomy Journal 58:99-102.

White, J.W. 1993. Implications of carbon isotope discrimination studies for breeding common bean under water deficits. Stable isotopes and plant carbon-water relations. Elsevier 387-398.

Wiggans, R. 1939. Influence of space and arrangement on the production of soybean plants. Journal of the American Society of Agronomy.

Wright, G., R. Rao, and G. Farquhar. 1994. Water-use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop science 34:92-97.

Xavier, A., B. Hall, A. A. Hearst, K. A. Cherkauer, and K. M. Rainey. 2017. Genetic architecture of phenomic-enabled canopy coverage in Glycine max. L. Merr Genetics 206(2):1081-1089.

Xiao, Y., Z. Qian, K. Wu, J. Liu, X. Xia, W. Ji, and Z. He. 2012. Genetic gains in grain yield and physiological traits of winter wheat in Shandong Province, China, from 1969 to 2006. Crop Sci. 52:44-56.

Yoda, K., T. Kira, H. Ogawa, and K. Hozumi. 1963. Self-thinning in overcrowded pure stands under cultivated and natural conditions. J. Institute of Polytech., Osaka City University. D14:107–129.

Yousfi, S., N. Kellas, L. Saidi, Z. Benlakehal, L. Chaou, D. Siad, F. Herda et al. 2016. Comparative performance of remote sensing methods in assessing wheat performance under Mediterranean conditions. Agricultural Water Management, 164, 137–147. https://doi.org/10.1016/j.agwat.2015.09.016.

Zapata, F., S. Danso, G. Hardarson, and M. Fried. 1987. Time course of nitrogen fixation in field-grown soybean using nitrogen-15 methodology 1. Agronomy Journal 79:172-176.

Zhao, B., M. Kondo, M. Maeda, Y. Ozaki, and J. Zhang. 2004. Water-use efficiency and carbon isotope discrimination in two cultivars of upland rice during different developmental stages under three water regimes. Plant and Soil 261:61-75.

2. CHAPTER: Physiological Characterization of the SoyNAM Parental Lines for Yield-related Traits (canopy coverage, radiation use efficiency, seed growth rate, effective filling period, harvest index, seed yield, and seed weight)

Abstract

As the largest producer of soybean *(Glycine max* L. Merr.) worldwide, the U.S. needs to maintain its soybean supply due to increasing demand for soybean as food and feed. The narrow genetic base of soybean in North America risks limiting future yield gain. The objective of this study was to identify the extreme genotypes among the 41 parental genotypes of the Soybean Nested Association Mapping (SoyNAM) population for yield-related traits that have not been previously or extensively mapped. SoyNAM is a population in which 40 diverse genotypes have been crossed with a common hub parent. Physiological traits identified as important for yield that were evaluated include: canopy coverage, radiation use efficiency, seed growth rate, effective filling period, harvest index, and seed weight. An experiment was conducted for three years in Fayetteville, Arkansas under irrigated conditions evaluating the 41 SoyNAM parents and at least one non-nodulating genotype for these physiological traits. Several genotypes differed statistically from the hub parent, some genotypes were also identified as common extreme parents for more than one trait. Genotypes LG04-6000 and LG03-3191 had a slower seed growth rate and a longer effective seed filling period than the hub parent; genotype LG04-6000 also had a significantly high yield in multiple years. Genotypes LG03-3191, PI 437169B, and S06-13640 established a canopy more quickly compared to the hub parent all three years. Genotypes Skylla and LG05-4464 were identified with the highest radiation use efficiency. Identified extreme genotypes in this study can lay the foundation for robust quantitative trait loci (QTL) mapping that could lead to the identification of more informative QTLs for physiological traits important for yield.

2.1 Introduction

Due to a growing population, shifting food choices, and increase in biofuel demands, the world food requirement is expected to double by 2050 (Godfray et al., 2010; Tilman et al., 2011; Ray et al., 2013). The yield increase rate necessary to meet this demand is 2.4%; however, current yearly increase (1.2%) is only half of this predicted rate (Ray et al., 2013). Maize (*Zea mays* L. Merr), wheat (*Triticum aestivum* L. Merr.), rice (*Oryza sativa* L. Merr), and soybean are currently the four major key crops worldwide, producing nearly two-thirds of the global agricultural calories (Tilman et al., 2011). Ray et al. (2013) indicated that the present yield increase rates of these chief crops are 1.6%, 0.9%, 1.0%, and 1.3% each year respectively, far less than the estimated 2.4% per year proposed to double the global production by 2050. At, current rates, the global production of these crops would increase by 67%, 38%, 42%, and 55% respectively, which would not meet the expected demands by 2050.

In the midst of the necessity of not only more food but also nutritionally-rich food, soybean is an important protein source. It also has multiple uses in the form of vegetable oil, animal feed and a wide variety of human food applications, ranging from center-of-the-plate protein, beverage, dried bean, baking flour, snack food, fresh green vegetable, cultured product, dessert, and baked goods (United Soybean Board, 2016). The United States is the world's largest producer of soybean, accounting for one-third of the global production with an export market that has grown from 14.3 million metric tonnes (MMT) to 56.2 MMT during the time period 1988 to 2017 (Soystats, 2018). The average soybean yield in 2017 in the U.S. was 3300 kg ha^{-1} (49.1 bu ac^{-1}) and Arkansas's average soybean yield in 2017 was 3400 kg ha^{-1} (51 bu ac^{-1}) (USDA, 2017).

Soybean yields in the U.S. have stagnated (Ray et al., 2012), and the narrow genetic base may further limit future yield gains. The SoyNAM consists of populations derived from the crosses amongst 41 diverse parental genotypes that were developed to map important traits of interest and that can serve as an important tool for increasing diversity in the North American soybean gene pool. An advantage of using a NAM over traditional biparental mapping is that it can contribute to increased genetic resolution, reduced linkage disequilibrium, and it controls the population structure through design (Rafalski, 2010). The first step in utilizing the SoyNAM is the characterization of phenotypic diversity among the parental genotypes.

This chapter reports results of yield and traits closely associated with yield among the SoyNAM parental genotypes. These traits include canopy coverage, radiation use efficiency, seed growth rate, effective seed filling period, harvest index, seed weight, and yield.

The objectives of this chapter were:

1. To characterize the SoyNAM parental lines for the yield-related traits: canopy coverage, radiation use efficiency, seed growth rate, effective filling period, harvest index, seed weight, and seed yield while grown under optimum growing conditions;
2. To identify the most extreme genotypes among the 41 SoyNAM genotypes based on the above traits.

2.2 Materials and Methods

2.2.1 Field Preparation and Experimental Design

2.2.1.1 Genotypes Evaluated

The 41 parental genotypes of the SoyNAM project (obtained from the University of Nebraska) were planted at University of Arkansas System Division of Agriculture's Agricultural Research and Extension Center, Fayetteville, Arkansas (36º05' N, 94º10' W) on a Captina silt

loam (fine-silty, siliceous, active, mesic Typic Fragiudult) soil. The 41 nodulating SoyNAM parental genotypes range from maturity group (MG) 1 through 5. Also, included in the experiment were non-nodulating near isolines of the genotypes Clark (MG4, 2015 and 2016), Lee (MG 6, 2017), and Harosoy (MG 2, 2016 and 2017), which were included for the determination of the amount of nitrogen (N) derived from the soil and from atmospheric N_2 fixation. A complete description and discussion of methods for determining N derived from the atmosphere are provided in Chapter 3.

2.2.1.2 Field Design

The fields in which these genotypes were planted each year had a winter cover crop of cereal rye (*Secale cereale* L. Merr.) planted the previous fall. The cereal rye crop was harvested after heading and removed from the field, with the expectation that it would lower inorganic soil-N level. The removal of residual soil N in the harvested cereal rye cover crop resulted in low shoot N concentrations in the non-nodulating genotypes and provided easy discrimination of soil derived and atmospherically fixed N_2, also making non-nodulating genotypes visually distinctive. The experiment was conducted during the summers of 2015, 2016, and 2017 utilizing a randomized complete block design and four replications. Each plot consisted of four rows, 9.14 m in length with row spacing of 0.46 m. Sowing dates were 3 June 2015, 7 June 2016, and 10 June 2017, and seeding density was 46 seed m^{-2}. Irrigation was applied using an overhead sprinkler system, managed through an irrigation scheduling program that estimated soil-water deficit, and irrigation was applied when the estimated soil-water deficit reached 30 mm (Purcell et al, 2007).

2.2.2 Data Collection (Sampling and Processing)

2.2.2.1 Canopy Coverage

A week following emergence, stand counts were determined for each plot by counting the number of plants in 1m of the two central rows and averaging the two measurements. Canopy coverage measurements were made either from an aerial platform or from the ground using a digital imaging method (Purcell, 2000).

In 2015 and 2016, canopy coverage was determined from ground images of each plot (Purcell, 2000) taken every week using a 3.2-megapixel digital camera (FujiFilm Fine Pix A330, Fujifilm, Tokyo, Japan) prior to canopy closure. These images were saved as Joint Photographic Experts Group (JPEG) files with dimensions of 640×480, reordered by FastStone Image Viewer 4.2 (FastStone Soft, http://www.faststone.org), and analyzed by Sigma Scan Pro 5.0 (SPSS, Inc., Chicago, IL) with hue values range of 30 to 115, and saturation values ranging from 0 to 100 to obtain canopy coverage values similar to that obtained from "Field Analyzer" software (Field Analyzer, https://www.turfanalyzer.com/field_analyzer.html). Canopy coverage measurements were used as an indirect measure of the fraction of radiation intercepted (FRI; Purcell, 2000).

In 2017, aerial images were obtained from a drone (DJI Phantom 4 Pro, Dà-Jiāng Innovations, Shenzhen, China, https://www.dji.com/) flown at 61m above ground level (AGL), which covered the entire width of the field. Captured images (Figure 2.1-A) were used to derive canopy coverage estimates for each plot using the "Field Analyzer" software (Figure 2.1-B and 2.1-C). The software identifies green pixels in the image from the hue, saturation, and brightness and eliminates the soil background. The software then calculates canopy coverage as the fraction of green pixels divided by the total number of pixels in the selected region of the image. Images were acquired three to four times before the canopy completely closed.

Phenological development of plants was recorded twice a week for each genotype beginning at the R1 growth stage (Fehr and Caviness,1977) and dates of first flower (R1), full flower (R2), beginning seed fill (R5), full seed (R6), and physiological maturity (R7) were recorded.

2.2.2.2 Radiation Use Efficiency (RUE)

Radiation use efficiency (g MJ^{-1}) is described as the ratio of the change in biomass to the amount of radiation intercepted between two sampling dates (Monteith, 1977; Ries et al., 2012). Once the canopy was completely closed (approximately 5 weeks after emergence), 1 m^2 of each plot was harvested at ground level; a similar sample was taken 2 weeks later. The samples were dried at 60°C, weighed, and the change in biomass between the two sampling dates was determined (g m^{-2}). Total intercepted radiation for each day was derived as the product of FRI (i.e., FRI = 1, at full canopy closure) and total incident radiation (Rs) for that day. Daily intercepted radiation values were then summed for the days between the two sampling dates to obtain the total amount of radiation intercepted between the two sampling dates (cumulative intercepted radiation). Change in biomass between the sampling dates, divided by the cumulative intercepted radiation provided RUE (Ries et al., 2012). This measurement was performed only in 2016. Biomass samples were based on a relatively small area of 1 m^2, and it was necessary to have a uniform stand. In 2015 and 2017 the stands were not uniform and a non-uniform stand would not have provided a representative sample; a decision was made against biomass sampling and RUE measurements for these years.

2.2.2.3 Seed Growth Rate (SGR) and Effective Filling Period (EFP)

During the linear phase of seed growth, three random plants from the two center rows were harvested at ground level at mid-R5 and then again after 7-10 days. The pods were

removed; leaves, stems, and pods were dried separately. Immature seeds were shelled, weighed, counted, and small and aborted seeds (stunted seeds or seeds as thin as a scale) were removed. To do this, shelled seeds were passed through a No. 4 sieve (allows particle size smaller than 4.76 mm through it) and then through a No. 6 sieve (allows particle size smaller than 3.36 mm through it). Everything obtained below the No. 6 sieve was discarded. Average seed weight (g $seed^{-1}$) was obtained by dividing the seed weight by the number of seeds. The difference in the average seed weight between the two harvests divided by the number of days between the two sampling dates was used to determine SGR (g $seed^{-1}$ day^{-1}, Figure 2.2). The EFP (days) was estimated by dividing average seed weight at maturity by the SGR (Figure 2.2).

2.2.2.4 Harvest Index (HI) and Seed Weight

At physiological maturity, three plants were harvested (taking care that the sample was uniform and representative of the average plants in a plot) to determine HI. The entire shoot weight was determined, and the total seed weight was obtained after threshing the samples. Harvest index was calculated as the ratio of the seed weight to the shoot weight. One-hundred seed weight was also obtained from the same sample, as an estimate of the average seed weight.

2.2.2.5 Seed Yield

Seed yield was determined by harvesting the central portion of the middle two center rows (4.2 m in 2015, 3.7 m in 2016, and 4.5 m in 2017) of each plot at maturity (R8) using a plot combine. The yields were corrected to 13% moisture content.

2.2.3 Statistical Analysis

Outliers were detected using studentized residuals (r*). A studentized residual is a residual divided by its estimated standard deviation (Belsley et al., 2005). Studentized residuals are described as:

$$r^* = [y_i - \hat{y}(i)] / s \quad [2.1]$$

where,

y_i is the observed value, $\hat{y}(i)$ is the predicted value, and s is the estimated standard deviation.

Studentized residuals depict unexpected values with respect to the standard deviation. In our study, the sample size was 172 (43 genotypes, 41 nodulating and 2 non-nodulating, and 4 replications; 43 × 4). Based on this sample size, the expected number of observations with studentized residual 2 (i.e., 2 standard deviations away from mean) would be 8 (172 × 4.6 %, as there are only 4.6% observations outside the 2 standard deviation range; Hordo et al., 2008). Similarly, if the studentized residual was 3 (which is 3 standard deviations away from mean), there should be no outliers (≈ 0.5; 172 × 0.25 %, as 0.25 % observations would be outside 3 standard deviation range; Hordo et al., 2008). In our study, any observation with a studentized residual of 3 or more was considered a potential outlier. Thus, we closely observed the data points that had a studentized residual of 3 or higher and removed the unusual data points.

After removing outliers, the data were analyzed using the GLIMMIX procedure in SAS 9.4 (Cary, NC, USA), as some response variables had non-normal distributions. For example, effective filling period had a gamma distribution. Identification of the distribution of the response variables began with normality testing of the residuals of each variable. The Shapiro-Wilk test was used to test the normality of the residuals of each response variable. The Shapiro-Wilk test was found significant only for effective filling period, indicating that the data were not

distributed normally. Then, to identify the correct distribution of this response variable, the data range was checked. The effective filling period had positive values above zero and hence a gamma distribution was identified.

All variables were analyzed with analysis of variance except canopy coverage in the year 2017. The model consisted of year, maturity group, genotypes nested within maturity groups, and year by genotype interaction as the fixed effects. Replication and replication nested within a year were considered as random effects.

Canopy coverage was analyzed as a repeated measure as there were multiple measurement dates each year. In 2017, for canopy coverage, analysis of covariance (ANCOVA) was performed instead of analysis of variance (ANOVA) with stand counts (no. of plants in $1m^2$ area of two center rows after emergence) as a covariate. The analysis made statistical adjustments based on the differences incurred by stand counts to account for the effect of stand count on canopy coverage.

2.3 Results

2.3.1 Analysis of Variance

Analysis of variance showed that there were significant main effects of year and genotype by year interactions for all physiological traits other than RUE (Table 2.1). The main effect of MG was significant for all traits except RUE. For most traits, genotypes differed significantly, but there were no significant differences among genotypes for RUE.

Analysis of canopy coverage was performed separately for each year, and repeated measurements on different dates were included in the model as the variable date. Table 2.2 shows that date as a factor was significant; however, the interaction of date with genotype was

not significant for any of the years. Thus, the canopy coverage increased with time but the genotypes responded similarly at each of the measurement dates. In 2017, an initial analysis found that stand count and canopy coverage were significantly correlated ($p \leq 0.05$, $r = 0.72$). To address this concern, stand count was included as a covariate. However, the covariate analysis identified stand count as non-significant. Genotypes differed significantly from each other every year. Extreme genotypes were identified for each year. Also, as expected, different maturity groups were statistically different for canopy coverage as well (Table 2.2).

2.3.2 Correlations

There was a strong negative correlation of SGR with EFP ($P \leq 0.01$) for all three years, with Pearson correlation coefficients of -0.79 (2015), -0.79 (2016) and -0.84 (2017) (Table A.6, A.7, and A.8). SGR and yield had a strong negative correlation in 2015 ($r = -0.61$) and 2017 ($r = -0.50$) at $P \leq 0.01$ (Table A.6 and A.8). In contrast, yield and EFP had a positive correlation for 2015 ($r = 0.47$, $P \leq 0.01$, Table A.6), and 2017 ($r = 0.39$, $P \leq 0.05$, A.8). Also, HI and EFP had a positive correlation in 2015 ($r = 0.32$, $P \leq 0.05$, Table A.6).

Seed yield and seed weight were negatively correlated in 2015 ($r = -0.42$, $P \leq 0.01$, Table A.6) and 2016 at ($r = -0.40$, $P \leq 0.01$, A.8). While, canopy coverage had a positive correlation ($r = 0.35$, $P \leq 0.01$) with seed weight in 2016 (Table A.7), and positive correlations with yield ($r = 0.38$) and EFP ($r = 0.33$) in 2017 at $P \leq 0.05$ (Table A.8).

2.3.3 Distribution of the SoyNAM Parental Genotypes

The repeated measure analysis for canopy coverage had no significant genotype by date interaction (Table 2.2); canopy coverage values, averaged over dates, varied from 0.59 to 0.75 in 2015, 0.57 to 0.82 in 2016, and 0.38 to 0.72 in 2017 (Table 2.4). The canopy coverage of the hub parent, averaged over dates, varied from 0.47 to 0.67 among years (Table 2.4). Kaler et al.

(2018) made canopy coverage measurements twice at Fayetteville, AR for 373, MG 4 plant introductions (PIs) and obtained a range of 0.06 to 0.33 for the first date, and 0.41 to 0.81 for the second measurement date. The range reported in Table 2.4 is similar to the range reported by Kaler et al. (2018), although that study had only MG 4 genotypes, while the current study included genotypes from MG 1 to 5.

Genotypes S06-13640, PI 437169B, and LG03-191 had high canopy coverage for all three years and differed significantly from the hub parent, IA3023 (Figure 2.3, Table 2.4). Genotypes LG05-4464 was a high canopy coverage genotype in 2016 and differed significantly from the hub parent. Genotype LG94-1128 had low canopy coverage in 2015 and genotype U03-100612 had low canopy coverage in 2017; each of them differed significantly from the hub parent (Figure 2.3, Table 2.4). No genotype had a low canopy coverage consistently for all the three years.

In 2016, the observed genotypic range of RUE was 0.46 to 1.06 g MJ^{-1} (Table 2.4). Pengelly et al., (1999) observed RUE of 0.89 g MJ^{-1} (based on total intercepted solar radiation) for soybean. Kitani and Horie (1988) observed maximum RUE of 1.2 g MJ^{-1} in soybean (based on total intercepted solar radiation). Sinclair and Muchow (1999) found that the RUE values for soybean varied from 1.32 to 2.52 g MJ^{-1} (photosynthetically active radiation (PAR) basis, or 0.66 to 1.26 g MJ^{-1} on the total solar radiation basis) from an average value from six different studies. Van Roekel and Purcell (2014) reported a range of RUE from 1.46 to 1.89 g MJ^{-1} (based on total intercepted solar radiation, for experiments conducted under maximum yield conditions). The RUE of the hub parent in this experiment was 0.63 g MJ^{-1} (Table 2.4). Genotypes SKYLLA and LG05-4464 had the highest RUE values, which differed significantly from the hub parent (Figure 2.4, Table 2.4).

The range of SGR among genotypes was similar for each year: in 2015 it ranged from 3.8 to 7.9 mg seed^{-1} day^{-1}, in 2016 SGR ranged from 3.4 to 7.3 mg seed^{-1} day^{-1}, and in 2017 it ranged from 3.4 to 8.8 mg seed^{-1} day^{-1} (Table 2.4). Earlier studies also reported significant differences in SGR among genotypes (Egli et al., 1978, 1981; Guldan and Brun, 1985), and reported very similar ranges of SGR as observed in the current study. Egli et al. (1981) reported SGRs from 3.9 to 10.8 mg seed^{-1} day^{-1}; Egli et al. (1978) reported SGRs from 3.19 to 9.38 mg seed^{-1} day^{-1} for three PIs; Guldan and Brun (1985) reported SGRs from 2.6 to 10.0 mg seed^{-1} day^{-1}; and Egli (1998) reported SGRs from 3.6 to 14.7 mg seed^{-1} day^{-1} for 12 soybean cultivars. In the current study, the SGR of the hub parent was 5.4 mg seed^{-1} day^{-1} (2015), 4.2 mg seed^{-1} day^{-1} (2016), and 3.6 mg seed^{-1} day^{-1} (2017) (Table 2.4) for the 3 years.

Genotypes LG03-3191, LG04-6000, and LD00-3309 had relatively slow SGR in 2015 and differed significantly from the hub parent (Figure 2.5, Table 2.4). Genotype LG04-6000 had slow SGR in 2016 and genotype LG03-3191 was the slowest SGR genotype in 2017, but they did not differ significantly from the hub parent (Figure 2.5, Table 2.4). Genotype SKYLLA had a high SGR in 2015 and 2016, genotype PI 437169B had a high SGR in 2015 and 2017, and genotype LG05-4464 had a high SGR in 2016. Each of these genotypes differed significantly from the hub parent (Figure 2.5, Table 2.4). As mentioned earlier MG had a significant effect on SGR. It was found that genotypes PI 437169B, SKYLLA, and LG05-4464 that had high SGR belonged to relatively early MGs. Genotype PI 437169B and SKYLLA were from MG 2, while LG05-4464 was from MG 3. Similarly, genotypes with slow SGR were of relatively later MGs. Genotype LG04-6000, LG03-3191, and LD00-3309 all belonged to MG 4. This seems reasonable as later MG genotypes mature later than early MGs, sown at the same time. However,

later MG genotypes generally have a longer vegetative phase but have seed growth/filling period (EFP) similar to early MG genotypes (Edwards and Purcell, 2005; Egli, 2017).

In 2015, EFP ranged from 20 to 43 days; in 2016 EFP ranged from 21 to 53 days, and in 2017 EFP ranged from 21 to 57 days (Table 2.4). Previous studies have reported soybean's EFP ranging from 13 to 57 days for 59 soybean genotypes (Swank et al., 1987), 31 to 46 days for 20 genotypes of MG 4, 6, and 7 (Boerma and Ashley, 1988), 19 to 41 days across two years (Egli et al., 1978), and 22 to 33 days for 20 soybean cultivars (Egli, 1998). Van Roekel et al. (2015) noted that EFP for most soybean cultivars was between 22 to 33 days. The EFP of the hub parent in the current study was 25 (2015), 34 (2016), and 47 days (2017) (Figure 2.6, Table 2.4). The average daily temperatures during seed-fill were lower in 2017 (Table A.5) compared to 2015 (Table A.3) and 2016 (Table A.4), and EFP increases with the decrease in temperature (Egli and Wardlaw, 1980).

Genotypes with long EFP have been associated with high yield (Daynard et al., 1971; Dunphy et al., 1979; Egli, 1975; Smith and Nelson, 1986), and it was of interest to identify genotypes with a long EFP among the SoyNAM parents. Genotype LD01-5907, LG04-6000, LG03-3191, S06-13640, and LD00-3309 had a long EFP relative to the hub parent in 2015 (Figure 2.6, Table 2.4) and differed significantly from the hub parent. Genotype LD01-5907 had the longest EFP in 2016, whereas genotypes LG94-1128, TN05-3027, LG05-4464, and LD02-9050 had a short EFP relative to the hub parent the same year, and each of them differed significantly from the hub parent (Figure 2.6, Table 2.4). Genotype LG03-3191 had a long EFP again in 2017 but did not differ significantly from the hub parent, and genotype PI 437169B had the shortest EFP the same year. It is noteworthy that genotype LD01-5907 had the longest EFP for two years (2015 and 2016) with a significant difference from the hub parent (Figure 2.6).

Genotypes from relatively late MG had longer EFP. Among genotypes LG04-6000, LG03-3191, S06-13640, LD00-3309, and LD01-5907 that had significantly longer EFP than the hub parent, all but LD01-5907 belonged to MG 4, and it belonged to MG 3. However, genotypes LG94-1128, TN05-3027, LG05-4464, LD02-9050, and PI 437169B that had significantly shorter EFP compared to hub parent, did not necessarily belong to early MGs and were anywhere from MG 2 to 5. While discussing SGR, later MG genotypes generally had slow SGR, and for the same reason, they tended to have longer EFP. That is, slow SGR leads to longer EFP. Egli et al. (1984) also reported that the later maturing cultivars (MG 4 and 5) had longer filling periods (as the duration from R5 to R7) than the MG 3 cultivars even though EFPs showed no significant differences. Egli et al. (1984) concluded that measurements of EFP always had large coefficients of variation, but that the duration of seed filling was an important determinant of yield.

Seed yield ranged from 2227 to 4520 kg ha^{-1} (2015), 2074 to 4581 kg ha^{-1} (2016), and 3377 to 6386 kg ha^{-1}(2017) (Figure 2.7, Table 2.4). The yield of the hub parent was 3769 kg ha^{-1} (2015), 3669 kg ha^{-1} (2016), and 5455 kg ha^{-1} (2017) (Table 2.4). Genotype LD02-9050 had the highest yield in 2015 and 2016 and differed significantly from the hub parent in these years (Figure 2.7, Table 2.4). Genotype LG04-6000 was a high yielding genotype in 2016 and had the highest yield in 2017, differing statistically from the hub parent. Genotypes LD01-5907 and LG05-4292 were the high yielding genotypes in 2016 and also differed significantly from the hub parent. Genotype PI 437169B and PI 507681B were low yielding genotypes all three years, differing statistically from the hub parent (Figure 2.7, Table 2.4). High yielding genotypes LD02-9050, LG04-6000, LG05- 4292 belonged to MG 4, and genotype LD01-5907 belonged to MG 3. Later MGs had relatively slower SGR and longer EFP, which have been related to higher yield (Egli et al., 1984; Daynard et al., 1971; Smith and Nelson, 1986).

Seed weight varied from 0.12 to 0.17 g seed $^{-1}$ (2015), from 0.13 to 0.20 g seed $^{-1}$ (2016), and from 0.14 to 0.20 g seed $^{-1}$ (2017) (Figure 2.8, Table 2.4). Swank et al. (1987) reported a range of seed weight from 0.07 to 0.36 g seed^{-1} for 59 soybean genotypes. Egli (1998) reported seed weight from 0.08 to 0.48 g seed^{-1} for 20 soybean cultivars. The seed weight of the hub parent was 0.15 g seed $^{-1}$ in 2015, 0.14 g seed $^{-1}$ in 2016, and 0.16 g seed $^{-1}$ in 2017 (Table 2.4).

As described earlier, yield and seed weight were negatively correlated (Table A.6 and A.8), and it was of interest to identify extremes for seed weight. Genotype LD00-3309 had the second lowest seed weight in 2015 and the lowest in 2017, and it differed significantly from the hub parent (Figure 2.8, Table 2.4). Genotype LG05-4832 had a low seed weight, significantly different from the hub parent in 2015 and 2017 (Figure 2.8, Table 2.4). Genotype LG97-7012 had a seed weight significantly higher than the hub parent for all 3 years (Figure 2.8, Table 2.4). Genotypes PI 574486 and PI 561370 had a seed weight significantly higher than the hub parent in both 2016 and 2017 (Figure 2.8, Table 2.4).

Harvest index varied from 0.39 to 0.54 (2015), from 0.24 to 0.40 (2016), and from 0.48 to 0.58 (2017) (Table 2.4). The range of HI observed by a previous study during a two-year experiment for 24 genotypes of soybean was from 0.43 to 0.65 (Schapaugh and Wilcox, 1980). The HI of the hub parent in this study was 0.53 (2015), 0.34 (2016) and 0.57 (2017) (Figure 2.9, Table 2.4). Harvest index and yield had a positive correlation of 0.68 (2015, $P \leq 0.01$, Table A.6) and 0.56 (2016, $P \leq 0.01$, A.7). Harvest index and yield were not significantly correlated in 2017, ($r = 0.14$, Table A.8). Genotypes LG02-4485 (MG 2), LD02-9050 (MG 4), and LG97-7012 (MG 3) had the highest HI in 2016 and were significantly different from the hub parent (Figure 2.9, Table 2.4). Genotype LG97-7012 also had the highest HI in 2017, but it did not differ significantly from the hub parent (Figure 2.9, Table 2.4). The HI of the hub parent was towards

the upper end of HI range in 2017 (Figure 2.9, Table 2.4). Genotype PI 437169B had consistently low HI all three years differing significantly from the hub parent (Figure 2.9, Table 2.4).

2.4 Discussion

A strong negative correlation between SGR and EFP ($P \leq 0.01$) for all three years, with Pearson correlation coefficients of -0.79 (2015), -0.79 (2016), and -0.84 (2017) (Table A.6, A.7, and A.8) indicated that genotypes with a slow SGR tended to have a longer EFP and vice-versa. Similarly, EFP and yield had a positive correlation for 2015 (0.47, $P \leq 0.01$, Table A.6) and 2017 (0.39, $P \leq 0.05$, Table A.8), while yield and SGR had a strong negative correlation for 2015 ($r = -0.61$, $P \leq 0.01$, Table A.6) and 2017 (-0.50, $P \leq 0.01$, Table A.8). Genotypic results agreed with the correlations; genotypes LD00-3309, LG04-6000, and LG03-3191 had significantly lower SGRs and longer EFPs compared to the hub parent in the same year (2015, Figure 2.5 and 2.6, Table 2.4). Genotype LG04-6000 also had a significantly higher yield than the hub parent in multiple years (Figure 2.7, Table 2.4). Genotype LD01-5907 had long EFP in 2015 and 2016, and high yield in 2016 (Figure 2.6 and 2.7, Table 2.4). Similarly, a low yielding genotype PI 437169B (all 3 years, Figure 2.7, Table 2.4) had a high SGR for two of the years (Figure 2.5, Table 2.4), and low EFP in 2017 (Figure 2.6, Table 2.4).

These findings agree with the self-destruct hypothesis (Sinclair and Dewitt, 1975). Due to a high protein concentration soybean has a high N demand that is greater than its ability to accumulate or fix N. Hence, soybean translocates a large amount of N from vegetative tissues to developing seeds, and in the process render a loss of overall physiological activity. This self-destructive characteristic of soybean limits the length of its seed development period (Sinclair and de Wit, 1976), which indicates that genotypes with a relatively slower SGR and a longer EFP tend

to have a higher yield and vice-versa. A longer EFP is commonly associated with a slow HI increase rate or low dry matter allocation coefficient (DMAC; day^{-1}; Salado-Navarro et al., 1986a); the DMAC is a measure of the rate of HI increase and has been suggested as an alternative measure of SGR (Spaeth and Sinclair, 1985). Thus, the relationship between slow SGR and longer EFP is explainable through the self-destruction hypothesis (Salado-Navarro et al. 1986a, 1986b, 1993), as it indicates that a slow SGR reduces the daily demand for C and N remobilization. Thus, a slow SGR increases the EFP due to the low demand of remobilized C and N from the vegetative tissues (Salado-Navarro et al. 1986a, 1986b, 1993). Egli et al. (1978) also reported a negative relationship between SGR and EFP.

Previous research has not identified a consistent relationship between SGR and yield. Egli (1975) found a negative (non-significant) correlation, while Egli et al. (1978) found a weak positive, non-significant correlation between SGR and yield. However, reports have found an association between EFP and yield. Hanway and Weber (1971) reported differences in yield for eight cultivars with similar SGR due to differences in their EFP. Egli and Leggett (1973) found that though there were differences in the SGR for the cultivars in their research, the yield differences found were more closely associated with EFP. Similar to the present study, past studies have often identified a positive association of EFP with yield (e.g., Daynard et al., 1971; Dunphy et al., 1979; Egli, 1975; Smith and Nelson, 1986).

A long EFP is also associated with low temperatures during the filling period, (Egli and Wardlaw, 1980). In our study, the average daily temperature was slightly lower during 2017 (29.7°C / 18.9°C, T_{max}/ T_{min}) than in 2015 (30.6°C / 19.7°C) and 2016 (31.1°C / 20.6°C), but was within the range of 19 to 30 ºC for which EFP was reported stable (Egli and Wardlaw, 1980; Hesketh et al., 1973). Our study noted that the average EFP was longest for the coolest year

(2017, 36.5 ± 4.1 days), followed by 2016 (32.8 ± 3.7 days), and 2015 (27.6 ± 3.0 days). An increase in EFP with cooler temperatures agrees with previous reports that long EFP is associated with lower temperatures (Egli and Wardlaw, 1980; Edwards and Purcell, 2005).

Yield and seed weight were negatively correlated in 2015 (-0.42, P≤ 0.01) and 2016 (r = -0.40, P≤ 0.01, Table A.7). However, no genotype with a significantly lower seed weight than the hub parent had significantly higher yield than the hub parent (Figure 2.7, Figure 2.8, Table 2.4). Gay et al. (1980), while comparing old and new cultivars, found that the yield advantage of the cultivar 'Williams' over 'Lincoln' was due to a combination of greater seed weight and longer EFP. Other studies have shown that as seed weight decreases, seed number increases, and seed number influences yield more than the seed weight (Board 1987; De Bruin and Pederson, 2008; Singer et al., 2004). Therefore, breeding efforts towards increasing seed weight have not led to increase in yield (Hartwig and Edwards, 1970), as reduced seed weight can result in greater seed number but not necessarily increased yield (Van Roekel et al., 2015). If a larger seed weight is due to high SGR, it would generally have no effect on yield, as seed number would decrease with larger seeds; however, if large seed weight is due to a long EFP (Swank et al., 1987), there would not be a resultant decrease in seed number, resulting in an increase in yield.

Yield and HI had a strong positive correlation in 2015 (r = 0.68, P≤ 0.01), and 2016 (r = 0.56, P≤ 0.01, Table A.7). Yield and HI were not significantly correlated in 2017 (r = 0.14, Table A.8). Genotypes with HI significantly higher than the hub parent had higher yield. For example, genotype LD02-9050 had a HI significantly higher than the hub parent in 2016 (Figure 2.9), in 2015 and 2017 it was amongst the highest HIs (Table 2.4), and was also significantly higher yielding than the hub parent for 2015 and 2016 (Figure 2.7 and 2.9, Table 2.4). Rotundo et al.

(2014) concluded that breeding for increased HI along with increased N use efficiency had the potential for increasing yield.

Seed weight and canopy coverage had a positive correlation in 2016 ($r = 0.36$, $P \leq 0.05$, Table A.7). Place et al. (2011a, b) reported a positive relationship between canopy coverage and seed weight in soybean; they found that in organically grown soybean, genotypes with large seed weight resulted in more robust canopies, resulting in better weed control. Kaler et al. (2018) also reported a positive relationship between canopy coverage and seed weight at 7 out of 10 locations.

Yield and canopy coverage were positively correlated in 2017 ($r = 0.38$, $P \leq 0.05$, Table A.8). If the canopy of a genotype closes early, it will intercept more sunlight during its lifecycle compared to other genotypes. Studies have found a positive relationship between cumulative intercepted photosynthetically active radiation (CIPAR) and yield (Edward et al., 2005; Kantolic et al., 2013). Koester et al. (2014) concluded that greater biomass and yield of soybean cultivars released between 1923 and 2007 was due to increased light interception. Similarly, Hall (2015) found that rapid canopy development or early canopy closure in soybean provides a foundation for greater biomass accumulation during the season, ultimately leading to greater grain yield. Genotype PI 437169B had a significantly greater canopy coverage than the hub parent for all 3 years (Figure 2.3, Table 2.4). This genotype was selected in the SoyNAM population for high yield under drought. However, PI 437169B is not an improved cultivar, so it is unlikely for it to have yield higher than the hub parent (which is an elite breeding line), despite having a greater canopy coverage.

The current study, as well as previous studies, have found association of SGR, EFP, and seed weight (negative, positive, and negative respectively) with yield. However, earlier attempts

on increasing soybean yield based on EFP, SGR, seed weight, and seed number have not been successful (Donald and Hamblin, 1976; Egli, 1998). Van Roekel et al. (2015) suggested that the compensation that occurs among these traits indicates that several paths might result in similar yields. They also suggested two major reasons for the past failures in increasing yield by selecting for SGR and EFP. First, a large number of alleles contribute to these traits leading to their complexity. Second, evaluations were performed under non-ideal conditions, leading to low heritability and large genotype by environment interactions.

2.5 Conclusions

Van Roekel et al. (2015) pointed out that the highest reported soybean yield is about three times greater than the highest reported U.S. average yield, indicating that there is still considerable scope for soybean yield improvement. However, the narrow genetic base of soybean in North America creates a risk of limiting future yield gains. The present research evaluated the 41 SoyNAM parental genotypes for different physiological traits that are important with respect to yield. Several genotypes were identified as being significantly different from the hub parent and some as common extreme parents for more than one physiological trait. Extreme genotypes identified can be used to select specific biparental populations from SoyNAM. As identified extreme genotypes and populations derived from them in SoyNAM, make ideal candidates for further genetic and physiological studies.

Tables and figures

Table 2.1 Analysis of variance for radiation use efficiency , seed growth rate , effective filling period , seed weight , harvest index , and yield evaluated on the SoyNAM parental genotypes for the effect of year, maturity group (MG), genotypes nested within maturity group and year.

Traits	Year		MG		Genotype (MG)		Year*Genotype (MG)	
	DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value
Radiation use efficiency	0	-	4	0.7189	36	0.5119	0	-
Seed growth rate	2	0.0174	4	<0.0001	36	<0.0001	72	<0.0001
Effective filling period	2	<0.0001	4	<0.0001	36	<0.0001	72	<0.0001
Seed weight	2	0.0001	4	<0.0001	36	<0.0001	72	0.0005
Harvest index	2	<0.0001	4	<0.0001	36	<0.0001	72	0.0209
Yield	2	<0.0001	4	<0.0001	36	<0.0001	72	0.0166

Table 2.2 Analysis of variance for canopy coverage evaluated on the SoyNAM parental genotypes for the effect of maturity group (MG), genotypes nested within maturity group and Date (repeated measurements of canopy coverage in a year) for 2015, 2016, and 2017.

Year	Date		MG		Genotype (MG)		Date * Genotype (MG)	
	DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value
2015	5	<0.0001	4	0.0007	36	<0.0001	180	0.3025
2016	2	<0.0001	4	<0.0001	36	<0.0001	72	0.1173
2017[†]	2	<0.0001	4	<0.0001	36	<0.0001	72	0.5415

[†] Stand count was a covariate; whose effect was non-significant (p = 0.0503).

Table 2.3 A list of genotypes that significantly differed from the hub parent.

Genotype	Characteristics found
LG04-6000	▪ Slow SGR in 2015 (Figure 2.5), ▪ long EFP in 2015 (Figure 2.6), and ▪ high yield for the years 2016 and 2017 (Figure 2.7).
LG03-3191	▪ Slow SGR in 2015 (Figure 2.5), ▪ long EFP in 2015 (Figure 2.6), and ▪ high canopy coverage in all the three years 2015, 2016, and 2017 (Figure 2.3).
S06-13640	▪ High canopy coverage all the three years (Figure 2.3), and ▪ long EFP in 2015 (Figure 2.6).
LD00-3309	▪ Slowest SGR genotype in 2015 (Figure 2.5), ▪ long EFP in 2015 (Figure 2.6), and ▪ low seed weight in 2015 and 2017 (Figure 2.8).
LG05-4464	▪ High canopy coverage in 2016 (Figure 2.3), ▪ high RUE in 2016 (Figure 2.4), ▪ short EFP in 2016 (Figure 2.6), and ▪ high SGR in 2016 (Figure2.5).
LG05-4832	▪ Low seed weight in 2015 and 2017 (Figure 2.8).
LD02-4485	▪ High HI in 2016 (Figure 2.9).
LG05-4292	▪ High yield in 2016 (Figure 2.7)
PI 437169B	▪ High canopy coverage all the three years (Figure 2.3), ▪ high SGR in 2015 and 2017 (Figure 2.5), ▪ short EFP in 2017 (Figure 2.6), ▪ low HI all three years (Figure 2.9), and ▪ low yield all three years (Figure 2.7).
LD01-5907	▪ High yield in 2016 (Figure 2.7), and ▪ long EFP in 2015 and 2016 (Figure 2.6).
SKYLLA	▪ High RUE in 2016 (Figure 2.4), and ▪ high SGR in 2015 and 2016 (Figure 2.5).
LD02-9050	▪ High yield in 2015 and 2016 (Figure 2.7), ▪ high HI in 2016 (Figure 2.9), and ▪ short EFP in 2016 (Figure 2.6).
LG97-7012	▪ High HI in 2016 (Figure 2.9), and ▪ high seed weight all three years (Figure 2.8).

RUE, SGR, EFP, and HI stand for radiation use efficiency, seed growth rate, effective filling period and harvest index respectively.

Table 2.4 Mean values of all genotypes along with least significant difference (LSD) for the respective physiological traits.

		Physiological Traits																	
		Canopy coverage			Radiation use efficiency (g MJ^{-1})	Seed growth rate (mg seed^{-1} day^{-1})			Effective filling period (days)			Yield (Kg ha^{-1})			Seed weight (g seed^{-1})			Harvest index		
		2015	2016	2017	2016	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Genotypes	**LSD**	**0.15**	**0.12**	**0.17**	**0.32**	**1.33**	**1.92**	**1.09**	**2.51**	**2.56**	**2.66**	**400.65**	**483.51**	**457.23**	**0.02**	**0.02**	**0.01**	**0.04**	**0.03**	**0.03**
	MG																			
U03-100612	1	0.66	0.57	0.57	0.74	7.11	5.09	4.93	20.74	33.91	35.27	2723.08	3123.72	4971.15	0.15	0.14	0.17	0.47	0.36	0.58
LD02-4485	2	0.66	0.71	0.71	0.63	4.59	4.13	4.97	31.79	32.94	31.95	3705.47	4314.22	5370.94	0.14	0.14	0.16	0.52	0.40	0.56
LG92-1255	2	0.65	0.67	0.67	0.70	5.65	5.22	5.96	31.34	34.09	35.83	2892.22	2903.20	4692.41	0.17	0.17	0.20	0.45	0.36	0.53
LG94-1128	2	0.59	0.67	0.67	0.89	7.17	6.34	4.59	19.49	24.80	35.71	2932.73	3291.69	4376.08	0.14	0.15	0.16	0.48	0.30	0.54
LG94-1906	2	0.63	0.66	0.66	0.63	6.15	5.30	5.62	24.95	31.67	30.03	3056.04	2945.68	4207.60	0.15	0.16	0.16	0.48	0.28	0.52
PI 404188A	2	0.73	0.78	0.78	0.86	6.04	4.75	4.23	23.81	26.15	38.17	2392.64	2565.99	3565.59	0.14	0.14	0.16	0.46	0.28	0.52
PI 437169B	2	0.73	0.81	0.81	0.76	6.85	5.24	8.79	25.93	33.81	20.48	2226.67	2074.27	3722.03	0.17	0.19	0.17	0.39	0.24	0.48
PI 507681B	2	0.62	0.65	0.65	0.47	5.83	4.58	5.44	27.38	35.89	30.15	2410.44	2493.41	3377.29	0.16	0.16	0.16	0.46	0.29	0.53
PI 518751	2	0.69	0.75	0.75	0.70	5.40	5.36	5.94	26.51	30.42	28.71	3050.51	2793.64	3980.50	0.14	0.16	0.17	0.49	0.31	0.55
PI 574486	2	0.68	0.77	0.77	0.77	6.31	6.19	5.58	27.25	30.96	36.68	3064.73	3687.23	4337.65	0.17	0.19	0.20	0.47	0.32	0.54
Skylla	2	0.69	0.72	0.72	1.06	7.99	6.12	4.63	20.78	31.11	38.24	2923.33	3408.07	4708.18	0.16	0.17	0.17	0.42	0.32	0.48
4J105-3-4	3	0.69	0.78	0.78	0.72	5.32	5.73	5.31	30.60	31.58	33.26	4342.62	4413.96	5075.64	0.16	0.17	0.17	0.52	0.34	0.55
5M20-2-5-2	3	0.69	0.75	0.75	0.56	5.22	5.38	5.70	29.45	29.88	31.75	3805.37	3634.33	4935.06	0.15	0.16	0.17	0.50	0.32	0.52
CL0J095-4-6	3	0.67	0.62	0.62	0.61	5.48	6.03	4.73	25.93	26.55	36.11	3767.14	4189.94	4600.83	0.14	0.15	0.17	0.53	0.35	0.53
CL0J173-6-8	3	0.68	0.75	0.75	0.68	5.72	6.46	4.97	27.67	25.96	36.74	4087.42	3800.93	4692.28	0.16	0.16	0.18	0.52	0.34	0.54
HS6-3976	3	0.65	0.74	0.74	0.58	5.43	6.54	5.27	27.75	25.00	35.34	3489.09	3364.92	4884.86	0.15	0.16	0.18	0.52	0.30	0.54

Table 2.4 (Cont.)

		Physiological Traits																	
		Canopy coverage			Radiation use efficiency (g	Seed growth rate (mg seed^{-1} day^{-1})			Effective filling period (days)			Yield (Kg ha^{-1})			Seed weight (g seed^{-1})			Harvest index		
		2015	2016	2017	2016	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Genotypes	**LSD**	**0.15**	**0.12**	**0.17**	**0.32**	**1.33**	**1.92**	**1.09**	**2.51**	**2.56**	**2.66**	**400.65**	**483.51**	**457.23**	**0.02**	**0.02**	**0.01**	**0.04**	**0.03**	**0.03**
	MG																			
IA3023	3	0.67	0.61	0.61	0.63	5.40	4.25	3.60	24.13	33.92	46.78	3769.46	3669.15	5455.66	0.15	0.14	0.16	0.53	0.34	0.57
LD01-5907	3	0.68	0.69	0.69	0.75	4.57	4.02	4.51	42.54	52.35	36.93	4053.98	4406.29	5292.77	0.15	0.15	0.16	0.52	0.37	0.55
LG00-3372	3	0.72	0.66	0.66	0.82	5.49	5.89	4.52	27.35	28.97	37.97	3418.95	3679.13	5520.63	0.14	0.15	0.17	0.45	0.31	0.51
LG03-2979	3	0.68	0.72	0.72	0.69	4.92	4.33	4.38	25.51	33.41	38.58	3535.14	4131.47	4906.39	0.12	0.14	0.16	0.52	0.35	0.54
LG04-4717	3	0.69	0.75	0.75	0.84	5.26	5.24	4.79	27.78	30.21	31.96	3546.15	4152.92	5178.37	0.15	0.15	0.15	0.51	0.34	0.54
LG05-4464	3	0.68	0.77	0.77	0.95	5.04	7.32	4.75	28.72	20.46	38.22	3256.31	3931.22	5586.00	0.14	0.15	0.18	0.48	0.34	0.54
LG05-4832	3	0.63	0.70	0.70	0.64	5.00	4.58	3.87	26.67	26.07	40.08	3761.03	2993.59	5233.77	0.13	0.13	0.15	0.49	0.28	0.53
LG90-2550	3	0.71	0.74	0.74	0.60	4.95	6.13	4.87	29.46	27.05	32.55	3316.12	3422.41	4714.99	0.14	0.14	0.15	0.52	0.34	0.56
LG97-7012	3	0.65	0.70	0.70	0.63	7.87	4.73	5.61	22.24	45.46	35.17	3142.25	3239.63	4720.29	0.17	0.19	0.19	0.47	0.38	0.58
LG98-1605	3	0.63	0.68	0.68	0.81	5.45	3.48	5.26	26.29	46.60	31.58	2841.33	3661.62	4688.19	0.14	0.15	0.16	0.50	0.35	0.55
Maverick	3	0.71	0.67	0.67	0.61	5.29	4.09	4.35	25.98	34.79	38.77	4009.79	4341.19	5497.30	0.14	0.14	0.17	0.50	0.32	0.54
NE3001	3	0.71	0.73	0.73	0.77	6.10	4.00	5.06	28.80	42.34	35.73	2646.68	3163.46	4810.32	0.17	0.17	0.18	0.46	0.34	0.57
PI 398881	3	0.74	0.74	0.74	0.72	6.13	4.36	5.23	24.93	38.75	33.54	2656.37	2803.17	3851.07	0.15	0.15	0.17	0.51	0.35	0.56
PI 427136	3	0.67	0.79	0.79	0.64	6.59	4.00	5.11	23.68	37.22	35.42	2660.19	3196.12	4403.89	0.16	0.16	0.17	0.48	0.30	0.54
PI 561370	3	0.71	0.74	0.74	0.66	6.67	5.85	5.30	22.04	35.59	38.65	2311.16	2723.61	3803.86	0.17	0.20	0.20	0.49	0.32	0.55
Prohio	3	0.70	0.72	0.72	0.81	5.17	3.41	3.69	27.26	44.75	43.74	3695.57	3857.31	5128.32	0.14	0.14	0.16	0.51	0.33	0.53

Table 2.4 (Cont.)

Genotypes	LSD	Physiological Traits																	
		Canopy coverage			Radiation use efficiency (g	Seed growth rate (mg seed^{-1} day^{-1})			Effective filling period (days)			Yield (Kg ha^{-1})			Seed weight (g seed^{-1})			Harvest index		
		2015	2016	2017	2016	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
		0.15	**0.12**	**0.17**	**0.32**	**1.33**	**1.92**	**1.09**	**2.51**	**2.56**	**2.66**	**400.65**	**483.51**	**457.23**	**0.02**	**0.02**	**0.01**	**0.04**	**0.03**	**0.03**
	MG																			
LD00-3309	4	0.61	0.63	0.63	0.60	3.83	4.14	3.76	33.45	35.19	38.93	4061.20	4056.49	5781.10	0.12	0.14	0.14	0.52	0.33	0.54
LD02-9050	4	0.70	0.67	0.67	0.82	5.68	6.94	5.48	26.16	20.40	33.06	4520.06	4581.44	5345.72	0.15	0.15	0.17	0.52	0.38	0.55
LG03-3191	4	0.75	0.80	0.80	0.65	4.07	5.53	3.41	34.82	27.00	56.96	3653.50	4095.28	5006.22	0.16	0.15	0.19	0.51	0.37	0.52
LG04-6000	4	0.67	0.65	0.65	0.64	3.91	3.93	4.37	37.06	37.57	38.70	3826.88	4367.77	6386.03	0.14	0.14	0.16	0.52	0.34	0.55
LG05-4292	4	0.66	0.66	0.66	0.61	5.73	3.98	4.40	24.70	39.57	39.95	4181.16	4549.59	5861.82	0.14	0.15	0.17	0.50	0.32	0.52
LG05-4317	4	0.64	0.68	0.68	0.66	4.85	5.04	5.54	28.22	29.73	32.01	3707.10	3863.25	5255.26	0.14	0.14	0.17	0.49	0.29	0.55
Magellan	4	0.64	0.74	0.74	0.71	5.22	4.76	3.42	28.16	32.95	47.46	3593.31	4518.09	5403.11	0.14	0.15	0.17	0.51	0.35	0.52
S06-13640	4	0.75	0.79	0.79	0.67	4.26	4.55	3.79	34.72	36.20	47.46	3802.82	3751.49	5461.22	0.15	0.16	0.17	0.46	0.31	0.51
TN05-3027	5	0.69	0.75	0.75	0.67	4.64	6.38	5.13	29.77	22.44	32.25	4223.19	3693.74	5965.92	0.14	0.13	0.16	0.54	0.31	0.55
Non-nodulating																				
Harsoy Non-Nod	2	-	0.49	0.52	0.32	-	-	-	-	-	-	-	1274.22	-	-	-	-	-	-	-
Clark Non-Nod	4	-	0.71	-	0.63	-	-	-	-	-	-	-	2802.67	-	-	0.14	-	-	0.29	-
Lee Non-Nod	6	-	-	0.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

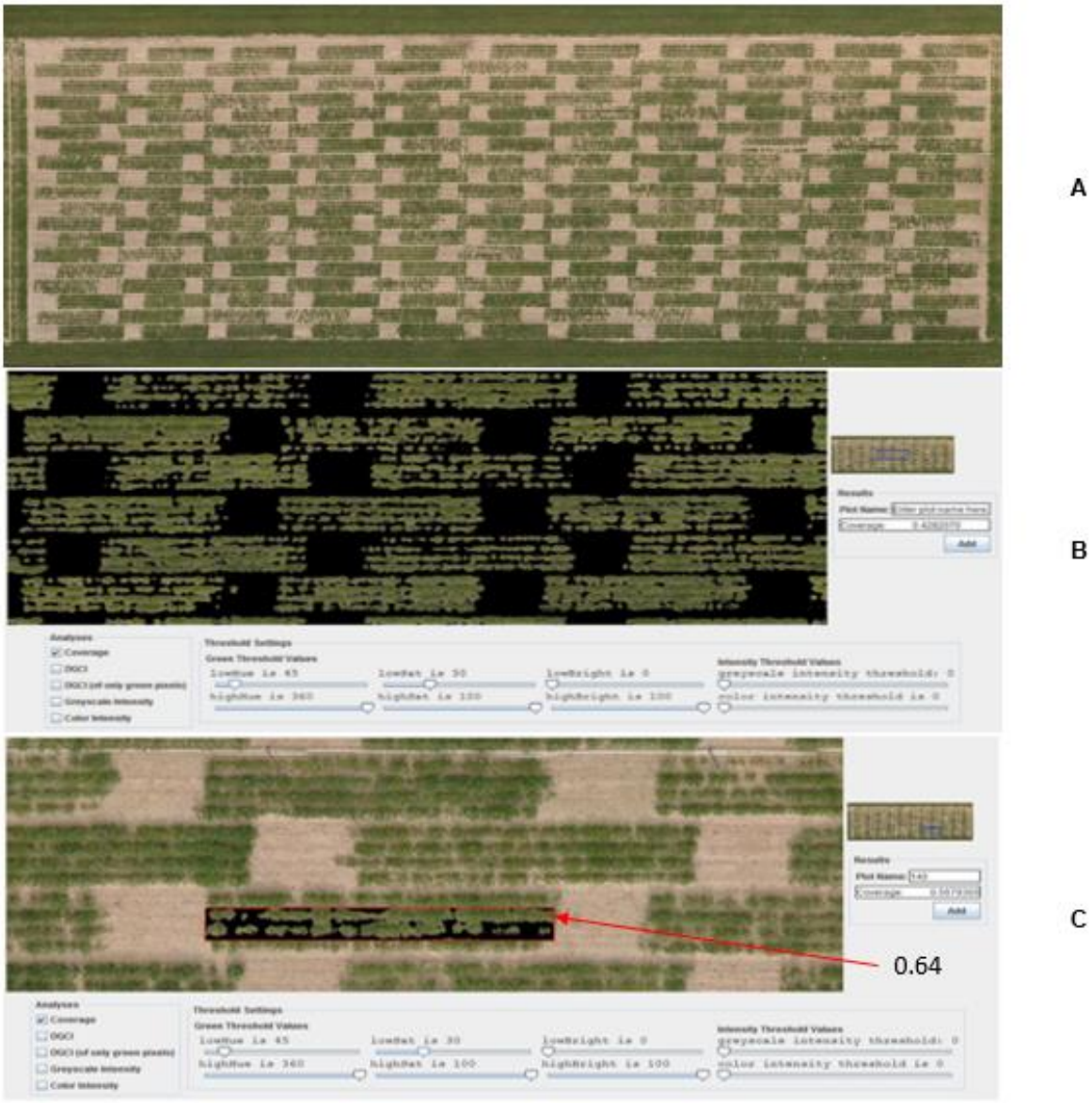

Figure 2.1 Method of estimation of canopy coverage: Snapshot of entire field was captured at once from the drone (A), once the image was opened in Fieldanalyzer (B) it was possible to zoom in, determine hue and saturation combinations that identify green tissue but eliminated the soil; (C) the two center rows along with the soil background of the surrounding inter-row space were selected, software eliminated the soil background and provided a fraction of green pixels relative to the total pixels for the selected area.

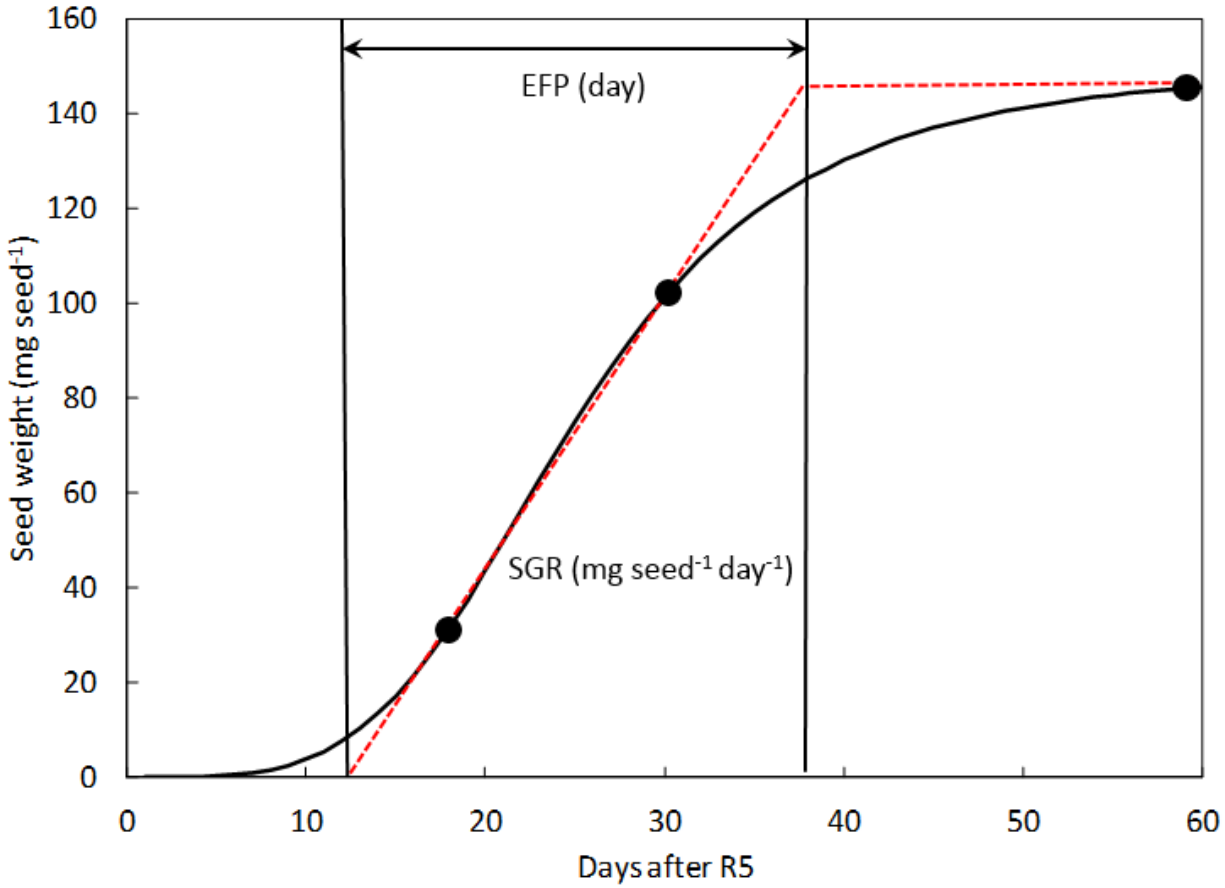

Figure 2.2 An example of a method of estimation of seed growth rate (SGR) and effective filling period (EFP): Two samples were taken during the linear phase of seed growth (mid-R5) 7-10 days apart indicated by the two center dots, and a final sample was taken at maturity indicated by the black dot at the top right corner. Seed growth rate (g seed^{-1} day^{-1}) was calculated as a difference of average seed weight between the two harvests, divided by the number of days between the two sampling dates. Effective filling period was obtained by dividing the final seed weight by SGR.

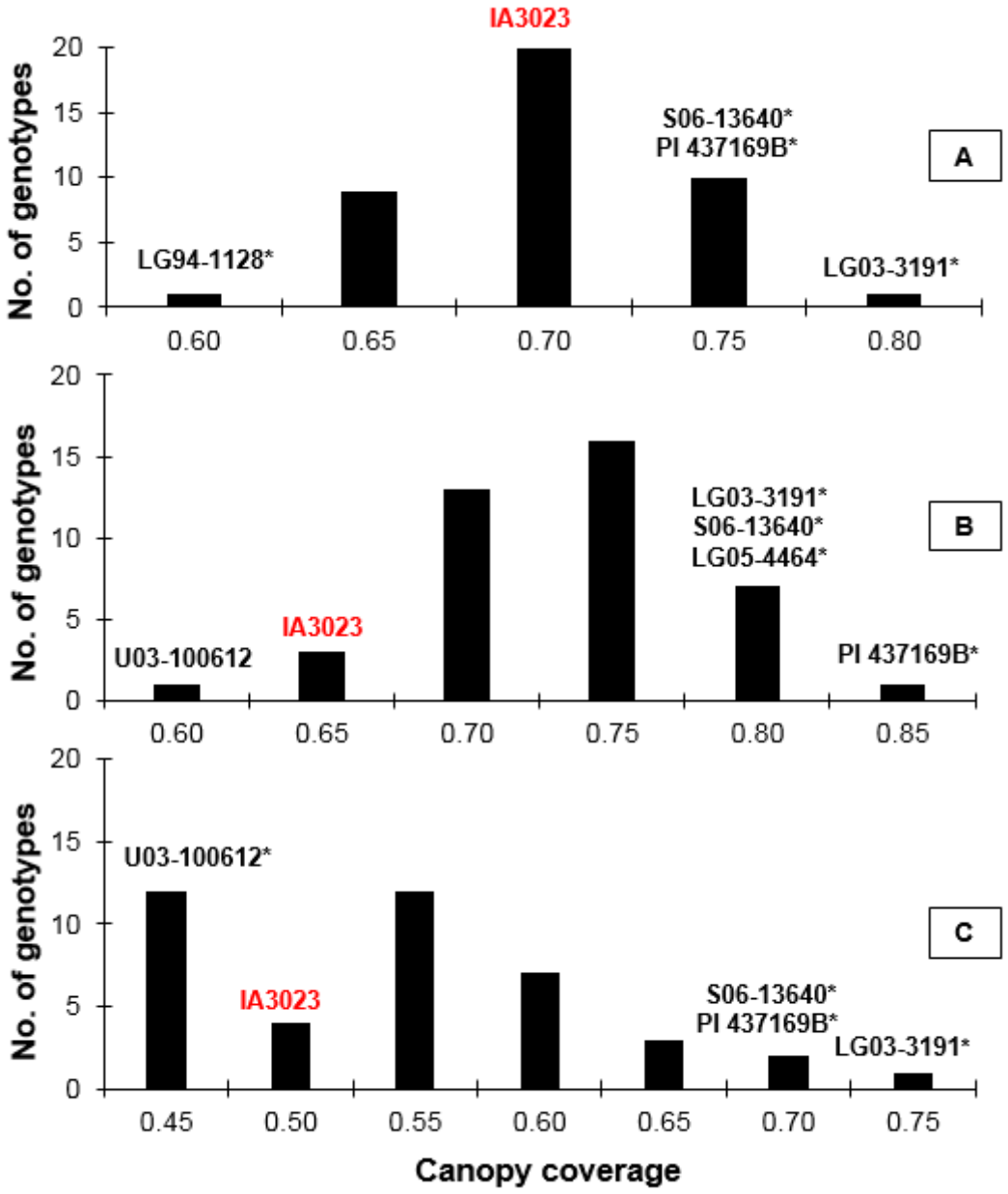

Figure 2.3 Frequency distribution graphs of SoyNAM parental genotypes for canopy coverage in years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

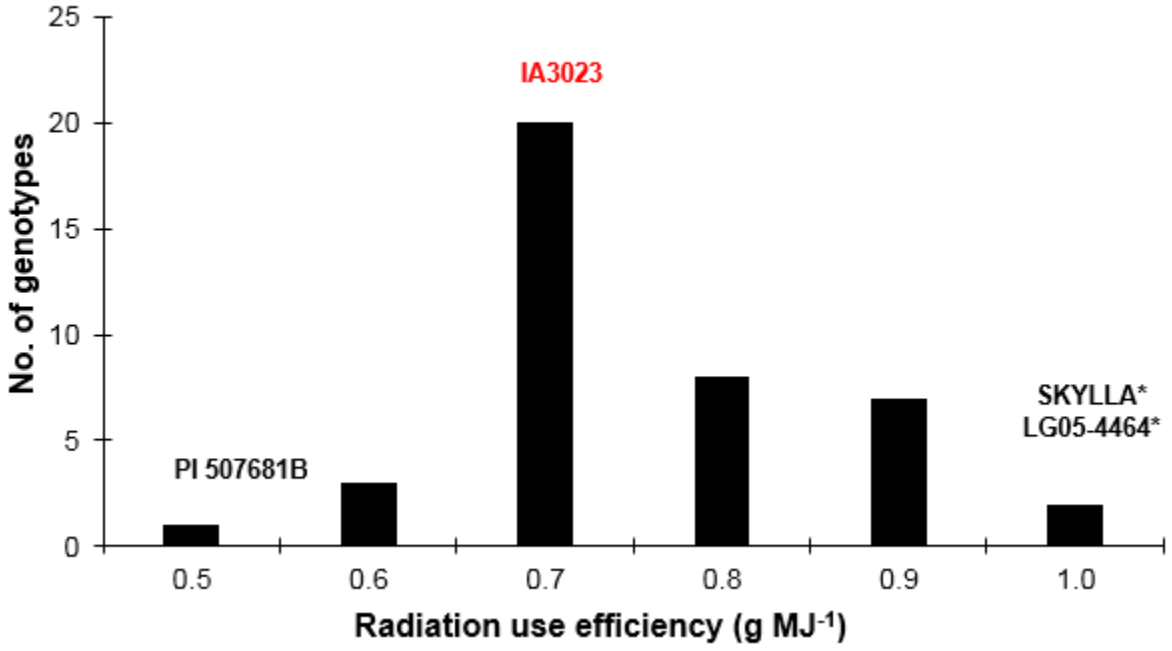

Figure 2.4 Frequency distribution graphs of SoyNAM parental genotypes for radiation use efficiency in 2016. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

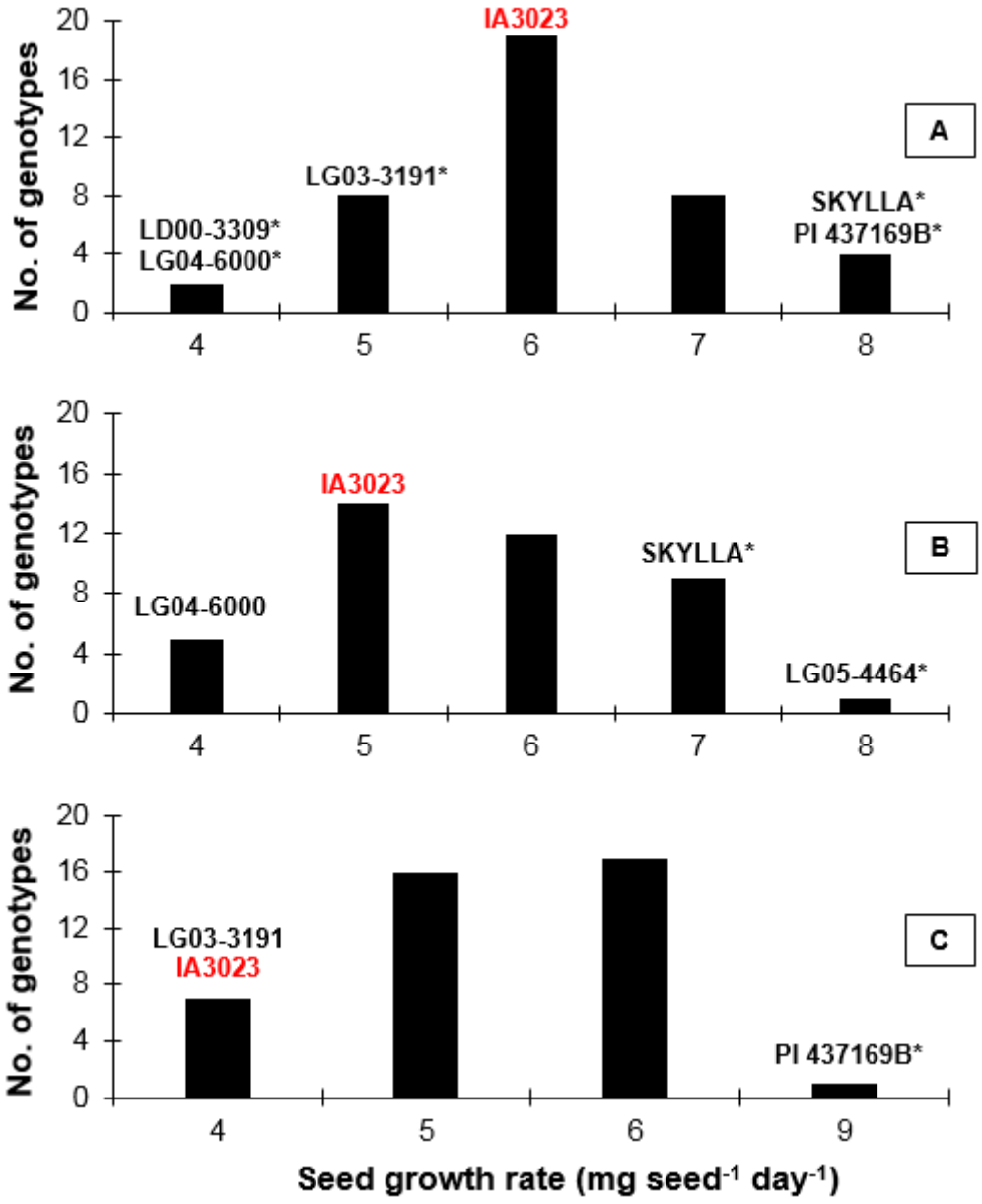

Figure 2.5 Frequency distribution graphs of SoyNAM parental genotypes for seed growth rate in years (A) 2015, (B) 2016, and (C) 2017, denoting extreme genotypes of both the ends and genotypes significantly different from the hub parent IA3023 (shown in red) represented by*.

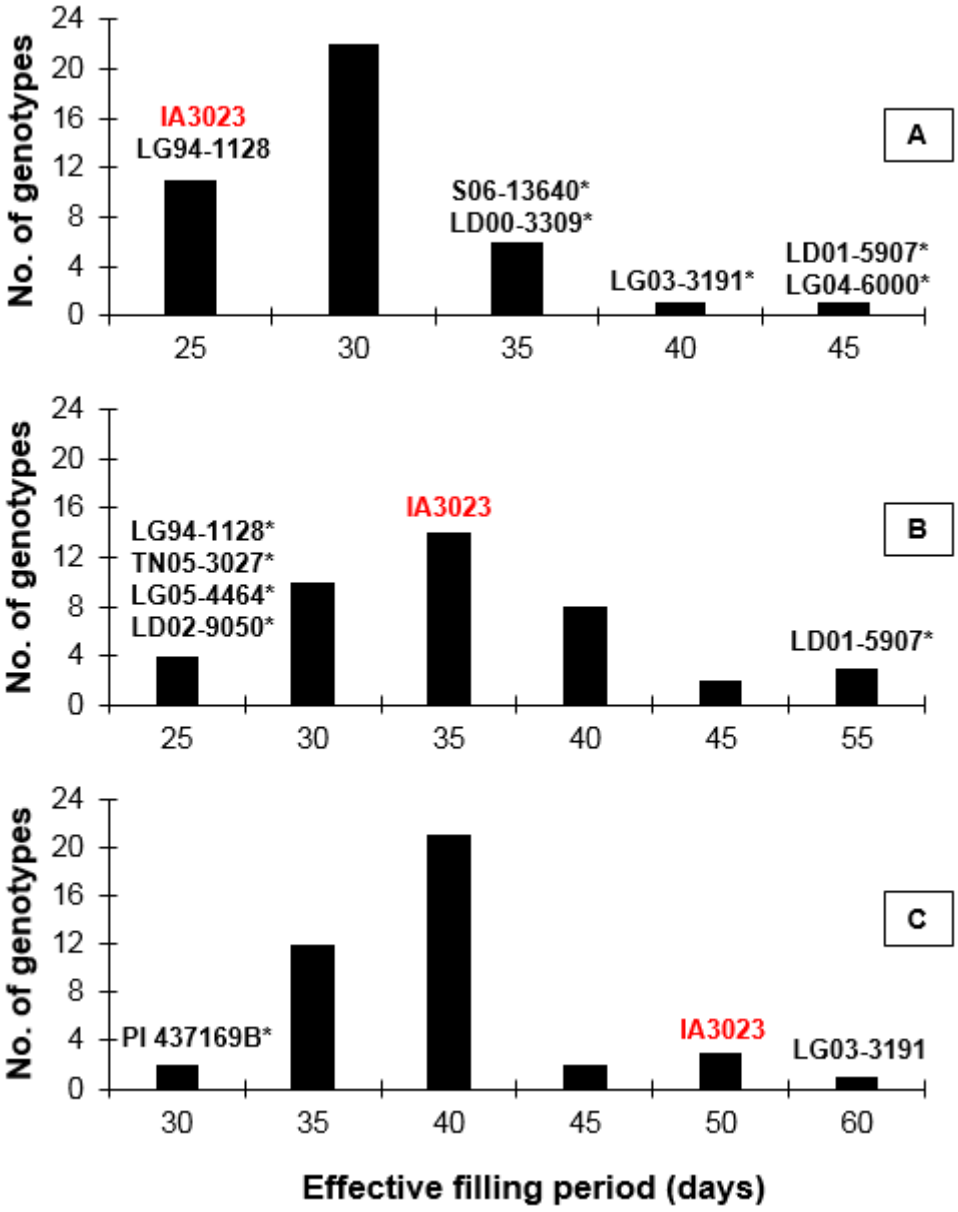

Figure 2.6 Frequency distribution graphs of SoyNAM parental genotypes for effective filling period in years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

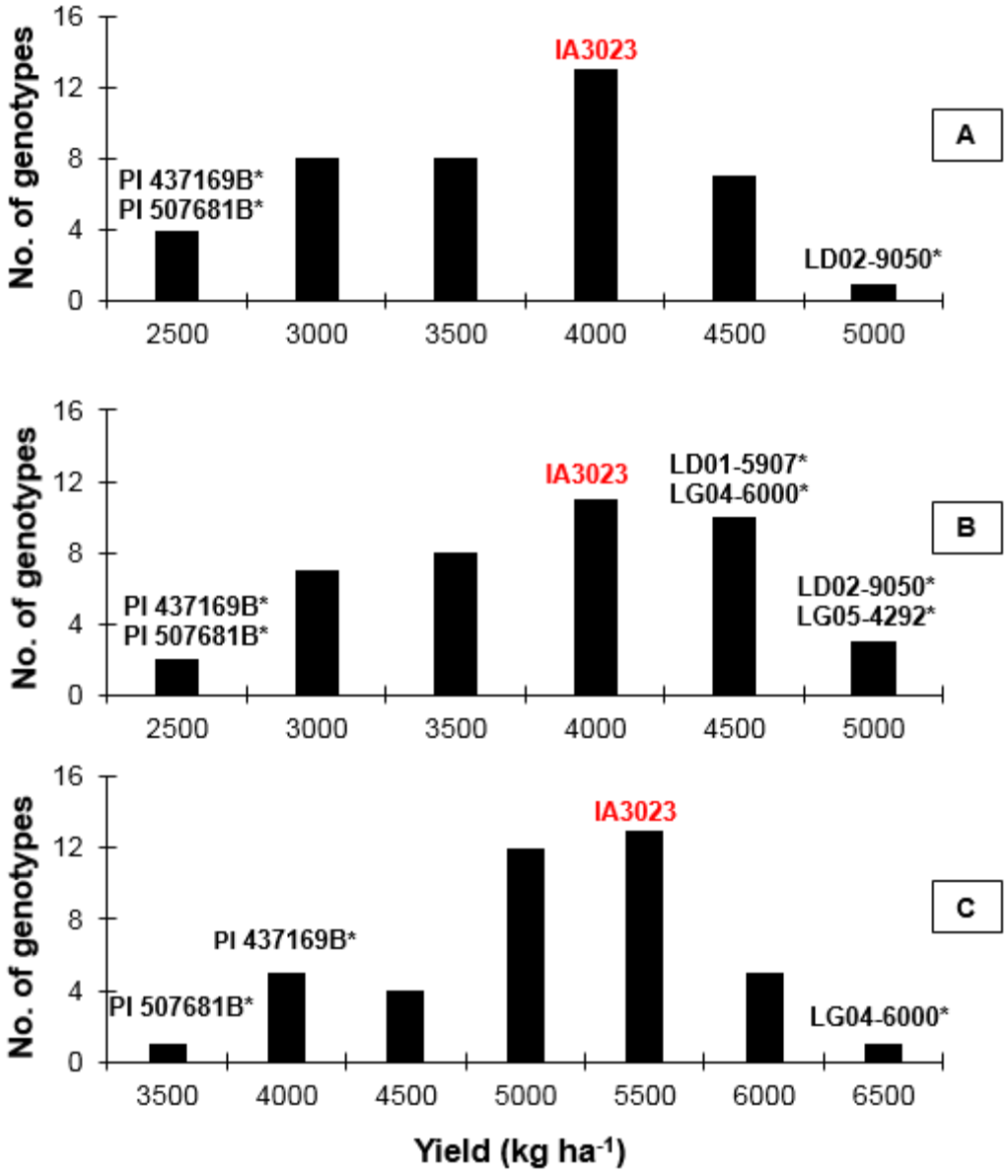

Figure 2.7 Frequency distribution graphs of SoyNAM parental genotypes for seed yield in years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

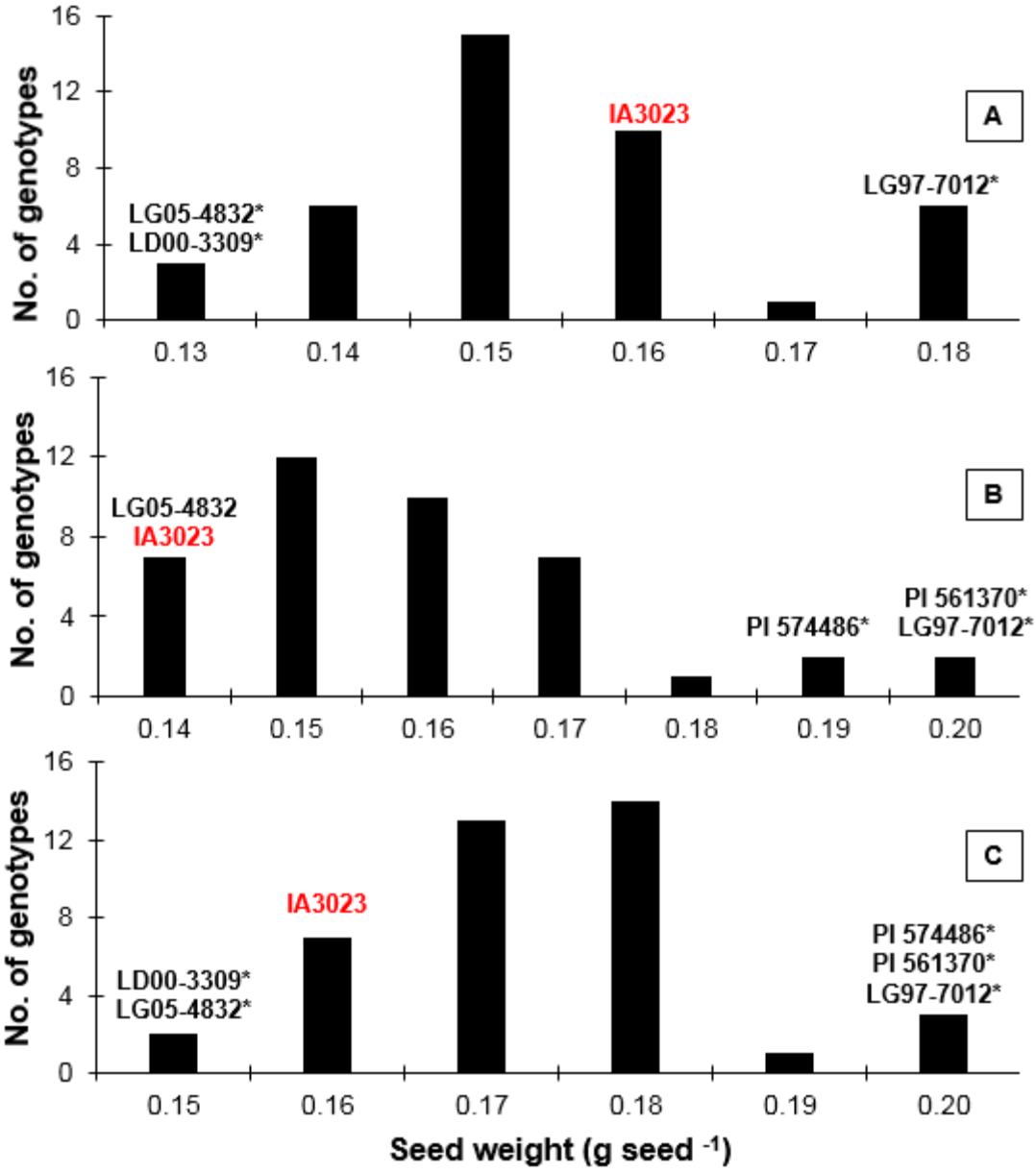

Figure 2.8 Frequency distribution graphs of SoyNAM parental genotypes for seed weight in years (A) 2015, and (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

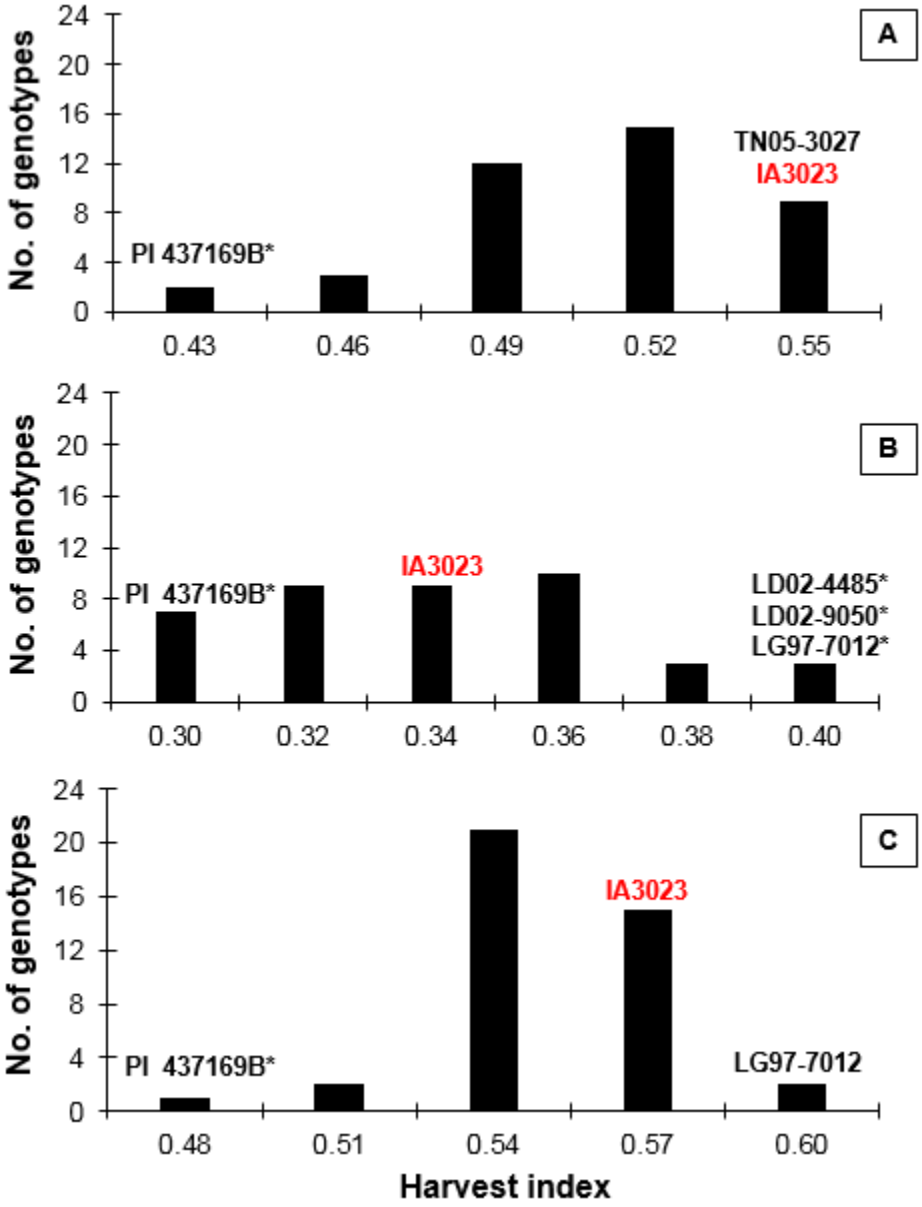

Figure 2.9 Frequency distribution graphs of SoyNAM parental genotypes for harvest index in years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

References

Belsley, D.A., E. Kuh, and R.E. Welsch. 2005. Regression diagnostics: Identifying influential data and sources of collinearity (Vol. 571). John Wiley and Sons.

Board, J.E. 1987. Yield Components Related to Seed Yield in Determinate Soybean 1. Crop Sci. 27(6):1296-1297.

Boerma, H.R. and D.A. Ashley. 1988. Canopy photosynthesis and seed-fill duration in recently developed soybean cultivars and selected plant introductions. Crop science. 28(1):137-140.

Daynard, T., J. Tanner, and W. Duncan. 1971. Duration of the grain filling period and its relation to grain yield in corn, Zea mays L. Crop Sci. 11:45-48.

De Bruin, J.L. and P. Pedersen. 2008. Soybean Seed Yield Response to Planting Date and Seeding Rate in the Upper Midwest All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Agronomy J. 100(3):696-703.

Donald, C. and J. Hamblin. 1976. The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Advances in agronomy 28:361-405.

Dunphy, E., J. Hanway, and D. Green. 1979. Soybean yields in relation to days between specific developmental stages. Agron. J. 71:917-920.

Edwards J.T., and L.C. Purcell. 2005. Soybean yield and biomass responses to increasing plant population among diverse maturity groups: I Agronomic characteristics. Crop Sci. 45(5):1770–1777.

Edward J.T., L.C. Purcell, and D.E. Karcher. 2005. Soybean yield and biomass responses to increasing plant population among diverse maturity groups. II Light interception and utilization Crop Sci. 45:1778–1785.

Egli, D.B. 1975. Rate of accumulation of dry weight in seed of soybeans and its relationship to yield. Canadian J. of Plant Sci. 55(1):215-219.

Egli, D.B. 2017. Seed biology and yield of grain crops. CABI.

Egli, D.B., and J. E. Leggett. 1973. Dry matter accumulation patterns in determinate and indeterminate soybeans 1. Crop Sci. 13(2): 220-222.

Egli, D.B., J.E. Leggett, and W.G. Duncan. 1978. Influence of N stress on leaf senescence and N redistribution in soybean. Agron. J. 70:43–47.

Egli, D B., J. Fraser, J. E. Leggett, and C. G. Poneleit. 1981. Control of seed growth in soya beans [Glycine max (L.) Merrill]. Annals of Botany. 48(2):171-176.

Egli, D.B., J. E. Leggett, and J. M. Wood. 1978. Influence of Soybean Seed Size and Position on the Rate and Duration of Filling1. Agron. J. 70(1):127-130.

Egli, D.B. 1998. Seed biology and the yield of grain crops. CAB International. Wallingford, UK.

Egli, D.B., J.H. Orf, and T.W. Pfeiffer. 1984. Genotypic Variation for Duration of Seedfill in Soybean 1. Crop Sci. 24(3):587-592. doi:10.2135/cropsci1984.0011183X002400030037x.

Egli, D.B. and I.F. Wardlaw.1980. Temperature Response of Seed Growth Characteristics of Soybeans1. Agronomy J. 72(3):560-564.

Fehr, W.R., and C. E. Caviness. 1977. Stages of soybean development. Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa.

Gay, S., D.B. Egli, and D.A. Reicosky. 1980. Physiological aspects of yield improvement in soybeans. Agron. J. 72:387–391.

Godfray, H.C., J. Beddington, J.R. Crute, I.R. Haddad, L. Lawrence, D. Muir et al. 2010. Food security: the challenge of feeding 9 billion people. Science. 327(5967):812-818.

Guldan, S. J., and W. A. Brun. 1985. Relationship of Cotyledon Cell Number and Seed Respiration to Soybean Seed Growth 1. Crop Sci. 25(5): 815-819.

Hall, B.P. 2015. Quantitative characterization of canopy coverage in the genetically diverse SoyNAM population Master's dissertation, Purdue University, West Lafayette, IN.

Hanway, J., and C. Weber. 1971. Dry matter accumulation in eight soybean (Glycine max (L.) Merrill) varieties. Agron. J. 63:227-230.

Hartwig, E.E. and C.J. Edwards. 1970. Effects of Morphological Characteristics Upon Seed Yield in Soybeans 1. Agron. J. 62(1):64-65.

Hesketh, J.D., D.L. Myhre, and C.R. Willey. 1973. Temperature Control of Time Intervals Between Vegetative and Reproductive Events in Soybeans 1. Crop Sci.13(2):250-254.

Hordo, M., A. Kiviste, A. Sims, and M. Lang. 2008 "Outliers and/or measurement errors on the permanent sample plot data." Proceedings of the sustainable forestry in theory and practice: recent advances in inventory and monitoring, statistics and modeling, information and knowledge management, and policy science. USDA For Serv Gen Tech Rep, PNW-688. Portland, OR, USA 15.

Kaler, A.S., J.D. Ray, W.T. Schapaugh, M.K. Davies, C.A. King, and L.C. Purcell. 2018. Association mapping identifies loci for canopy coverage in diverse soybean genotypes. Molecular Breeding. 38(5):50.

Kantolic, A.G., G.E. Peralta, and G.A. Slafer. 2013. Seed number responses to extended photoperiod and shading during reproductive stages in indeterminate soybean. European journal of agronomy. 51:91-100.

Koester, R.P., J.A. Skoneczka, T.R. Cary, B.W. Diers, and E.A. Ainsworth. 2014. Historical gains in soybean (Glycine max L. Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. Journal of experimental botany. 65(12):3311-3321.

Place, G.T., S.C. Reberg-Horton, D.A. Dickey, T.E. Carter. 2011a. Identifying soybean traits of interest for weed competition. Crop Sci 51:2642-2654.

Place, G.T., S.C. Reberg-Horton, T E. Carter, and A.N. Smith. 2011b. Effects of soybean seed size on weed competition. Agron. J. 103(1):175-181.

Purcell, L.C. 2000. Soybean canopy coverage and light interception measurements using digital imagery. Crop Sci. 40:834-837.

Purcell, L.C., J.T. Edwards, and K.R Brye. 2007. Soybean yield and biomass responses to cumulative transpiration: Questioning widely held beliefs. Field Crops Res. 101(1):10-18.

Pengelly, B., F. Blamey, and R. Muchow. 1999. Radiation interception and the accumulation of biomass and nitrogen by soybean and three tropical annual forage legumes. Field Crops Research 63:99-112.

Ray, D. K., N. Ramankutty, N.D. Mueller, P.C. West, and J.A. Foley. 2012. Recent patterns of crop yield growth and stagnation. Nature communications 3:1293.

Ray, D.K., N.D. Mueller, P.C. West, and J.A. Foley. 2013. Yield trends are insufficient to double global crop production by 2050. PLOS ONE 8: e66428. doi: 10.1371/journal.pone.0066428.

Rafalski, J. A. 2010. Association genetics in crop improvement. Current opinion in plant biology. 13(2):174-180.

Ries, L.L., L.C. Purcell, T.E. Carter, J.T. Edwards, and C.A. King. 2012. Physiological traits contributing to differential canopy wilting in soybean under drought. Crop science. 52(1): 272-281.

Rotundo, J.L., L. Borrás, J. De Bruin, and P. Pedersen. 2014. Soybean nitrogen uptake and utilization in Argentina and United States cultivars. Crop Sci. 54(3):1153-1165.

Salado-Navarro, L.R., T.R. Sinclair, and K. Hinson 1986a. Yield and Reproductive Growth of Simulated and Field-Grown Soybean I. Seed-Filling Duration 1. Crop science. 26(5):966-970.

Salado-Navarro, L.R., T.R. Sinclair, and K. Hinson. 1986b. Yield and Reproductive Growth of Simulated and Field-Grown Soybean. II. Dry Matter Allocation and Seed Growth Rates 1. Crop Sci. 26(5):971-975.

Salado-Navarro, L.R., T.R. Sinclair, and K. Hinson. 1993. Changes in yield and seed growth traits in soybean cultivars released in the Southern USA from 1945 to 1983. Crop Sci. 33:1204–1209.

Schapaugh, W., and J. Wilcox. 1980. Relationship between harvest indices and other plant characteristics in soybean. Crop Sci. 20:529-533.

Sinclair, T.R. and C.T. de Wit. 1975. Photosynthate and nitrogen requirements for seed production by various crops. Science. 189(4202):565-567.

Sinclair, T.R. and C.T. De Wit. 1976. Analysis of the Carbon and Nitrogen Limitations to Soybean Yield 1. Agron. J. 68(2):319-324.

Sinclair, T.R., and R.C. Muchow. 1999. Radiation use efficiency. Advances in agronomy 65:215-265.

Singer, J.W., R.W. Malone, D.W. Meek, and D. Drake. 2004. Predicting yield loss in indeterminate soybean from pod density using simulated damage studies. Agron. J. 96(2):584-589.

Smith, J.R., and R.L. Nelson. 1986. Relationship between seed-filling period and yield among soybean breeding lines. Crop Sci. 26:469-472.

Soystats. 2018. U.S. export history. http://soystats.com/wp-content/uploads/SoyStats2018.pdf

Spaeth, S.C. and T.R. Sinclair. 1985. Linear Increase in Soybean Harvest Index during Seed-Filling 1. Agronomy Journal, 77(2):207-211.

Swank, J.C., D.B. Egli, and T.W. Pfeiffer. 1987. Seed growth characteristics of soybean genotypes differing in duration of seed fill 1. Crop Sci. 27(1):85-89.

Tilman, D., C. Balzer, J. Hill, and B L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences. 108(50):20260-20264.

United Soybean board. 2016. The Soy connection for health professionals. Health and nutrition – Spring 2016 - Vol 24, No 2 Soy and Global Culture. http://www.soyconnection.com/newsletters/soy-connection/health-nutrition/soy-plays-growing-role-feeding-hungry-world (Accessed Sept. 20, 2018.)

USDA, NASS. 2017. United States Department of Agriculture, National Agricultural Statistics Service, Delta Regional Office: Arkansas. http://www.nass.usda.gov/Statistics_by_State/Arkansas /index.php (Accessed 1 June, 2018).

Van Roekel, R.J. and L.C. Purcell. 2014. Soybean biomass and nitrogen accumulation rates and radiation use efficiency in a maximum yield environment. Crop Science 54(3):1189-1196.

Van Roekel, R.J., L.C. Purcell, and M. Salmerón. 2015. Physiological and management factors contributing to soybean potential yield. Field Crops Research 182:86-97.

3. CHAPTER: Physiological Characterization of the SoyNAM Parental Lines for Drought-Related Traits (canopy wilting, canopy temperature, nitrogen concentration, nitrogen fixation rate, nitrogen derived from atmosphere, ureide concentration, and carbon isotope ratio)

Abstract

Drought is one of the most important sources of stress, limiting soybean yield. However, different soybean (*Glycine max* L. Merr.) genotypes differ in their ability to tolerate drought. There are relatively few traits that can be used as an effective measure of the drought tolerance ability of plants; some of these include: canopy temperature, canopy wilting, shoot nitrogen (N) and ureide concentrations, N_2 fixation, and carbon (C) isotope ratio. The objective of this study was to identify extreme genotypes among the Soybean Nested Association Mapping (SoyNAM) population parental lines for drought-related traits that have not been previously or extensively mapped. The experiment was conducted for three years in Fayetteville, Arkansas as a randomized complete block, with four replications that utilized all 41 SoyNAM parental genotypes and at least one non-nodulating genotype each year. Experiments were irrigated, but towards the end of each irrigation cycle, when drought symptoms started to appear, canopy wilting ratings and canopy temperature measurements were made. Once the canopy closed, during the late vegetative or early reproductive stage, shoot samples were taken and used to determine N_2 derived from the atmosphere (NDFA), shoot N and ureide concentrations, and C isotope ratio ($\delta^{13}C$; an indirect measure of water use efficiency). Several genotypes differed statistically from the hub parent. Genotype S06-13640 had relatively slow canopy wilting and was also reported to have high canopy coverage in the previous chapter. Genotype PI 398881 was a slow wilting genotype and had high canopy temperature under sufficient moisture conditions. Genotype LD01-5907 had high N_2 fixation and a long EFP (Chapter2). This information will prove useful for future physiological studies and also for selecting specific recombinant populations from the SoyNAM population for quantitative trait loci (QTL) mapping studies.

3.1 Introduction

Soybean is an important source of plant protein and oil worldwide, but just like several other crops, it is sensitive to drought. There is a significant decrease in soybean's N_2 fixation capacity even with moderate soil drying (Serraj et al., 1999a). This sensitivity of N_2 fixation to water deficit can greatly impact the yield potential of soybean (Purcell and King, 1996). Soybean is particularly sensitive to drought during the reproductive stage (Lessen, 2012). A visible moisture stress of just four days during the 3rd week of pod development can result in as much as 36% yield loss, which increases to about 39-45% in the 2nd - 4th week of seed fill.

The latest climate change scenarios depict that the 20-year extreme annual daily maximum temperature is likely to increase by about 1 to 3°C by the mid-21st century and by about 2 to 5°C by the late 21st century, depending on the region and emissions scenario (IPCC, 2012). Also, the world food and feed crop demands will double by 2050 (Foley et al., 2011). As per the historical data collection in Africa based on more than 20,000 trials (1999–2007), each "degree day" spent above 30°C caused a yield reduction of 1% for corn (*Zea mays* L. Merr) under optimal conditions; under water-deficit conditions, this loss was 1.7% (Lobell et al., 2011). However, the temperature increase is not the only impact of changing climate; disrupted rainfall pattern and distribution also have severe impacts (Feng et al., 2013). Together, increased temperature and disrupted rainfall patterns point toward a future with more frequent droughts. From an agronomic perspective, drought refers to a reduction in soil water content due to decreased rainfall or irrigation, leading to abnormal plant development and yield reduction at the field level (Passioura, 2007; Purcell and Specht, 2004). Drought stress differs from the water-deficit stress, which generally refers to the treatments inside a growth chamber or a greenhouse (Purcell and Specht, 2004).

Adequate irrigation could be a solution for alleviating drought, but it is not an option in many regions due to restrictive costs, decreasing ground-water level, or lack of available water sources (Shevah, 2015). For example, in 2012, only 28% of the total US cropping area was under irrigation (USDA, ERS, 2018). According to FAOSTAT (2011), 80% of the global agricultural area is under rain-fed cultivation but this produces 62% of the world's staple food products. All these data make water-deficit for agricultural production one of the biggest concerns. Tuberosa (2013), Tuberosa and Salvi (2006), and Waseem (2011) considered drought to be the most influential factor responsible for the reduction in crop yield worldwide.

In 2016, 33.87 million hectares in the U.S. were planted with soybean, accounting for 26% of the U.S. crop production area, and soybean's acreage has continued to grow (USDA, ERS, 2017). The United States is the world's largest soybean producer and exporter, and soybean provides 90% of the U.S. oilseed production (Soystats, 2018). Within the last 25 years, agricultural productivity of the United States was most affected by the drought in 2012 (USDA, 2013). The USDA (2013) reported that crop, livestock, as well as food retail prices, were affected by the drought in 2012. July 2012 was the hottest July for the USA since 1988; soybean yields decreased to 2663 kg/ha, a decrease of approximately 6% from 2011. This was the largest decrease since 2003 (Westcott and Jewison, 2013).

Due to world population growth and economic development, water demand by industry is significantly increasing. This will further affect the water availability for agriculture. Several studies have pointed towards an emerging water-scarcity in the mid-21st century (Boyer et al., 2013; Zipper et al., 2016). This chapter characterizes the SoyNAM parental genotypes for traits that are of importance with respect to understanding the impact of drought.

Soybean is a high protein content crop; therefore, N accumulation has been identified as an important constraint on final seed yield (Muchow and Sinclair, 1986). Nitrogen fixation provides a sustainable supply of N to the plant, eliminating N fertilization; however, N_2 fixation is sensitive to drought (King and Purcell, 2006). The natural abundance method (Shearer et al., 1980) using ^{15}N determines the N derived from atmosphere, providing a measure of the fraction of N derived from N_2 fixation. Shoot N and ureide concentrations are also informative regarding the sensitivity of N_2 fixation to drought (King and Purcell, 2006; King et al. 2014; Serraj et al., 1999). Canopy wilting is a visual assessment of drought and is a promising drought evaluation trait, with genotypes depicting large differences in their extent of wilting (King et al., 2009) and slow wilting genotypes depicting yield advantages under drought conditions (Cater et al., 2016). Carbon isotope ratio is a substitute measure of crop's water use efficiency (WUE) and is a potential method for evaluating a large number of genotypes under field conditions (Bai and Purcell, 2018b; Condon et al., 1990). Similarly, canopy temperature - a quantitative measurement of drought, is closely associated with stomatal conductance (Yousfi et al., 2016), and canopy temperature depression has been found positively associated with yield (Amani et al., 1996; Yousfi et al., 2016).

Thus, the objectives of this chapter were:

1. To characterize and evaluate the SoyNAM parental genotypes for drought-associated traits: wilting, canopy temperature, N concentration, N fixation rate, N derived from atmosphere, ureide concentration, and C isotopic ratio
2. To identify the most extreme genotypes among the 41 SoyNAM genotypes for each of the above traits.

3.2 Materials and Methods

3.2.1 Genotypes Evaluated

Forty-one genotypes that are parental genotypes of the SoyNAM project were obtained from the University of Nebraska and planted at the University of Arkansas (UA) System Division of Agriculture's Agricultural, Research and Extension Center, Fayetteville, Arkansas (36º05' N, 94º10' W) on a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudult) soil. In addition to the 41 SoyNAM parental genotypes (ranging from maturity group (MG) 1 through 5), non-nodulating isolines of Harosoy (MG 2, 2016 and 2017), Lee (MG 6, 2017), and Clark (MG 4, 2015 and 2016) were also included, to be able to distinctively determine the amount of N derived from soil and through atmospheric N_2 fixation. Thus, the total number of genotypes studied was 42 in 2015, and 43 in 2016 and 2017.

3.2.2 Field Design

The fall before planting soybean in the spring, cereal rye (*Secale cereale*) was planted as a cover crop. After heading, cereal rye was mown and removed from the field to decrease residual soil inorganic-N and increase the soybean's dependence on N_2 fixation. The experiment was conducted for three consecutive years during 2015, 2016 and 2017 as a randomized complete block design with four replications. Each plot consisted of four rows, 9.14 m in length with an inter-row spacing of 0.46 m. Experiments were planted on 3 June 2015, 7 June 2016, and 10 June 2017 at a density of 46 seeds m^{-2}. Weather data were obtained for a weather station "UA Turf Science KARFAYET50" from Weather Underground website (The Weather Company, Brookhaven, GA, https://www.wunderground.com/). An overhead sprinkler irrigation system was installed and the experiment was irrigated when the estimated soil-water deficit reached 30 mm (Purcell et al, 2007). However, approximately three times each year, the soil was allowed to dry for an additional one or two days to provide an opportunity to rate wilting and collect canopy

temperature data. Phenological development of all genotypes was accessed using the staging method of Fehr and Caviness (1977).

3.2.3 Nitrogen Concentration, Nitrogen Fixation Rate, Ureide Concentration, Nitrogen Derived from Atmosphere (NDFA), and Carbon Isotope Ratio ($\delta^{13}C$)

In 2016 two biomass samples were taken two weeks apart from $1m^2$ of each plot once the canopy closed completely. In 2015 and 2017 a three-plant sample was taken once at the R1 growth stage, but no biomass samples were taken due to non-uniform stands.

After harvesting the above ground biomass, samples were dried at 60°C until the weight was constant and ground using a Wiley Mill (Thomas Model 4 Wiley® Mill, Thomas Scientific, NJ, USA) to pass through a 6 mm screen. About 0.5 g of thoroughly mixed, course-ground material was ground to a fine powder using a SPEX Sample Pre Geno grinder (SPEX CentriPrep, Inc., NJ, USA) in a 15 ml centrifuge tube–conical bottom (part# 2252-PC-30; SPEX CentriPrep, Inc., NJ, USA) containing two 9.52 mm diameter stainless ball (440C Stainless Steel Ball, Tolerance/ Grade: 100, Abbott Ball Company, Inc., CT, USA), and shaken at 1500 rpm for 10 min. The geno-ground material (100 to 125 mg) was analyzed for total N concentration using the Dumas method (Bremner, 1965) with a Leco FP-428 Determinator (Leco Corporation, St. Joseph, MO), at the University of Arkansas Diagnostic Laboratory, University of Arkansas, Fayetteville, AR. A similar sub-sample (115-135 mg) was used to determine ureide concentration using colorimetric procedure by Young and Conway (1942). For N (^{15}N: ^{14}N) and C (^{13}C: ^{12}C) isotope analysis, 3 to 5 mg of finely-ground samples were packed in tin capsules, arranged in 96-well plates (Costech Analytical Technologies Inc., CA, USA), and sent for isotope analysis at the UC Davis Stable Isotope Facility (http://stableisotopefacilty.ucdavis.edu/13cand15n.html). The isotope analysis was performed using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer

(IRMS), and the final values of $\delta^{13}C$ isotope were expressed relative to the international standard V-PDB (Vienna PeeDEE Belemnite). Further information about the isotope analysis can be found at the U.C. Davis Isotope Laboratory website (https://stableisotopefacilty.ucdavis.edu).

The non-nodulating genotypes obtain all of their N from the soil, while nodulating genotypes obtain N from the soil as well as fix atmospheric N_2. The soil is relatively rich in ^{15}N compared to the atmosphere; hence, non-nodulating genotypes have a higher ^{15}N:^{14}N ratio than nodulating genotypes. There is a dilution of ^{15}N in the plant tissue when N_2 fixation occurs. Since nodulating genotypes fix atmospheric N_2 and non-nodulating genotypes do not, there is a difference in the N isotopic ratios of the two plant types. Non-nodulating genotypes were therefore included in this experiment to account for N obtained from the soil; this made it possible to determine the amount of N derived from the atmosphere. This method was introduced by Shearer et al. (1980) to evaluate differences among cultivars for N_2 fixation based on the natural abundance of the ^{15}N isotope relative to ^{14}N ($\delta^{15}N$). Nitrogen derived from the atmosphere (NDFA) is expressed as follows (Kohl and Shearer, 1981):

$$NDFA = ((\delta^{15}N_{ref} - \delta^{15}N_{samp}) / (\delta^{15}N_{ref} - \delta^{15}N_0)) \times 100 \quad [3.1]$$

Here, $\delta^{15}N_{ref}$ refers to the composition of a plant completely dependent on soil N, $\delta^{15}N_{samp}$ signifies the composition of the individual samples, and $\delta^{15}N_0$ refers to the composition of a sample, totally dependent on N_2 fixation. $\delta^{15}N_{ref}$ is determined by measuring the $\delta^{15}N$ of a non-nodulating reference crop, and $\delta^{15}N_0$ is a constant (-1.30) as determined for soybean by Bergersen et al. (1989).

In 2016, N_2 fixation rate was also calculated from the biomass (BM) samples. To do this, N content (gN m^{-2}) was determined as a product of N concentration and biomass weight of each

sample (g m^{-2}). The difference in the N content of the samples between two harvest dates, divided by the number of days between the harvests, provided the N accumulation rate (NAR, g N m^{-2} day^{-1}).

$$\text{NAR } (gNm^{-2}day^{-1}) = \left(\frac{(\text{BM weight of H2} \times \text{N ratio of H2}) - (\text{BM weight of H1} \times \text{N-ratio of H1})}{\text{days between harvest}}\right) \quad [3.2]$$

Nitrogen accumulation rate of non-nodulating genotypes was also calculated based on Eq. 3.2. The difference in the NAR for each nodulating genotype and the average of the NAR of non-nodulating genotypes provided a measure of N_2 fixation rate (Eq. 3.3).

$$\text{NFR } (gNm^{-2}day^{-1}) = \big((\text{NAR of nodulating genotypes}) - (\text{Average NAR of non} - \text{nodulating genotypes})\big) \quad [3.3]$$

3.2.4 Canopy Temperature

Aerial thermal infrared image analysis was performed to evaluate the canopy temperature of genotypes. Images were taken once the canopy was closed, towards the end of each irrigation cycle when there were visible drought symptoms. In 2016 and 2017, an infrared camera (FLIR Tau 640, Goleta, CA) was mounted on a DJI Phantom 4 Pro (https://www.dji.com/, Dà-Jiāng Innovations, Shenzhen, China) and flown at a height of 123 m. The camera detects wavelengths from 8 to 14 µm with a 640 × 512 resolution, and a pixel size of 17 microns. The lens used was 13 mm and 25 mm in 2016 and 2017, respectively. The camera is lightweight (110 g) and records temperature for each pixel on a relative scale from 0 to 255, as it distinguishes 256 shades of gray from white to black in the images. Sequential differences in the shades of gray differ by approximately 0.05°C, and the camera's temperature range is 12.8°C (256 × 0.05°C). The video stream from the camera was captured on a digital video recorder and individual picture frames were extracted from the video for analysis. The captured images were opened in GNU Image Manipulation Program (GIMP, https://www.gimp.org), lens distortion was removed,

images were rotated to align the plots horizontally on the screen, and the field area was squared using the perspective tool. The modified images were analyzed using "Field Analyzer" software (https://www.turfanalyzer.com/field_analyzer.html), which derived an average value of pixels within each plot as a measure of relative canopy temperature (Bai and Purcell, 2018a). The differences in the relative temperature values of the captured images was used to distinguish genotypes with relatively cooler and warmer canopies (Figure 3.1).

In 2015, a tethered balloon, 2 m-diameter flown approximately 75 m above the canopy was used as an aerial platform with the same camera system described previously (Bai and Purcell, 2018a) instead of a drone. The irregular flight achieved through a balloon did not give uniformity in the video stream obtained, as the camera angle changed very often. Hence, there were variations (gradient) both within a single image as well as across images. Therefore, the data obtained were normalized as described by Jones et al. (2009). First, the average of relative canopy temperature values of all the plots within a single image were determined. This was done for each image. Then, a correction for the gradient within an image was made, for this, the individual value of each plot was subtracted from the average value of that image. Corrections for each image were then averaged to obtain individual relative canopy temperature values for each plot for a particular date.

3.2.5 Canopy Wilting

Wilting ratings were made just before each irrigation whenever there were sufficient soil moisture deficit and visible drought symptoms, between 1200 and 1500 h. These measurements were visual ratings on a scale from 0 to 100, 0 representing no wilting; 20 representing slight wilting seen as leaf wilting and rolling in the top of the canopy; 40 representing moderate leaf rolling in the top of the canopy, relatively more wilted leaves throughout the canopy, and slight

loss of petiole turgidity; 60 representing intense wilting of leaves throughout the canopy, with enhanced loss of petiole turgidity; 80 representing plants with petioles extensively wilted and dead leaves throughout much of the canopy; and 100 representing plant death (King et al. 2009). There were two rating dates, August 3 and August 14 in 2015 (55 and 66 DAE), two rating dates, August 2 and August 11 in 2016 (52 and 60 DAE), and four rating dates from July 20 to August 25 in 2017 (34, 40, 49, and 70 DAE).

3.2.6 Statistical Analysis

Outliers were detected using studentized residuals as described in Chapter 2, and data points with a studentized residual >=3 were evaluated closely and removed if determined to be unusual.

Distribution of all the response variables was checked. The Shapiro-Wilk test was found significant for ureide concentration and nitrogen fixation rate, and hence their actual distribution was assessed. All other variables were normally distributed. The ureide concentration was identified to have a gamma distribution as it had all positive values above 0, and nitrogen fixation rate had a beta distribution with values between 0 and 1.

Data were then analyzed, using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc. Cary, NC). Variables other than canopy wilting and canopy temperature were analyzed with analysis of variance. The model consisted of year, maturity group, genotypes nested within maturity groups, and year by genotype interaction as the fixed effects, and replications and replications nested within a year as random effects.

For canopy wilting and canopy temperature, the data were analyzed by year using analysis of covariance (ANCOVA) instead of analysis of variance (ANOVA), with days after growth stage R1 being the covariate. The physiological maturity of the plant can confound

canopy temperature and the visual extent of wilting. Hwang et al. (2015) found that the genotypes that were at an advanced physiological stage showed relatively more severe wilting than the ones in the early physiological stage. Thus, the ANCOVA made statistical adjustments based on the differences in crop development at the time of measurement.

3.3 Results

The ANOVA for N concentration indicated that the main effect of year was significant but the interaction year by genotype (MG) was not significant. There were differences among genotypes for the trait, and MG did not have a significant effect (Table 3.1). Dhanapal et al. (2015a) also reported significant differences among genotypes based on N concentration. Averaged over years, N concentration among genotypes ranged from 2.94 to 3.62 g N $100g^{-1}$ (Figure 3.2, Table 3.4). The range was very similar each year; in 2015 the N concentration varied from 2.82 to 3.60 g N $100g^{-1}$, in 2016 it varied from 2.60 to 3.60 g N $100g^{-1}$, and in 2017 it varied from 3.04 to 3.70 g N $100g^{-1}$ (Table 3.4). This is similar to the range of N concentration observed in other reports (Dhanapal et al., 2015a; Hwang et al., 2013; King and Purcell, 2006). King and Purcell (2006) reported a range of 2.5 to 3.3 g N 100 g^{-1} for 15 soybean genotypes under well-watered conditions. Dhanapal et al. (2015a) reported a range of 1.51 to 3.65 g N $100g^{-1}$ for their evaluations on 373 diverse soybean genotypes for two years and at two locations, and Hwang et al. (2013) reported a range of 1.97 to 3.46 g N 100 g^{-1} for 97 soybean recombinant inbred lines (RILs) evaluated across 4 years. Genotype LG90-2550 had the highest N concentration (Figure 3.2), and differed significantly from the hub parent. Genotype S06-13640, LG94-1128, and SKYLLA had the lowest N concentrations, and each of them differed significantly from the hub parent (Figure 3.2, Table 3.4).

The ANOVA for ureide concentration indicated that both the main effect of year as well as the interaction of year and genotype was significant. Genotypes differed significantly among

themselves, and the effect of MG was significant (Table 3.1). There was great variation in the range of ureide concentration each year. In 2015, ureide concentration ranged from 44.84 to 94.68 µM g^{-1}, from 21.96 to 44.01 µM g^{-1} in 2016, and from 13.14 to 28.46 µM g^{-1} in 2017 (Figure 3.3, Table 3.4). Ureide concentration of the hub parent varied from 19.41 to 55.53 µM g^{-1} (Table 3.4). There is no specific explanation for the different range of ureide concentration in 2015 than in 2016 and 2017. However, previous studies that involved multiple years of experimentation have also reported large ranges for ureide concentration, and the range of the current study lies within the earlier reported ranges. Ray et al. (2015) reported average shoot ureide concentration ranging from 12.4 to 33.1 µM g^{-1} across four environments for 374 MG 4 soybean accessions. Hwang et al. (2013) reported a range from 9.8 µM g^{-1} to 64.0 µM g^{-1} for a set of 96 RILs, for a 4-year study.

King and Purcell (2001) found that drought tolerance for N_2 fixation was associated with low ureide concentration, but no genotype among the SoyNAM parental genotypes had a consistently low ureide concentration for all three years. Genotype S06-13640 had the lowest ureide concentration in 2015, PI 437169B in 2016, and Skylla in 2017 (Figure 3.3); each of these genotypes differed significantly from the hub parent. Genotype LG92-1255 had high ureide concentration consistently all three years, differing significantly from the hub parent (Figure 3.3). PI 437169B and SKYLLA are MG 2 genotypes, whereas, S06-13640 is an MG 4 genotype. Although the effect of MG was significant in the ANOVA (Table 3.1), genotypes ranging from MG 2 to MG 4 had low ureide concentration. There was not a consistent pattern between ureide concentration and MG.

For NDFA, the year, as well as the interaction of year with genotype were significant (Table 3.1), but MG was not a significant factor. There were large differences in the NDFA

values between years. In 2016, there was a relatively narrow NDFA range of 64.7 to 84.5%, while in 2017, NDFA ranged from 39.1 to 75.1% (Figure 3.3, Table 3.4). There is no clear explanation for the greater NDFA range in 2017; however, 2017 was an overall wet year. The NDFA value of the hub parent was 72.2 % in 2016 and 52.2 % in 2017 (Table 3.4). Dhanapal et al. (2015a) also identified differences among genotypes for NDFA and reported a range from 2.16 to 90.75% for the NDFA evaluated on 373 diverse soybean genotypes.

Genotype LD01-5907 consistently differed significantly from the hub parent for NDFA and had a high NDFA for both 2016 and 2017 (Figure 3.4). In 2017, other than LD01-5907, genotypes LG03-3191, LD02-9050, LG05-4292, and LD02-4485 also had high NDFA and were significantly different from the hub parent (Figure 3.4, Table 3.4). No genotype differed significantly from the hub parent at the lower end; however, genotypes LD02-4485 and SKYLLA had the lowest NDFA values in 2016 and 2017, respectively (Figure 3.4, Table 3.4). While NDFA provides a snapshot of the fraction of N derived from N_2 fixation, it does provide information regarding the quantity of N from N_2 fixation (g N m^{-2}). That is, genotypes may differ greatly in NDFA but could fix similar quantities of N_2 provided that differences in biomass (g BM m^{-2}) compensated for the difference in NDFA.

The ANCOVA of wilting data indicated that the covariate days after R1 (DAR1) was non-significant for all the years, and the genotypes differed significantly amongst themselves each year (Table 3.2). There was also a significant effect of MG in 2017 (Table 3.2). The wilting ratings varied from 25 to 35 (date-wise average) in 2015, 13 to 29 in 2016, and 20 to 32 in 2017 (Table 3.4). The average wilting rating of the hub parent across dates varied from 32 in 2015, to 22 in 2016, and 26 in 2017 (Table 3.4). The range of the wilting scores as reported by Charlson

et al. (2009) were from 19 to 68 for 79 F_5-derived soybean lines. The range of the current study falls within the range reported by Charlson et al. (2009).

Genotype S06-13640 was identified as a slow wilting genotype differing significantly from the hub parent all the 3 years (Figure 3.5). Genotype PI 398881 also had slow wilting in 2016 and 2017 and differed significantly from the hub parent for both years (Figure 3.5, Table 3.4). Genotypes on the other end were also identified; genotypes LG05-4292 and LG04-4717 had the highest wilting in 2015 and 2017, respectively, but did not differ significantly from the hub parent (Figure 3.5, Table 3.4). Genotype PI 437169B had high wilting in 2016 and differed significantly from the hub parent (Figure 3.5, Table 3.4).

The ANCOVA for canopy temperature data indicated that the covariate DAR1 (days after R1) was non-significant for the 3 years, and the genotypes differed significantly amongst themselves every year (Table 3.2). In 2015 and 2016 there was a significant effect of MG, but 2017 showed no significant effect of MG (Table 3.2). The relative canopy temperature ranged from -17.1 to 14.1 in 2015, 111.7 to 169.7 in 2016, and 55.5 to 68.8 in 2017 (Figure 3.6, Table 3.4). Higher canopy temperatures were observed during 2016 (Figure 3.6) when all the measurement dates showed water deficit of 30 mm and above (Table 3.5), while lower canopy temperatures were observed during 2015 (non- normalized data not shown) and 2017 (Figure 3.6) when measurement dates had minimal stress (Table 3.5). The canopy temperature values of the hub parent were -1.6 in 2015, 136.8 in 2016, and 60.8 in 2017 (Figure 3.6, Table 3.4). The relative canopy temperature values reported by Bai and Purcell (2018a) varied from 34 to 128 under fully irrigated conditions, and increased to 148 for the water deficit treatment, for their evaluations on five fast and five slow wilting genotypes. The current study was performed

completely under irrigated conditions and canopy temperature data were collected just prior to each irrigation event.

In 2015 and 2017, all measurement dates for canopy temperature occurred when the soil-moisture deficit was well below the threshold for irrigation, 30 mm (Table 3.5). For measurements in 2015 and 2017, genotypes LG90-2550 and PI398881 differed significantly and had higher canopy temperature than the hub parent (Figure 3.6, Table 3.4). Genotype 4J105-3-4 had a low canopy temperature and differed significantly from the hub parent in 2015 (Figure 3.6, Table 3.4). In 2017, no genotype had canopy temperature significantly lower than the hub parent, but genotype LG05-4292 had the lowest numerical value of canopy temperature (Figure 3.6, Table 3.4).

In 2016, the year when there was water-deficit for both the measurement dates (Table 3.5), PI 437169B had the highest canopy temperature and differed significantly from the hub parent, and TN05-3027 numerically had the lowest canopy temperature, but did not differ significantly from the hub parent (Figure 3.6, Table 3.4).

The ANOVA results of $\delta^{13}C$ indicated that year was non-significant, but genotype, MG, and the interaction of year and genotype was significant (Table 3.1). The $\delta^{13}C$ values varied from -28.7 to -29.5 in 2016 and from -28.8 to -29.5 in 2017 (Figure 3.7, Table 3.4). The $\delta^{13}C$ of the hub parent was similar for the 2 years, being -29.0 in 2016 and -29.2 in 2017. Dhanapal et al. (2015b) reported that the $\delta^{13}C$ values varied from -27.7 to -30.5 across two locations during 2 years of study for a 373 GWAS panel. The range of the current study lies within this range and is narrower as the current study evaluated a lesser number (41) of genotypes and was conducted only at a single location.

The $\delta^{13}C$ of the genotypes did not differ significantly from the hub parent at the higher end; however, genotypes LG94-1128 and LG92-1255 had the highest numerical $\delta^{13}C$ values in 2016 and 2017, respectively (Figure 3.7, Table 3.4). Genotype LG04-6000 had the lowest $\delta^{13}C$ in 2016 and differed significantly from the hub parent. Genotypes PI 518751, LG05-4832, and LG05-4292 had the lowest $\delta^{13}C$ in 2017 differing significantly from the hub parent (Figure 3.7, Table 3.4).

Nitrogen fixation rate was measured only for 1 year, and ANOVA indicated that the genotypes did not differ significantly among themselves (Table 3.1). The range of N_2 fixation rate in 2016 was from 0.17 to 0.51 g N m^{-2} d^{-1} (Figure 3.8, Table 3.4). The N_2 fixation rate of the hub parent was 0.33 g N m^{-2} d^{-1} (Table 3.4). Genotype SKYLLA numerically had the highest N_2 fixation rate for 2016 but did not differ significantly from the hub parent; similarly, LG94-1906 had the lowest N_2 fixation rate but did not differ significantly from the hub parent (Figure 3.8, Table 3.4).

There were several important significant ($P \leq 0.05$) correlations observed in the current study that merit attention. Nitrogen concentration and ureide concentration had a positive correlation in 2015 (r = 0.61) and 2017 (r = 0.44) (Table A.6 and A.8). Canopy temperature and wilting were negatively correlated in 2017 (r = -0.50, Table A.8). In 2016, N concentration and N_2 fixation rate were positively correlated (r = 0.42, Table A.7). Carbon isotope ratio and ureide concentration showed a positive correlation in 2017 (r = 0.38, Table A.8). Ureide concentration was positively correlated with yield in 2017 (r = 0.36, Table A.8). Nitrogen derived from the atmosphere was negatively correlated with yield in 2016 (r = -0.30, Table A.7), and positively correlated with canopy coverage in 2017 (r = 0.54, Table A.8). In 2017, $\delta^{13}C$ was positively correlated with canopy coverage (r = 0.39, Table A.8), yield (r = 0.31, Table A.8), and ureide

concentration (r = 0.38, Table A.8). Genotypes that differed significantly from the hub parent and possessed multiple drought and yield-related traits have been summarized in Table 3.3.

3.4 Discussion

Canopy temperature and wilting were significantly negatively correlated in 2017 (r = -0.50, $P \leq 0.01$, Table A.8), i.e. the genotypes with high canopy temperature under sufficient moisture conditions had low wilting scores under drought. In 2017, all the measurement dates for canopy temperature had sufficient soil moisture (Table 3.5), while all the wilting measurements were made under moisture deficit (Table 3.6). In agreement with the above correlation, genotype PI 398881 had high canopy temperature and low wilting in 2017 (Figure 3.6 and 3.5). King et al. (2009) hypothesized that there could be two reasons why some soybean genotypes are slow-wilting: 1) they either extract more water from the soil due to deeper rooting, or 2) they conserve more water relative to fast-wilting genotypes before the onset of severe drought. Ries et al. (2012) also hypothesized that soybean genotypes having a slow wilting characteristic conserve soil moisture by restricting transpiration, resulting in decreased RUE and/or improved water use efficiency (WUE). The current study is consistent with this hypothesis, as genotype PI 398881 had high canopy temperature under sufficient soil moisture conditions, and wilted slowly under drought. These responses indicate that the genotype conserved water by restricting transpiration when soil moisture was plentiful, resulting in high canopy temperature and then utilized that conserved soil moisture under drought, resulting in slow wilting. However, we did not observe a relationship of canopy temperature with carbon isotope ratio or RUE, or a relationship of wilting with carbon isotope ratio or RUE. Other reports have evaluated canopy temperature under drought condition and not under sufficient moisture availability, and they have found a positive correlation between canopy temperature and wilting (Bai and Purcell, 2018a; Kaler et al., 2018).

Yield and canopy temperature had a significant ($P \leq 0.01$) negative correlation in 2015 (r = -0.72, Table A.6), 2016 (r = -0.70, Table A.7), and 2017 (r = -0.42, Table A.8). Bai and Purcell (2018a) also reported a negative correlation between canopy temperature and yield for eight out of 12 measurement dates that included both full irrigation and water-deficit treatments. As mentioned earlier in the Materials and Methods section, the current experiment was performed under fully irrigated conditions, and canopy temperature measurements were taken during the end of each irrigation cycle when drought symptoms began to appear. For years 2015 and 2017, the measurement dates of canopy temperature had no soil water deficit which would be similar to the fully irrigated condition of Bai and Purcell (2018a), whereas for 2016 there were soil water-deficits on measurement dates similar to the water-deficit treatment of Bai and Purcell (2018a). One interpretation of these responses is that cool temperatures represent high rates of transpiration, and presumably photosynthesis, which would result in high yields.

Canopy temperature and seed weight had a significant ($P \leq 0.05$) positive correlation in 2015 (r = 0.39, Table A.6) and 2016 (r = 0.39, Table A.7). Canopy temperature also had a significant ($P \leq 0.05$) negative correlation with HI in 2015 (r = -0.45, Table A.6) and 2016 (r = -0.34, Table A.7). Genotype PI 437169B had significantly higher canopy temperature in 2016, and significantly lower HI than the hub parent (Figure 3.6 and 2.9). Generally, higher seed number (Egli, 1993, 1997; Sharma et al., 1996) and a low canopy temperature (Bai and Purcell, 2018a) are associated with high yield.

Shoot N concentration and ureide concentration were significantly ($P \leq 0.01$) and positively correlated in 2015 (r = 0.61, Table A.6) and 2017 (r = 0.44, Table A.8), indicating that genotypes with low shoot N concentration also had low ureide concentration, both of which have been associated with drought-tolerant N_2 fixation (King and Purcell, 2006; Purcell et al., 1998,

2000). Similarly, genotype SKYLLA and S06-13640 possessed significantly lower N concentration and lower ureide concentration than the hub parent (Figure 3.2 and 3.3). King and Purcell (2006) reported a positive relationship between N concentration and ureide concentration ($R^2 = 0.59$). Hwang et al. (2013) also found a strong positive correlation between ureide concentration and N concentration for both irrigated as well as drought treatments.

In 2016 (the only year when N_2 fixation rate data could be collected), N concentration and N_2 fixation rate were positively correlated, ($r = 0.42$, $P \leq 0.01$, Table A.7). However, genotype SKYLLA that had significantly lower N concentration than the hub parent was at the upper end of N_2 fixation rate in 2016, though not significantly different from the hub parent (Figure 3.2 and 3.8). Dhanapal et al. (2015a) found a negative correlation between NDFA and N concentration for three out of the four environments involved in their study. The current study identified no significant correlation between NDFA and N concentration, or NDFA and N_2 fixation rate, but did find a positive relationship between N concentration and N_2 fixation rate.

3.5 Conclusions

Increasing annual temperatures around the world and sensitivity of soybean yield to drought (Heatherly and Elmore, 2004) are important limitations with respect to soybean yield that need to be addressed. Irrigation should not be considered a complete solution as currently only 28% of the US cropland is under irrigation (USDA – ERS, 2018), and 59% of the U.S. irrigation is through underground water sources (FAO, 2018). Increasing demand for water due to urbanization, industrialization, and depleting groundwater resources will make further expansion of irrigated crop area a rare possibility. Breeding for increased drought tolerance could be of great importance in the current scenario when other approaches seem less reliable. Thus, this study characterized the SoyNAM parental genotypes for different drought-associated traits to evaluate the genotypes for their drought responses. Several genotypes significantly differed

from the hub parent for different physiological traits. Genotype PI 398881 was a slow wilting genotype under drought and had high canopy temperature under sufficient soil moisture conditions. Genotype S06-13640 had slow canopy wilting, early canopy coverage (as mentioned in the previous chapter), low shoot N and ureide concentrations, and long EFP. Similarly, genotype SKYLLA had high N fixation rate, low shoot ureide concentration, and high RUE (Chapter 2).

Tables and figures

Table 3.1 Analysis of variance for (nitrogen) N concentration, ureide concentration, nitrogen derived from atmosphere (NDFA), carbon (C) isotope ratio, and N_2 fixation rate evaluated on the SoyNAM parental genotypes for the effect of year, maturity group (MG), genotypes nested within maturity group and year.

Trait	Year		MG		Genotype (MG)		Year*Genotype (MG)	
	DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value
N concentration	2	0.0006	4	0.0841	36	<0.0001	72	0.4063
Ureide concentration	2	<0.0001	4	<0.0001	36	<0.0001	72	0.0356
NDFA	1	0.0008	4	0.2464	36	0.0566	36	0.0012
C isotope ratio	1	0.1926	4	0.0249	36	<.0001	36	0.0047
N_2 fixation rate	0	-	4	0.9191	36	0.6352	-	-

Table 3.2 Analysis of variance for canopy temperature and wilting evaluated on the SoyNAM parental genotypes for the effect of maturity group (MG), and genotypes nested within maturity group for the years 2015, 2016, and 2017 with days after R1 (DAR1) as a covariate.

Trait	Year	MG		Genotype (MG)		DAR1	
		DF (NUM)	p-value	DF (NUM)	p-value	DF (NUM)	p-value
Wilting	2015	4	0.2218	36	<0.0001	1	0.0614
Wilting	2016	4	0.1516	36	<0.0001	1	0.6767
Wilting	2017	4	0.0002	36	<0.0001	1	0.5949
Canopy temperature	2015	4	0.0079	36	<0.0001	1	0.7823
Canopy temperature	2016	4	<0.0001	36	<0.0001	1	0.1731
Canopy temperature	2017	4	0.2890	36	0.0039	1	0.5946

Table 3.3 A list of genotypes that differed significantly from the hub parent.

Genotype	Characteristics observed
LG90-2550	1. High N concentration all the three years (Figure 3.2). 2. High canopy temperature during 2015 and 2017 (low to no soil water deficit on measurement dates) (Figure 3.6).
LD01-5907	1. High NDFA for both the years of data collection, 2016 and 2017 (Figure 3.4). 2. Long EFP in 2015 and 2016 (Figure 2.6). 3. High yield in 2016 (Figure 2.7).
LG03-3191	1. High NDFA 2017 (Figure 3.4). 2. Longer EFP, slow SGR and high canopy coverage (Figure 2.6, 2.5, and 2.3).
LD02-9050	1. High NDFA in 2017 (Figure 3.4). 2. High seed yield and high HI (Figure 2.7 and 2.9).
LG05-4292	1. High NDFA in 2017 (Figure 3.4). 2. High yield in 2016 (Figure 2.7).
LD02-4485	1. High NDFA in 2017 (Figure 3.4). 2. High HI (Figure 2.9).
LG94-1128	1. Low shoot N concentration all three years (Figure 3.2). 2. Low canopy coverage in 2015 (Figure 2.3). 3. Short EFP in 2016 (Figure 2.6).
S06-13640	1. Slow wilting all three years (Figure 3.5). 2. High canopy coverage all three years ((Figure 2.3), and long EFP in 2015 (Figure 2.6). 3. Low N concentration all three years (Figure 3.2). 4. Low ureide concentration in 2015 (Figure 3.3)
PI398881	1. Low wilting for the years 2016 and 2017 (Figure 3.5). 2. High canopy temperature genotype in 2015 and 2017 (low to no soil water deficit on all measurement dates) (Figure 3.6).
PI 437169B	1. High wilting in 2016 (Figure 3.5). 2. High canopy temperature in 2016 (Figure 3.6). 3. High canopy coverage all the three years (Figure 2.3). 4. High SGR in 2015 and 2017 (Figure 2.5), low EFP in 2017 (Figure 2.6), low yield all the three years (Figure 2.7), low HI both the years of data collection (Figure 2.9), and low ureide concentration in 2016 (Figure 3.3).
SKYLLA	1. High RUE (Figure 2.4). 2. Low shoot ureide concentration in 2017 (Figure 3.3). 3. Low N concentration all the three years (Figure 3.2).

N, NDFA, EFP, HI, and RUE stand for nitrogen, N derived from atmosphere, effective filling period, harvest index, and radiation use efficiency respectively.

Table 3.4 Mean values of all genotypes along with least significant difference (LSD) for the respective physiological traits.

		Physiological Traits																	
		N concentration (gN 100g^{-1})				Ureide concentration (μM g^{-1})			N_2 derived from atmosphere (%)		Wilting			Canopy temperature			C isotope ratio			N_2 fixation rate (gN m^{-2} d^{-1})
		2015	2016	2017	3-year mean	2015	2016	2017	2016	2017	2015	2016	2017	2015	2016	2017	2016	2017	2-year mean	2016
Genotypes	LSD	0.25	0.24	0.23	0.23	3.25	1.48	1.54	9.78	16.45	4.51	5.95	3.25	13.11	23.42	7.38	0.32	0.36	0.33	0.29
	MG																			
U03-100612	1	3.40	3.17	3.52	3.36	73.46	41.47	24.52	79.55	56.13	32.88	25.31	25.90	-0.38	144.24	58.03	-29.43	-29.47	-29.45	0.41
LD02-4485	2	3.48	3.28	3.41	3.39	68.35	34.04	20.80	64.68	70.64	26.52	17.22	22.80	-16.16	128.40	58.56	-29.01	-29.22	-29.11	0.36
LG92-1255	2	3.49	3.20	3.66	3.45	88.23	44.01	28.47	76.04	57.24	28.13	15.22	21.48	3.06	122.90	59.04	-29.07	-28.88	-29.04	0.24
LG94-1128	2	3.00	2.63	3.31	2.98	72.13	32.29	20.34	80.76	50.72	33.02	25.25	27.20	8.03	131.66	60.31	-28.74	-29.50	-28.97	0.19
LG94-1906	2	3.26	2.91	3.44	3.21	70.09	34.38	19.47	73.87	57.24	28.27	24.00	25.85	1.02	166.79	58.98	-29.13	-29.30	-29.12	0.17
PI 404188A	2	3.25	3.18	3.52	3.32	70.23	32.30	17.24	84.50	52.14	29.32	16.81	20.59	8.49	161.01	64.33	-29.01	-29.47	-29.38	0.50
PI 437169B	2	3.19	3.17	3.24	3.20	70.80	21.96	18.15	72.75	58.44	32.26	28.95	24.67	14.10	169.69	61.25	-29.36	-29.40	-29.21	0.47
PI 507681B	2	3.50	3.14	3.19	3.28	80.00	34.16	13.77	78.98	54.40	28.67	14.72	19.79	4.67	166.31	65.08	-28.98	-29.43	-29.21	0.27
PI 518751	2	3.40	3.26	3.45	3.37	67.30	37.98	18.09	74.58	59.24	33.21	22.65	21.37	7.83	168.00	64.71	-29.27	-29.53	-29.24	0.37
PI 574486	2	2.78	2.98	3.50	3.08	63.75	33.13	18.29	73.48	48.54	30.65	21.62	25.00	7.94	146.28	63.17	-29.23	-29.35	-29.29	0.31
Skylla	2	2.96	2.81	3.05	2.94	58.14	35.03	13.14	70.91	39.13	26.77	21.53	22.58	-9.43	135.17	56.63	-29.30	-29.23	-29.26	0.51
4J105-3-4	3	3.38	3.06	3.50	3.31	75.23	36.99	27.65	74.42	69.24	25.82	16.94	24.62	-17.11	135.13	60.00	-29.13	-29.14	-29.13	0.36
5M20-2-5-2	3	3.18	3.02	3.26	3.15	71.51	38.08	23.76	81.57	54.14	24.88	18.57	22.25	-9.57	139.96	59.47	-29.16	-29.27	-29.21	0.28
CL0J095-4-6	3	3.60	3.39	3.39	3.46	63.96	34.42	17.59	74.83	62.14	29.77	23.03	23.61	-0.57	133.81	58.20	-29.29	-29.48	-29.38	0.34
CL0J173-6-8	3	3.37	2.84	3.30	3.17	67.04	36.52	20.17	76.67	63.37	27.57	17.81	22.98	-7.35	147.40	60.28	-29.02	-29.27	-29.14	0.26
HS6-3976	3	3.03	2.90	3.27	3.07	71.08	29.35	23.64	77.08	55.89	26.65	19.19	21.16	-6.41	149.10	57.68	-28.97	-29.00	-28.98	0.34

Table 3.4 (Cont.)

	Physiological Traits																		
		N concentration (gN 100g^{-1})				Ureide concentration (μM g^{-1})			N_2 derived from atmosphere (%)		Wilting			Canopy temperature			C isotope ratio			N_2 fixation rate (gN m^{-2} d^{-1})
		2015	2016	2017	3-year mean	2015	2016	2017	2016	2017	2015	2016	2017	2015	2016	2017	2016	2017	2-year mean	2016
Genotypes	**LSD**	**0.25**	**0.24**	**0.23**	**0.23**	**3.25**	**1.48**	**1.54**	**9.78**	**16.45**	**4.51**	**5.95**	**3.25**	**13.11**	**23.42**	**7.38**	**0.32**	**0.36**	**0.33**	**0.29**
	MG																			
IA3023	3	3.23	3.15	3.43	3.27	55.53	29.78	19.42	72.21	52.23	31.88	21.13	26.39	-1.59	136.77	60.85	-29.04	-29.16	-29.09	0.33
LG03-3191	4	3.13	3.14	3.44	3.24	61.20	26.13	23.91	77.11	68.92	27.07	16.61	23.27	-0.75	128.12	58.78	-28.99	-29.15	-29.07	0.46
LG04-6000	4	3.29	3.04	3.50	3.28	65.62	30.22	27.16	73.77	52.41	32.32	23.00	23.83	-0.48	126.76	59.99	-29.47	-29.40	-29.43	0.38
LG03-2979	3	3.52	3.36	3.65	3.51	74.70	34.44	24.73	69.67	65.26	32.27	26.56	31.80	-6.53	148.76	57.27	-29.35	-29.15	-29.32	0.34
LG04-4717	3	3.15	3.04	3.24	3.15	71.00	33.71	25.58	80.51	63.62	34.13	24.44	32.17	-5.54	130.54	60.12	-29.23	-29.03	-29.25	0.50
LG05-4464	3	2.84	2.89	3.24	2.99	50.60	27.99	21.32	75.75	55.04	31.38	25.19	27.21	0.39	135.51	59.53	-29.25	-29.05	-29.02	0.39
LG05-4832	3	3.12	3.15	3.12	3.13	61.57	23.33	15.88	77.97	58.86	29.34	21.76	22.44	-13.87	150.14	62.43	-29.32	-29.52	-29.15	0.33
LG90-2550	3	3.61	3.56	3.70	3.62	66.11	35.58	20.18	71.21	55.21	26.21	19.37	22.79	9.96	156.47	68.79	-28.83	-29.27	-29	0.40
LG97-7012	3	3.53	3.18	3.34	3.35	87.41	36.26	20.49	74.66	49.19	32.63	25.50	28.92	6.31	168.42	59.69	-28.93	-29.05	-29.22	0.32
LG98-1605	3	3.61	3.00	3.34	3.32	94.68	34.54	27.66	70.00	50.95	28.07	14.37	22.28	2.98	146.65	64.42	-29.08	-29.40	-29.23	0.36
Maverick	3	3.34	3.06	3.34	3.24	67.52	33.54	26.10	66.62	75.08	33.13	25.00	28.58	-5.47	112.75	58.59	-29.34	-29.51	-29.42	0.31
NE3001	3	3.47	3.35	3.56	3.46	74.72	39.45	23.53	79.78	60.83	29.13	24.64	26.60	11.47	155.27	57.82	-29.24	-29.45	-29.34	0.47
PI 398881	3	3.42	3.22	3.31	3.32	74.61	33.87	26.38	79.15	58.39	28.92	14.24	21.32	11.97	148.60	67.51	-29.18	-29.25	-29.35	0.43
PI 427136	3	3.27	2.59	3.36	3.08	65.84	29.82	21.67	77.87	62.23	29.67	19.31	21.95	8.28	145.53	65.08	-29.38	-29.33	-29.35	0.18
PI 561370	3	3.12	3.02	3.42	3.19	64.99	36.95	20.51	72.35	55.31	27.13	16.34	22.60	4.08	145.19	65.39	-29.33	-29.37	-29.4	0.27
Prohio	3	3.12	2.93	3.25	3.10	62.07	31.07	21.11	76.88	61.32	26.42	19.43	23.28	-4.57	141.68	61.41	-28.85	-29.25	-29.05	0.32

Table 3.4 (Cont.)

	Physiological Traits																		
		N concentration (gN 100g^{-1})				Ureide concentration (μM g^{-1})			N_2 derived from atmosphere (%)		Wilting			Canopy temperature			C isotope ratio			N_2 fixation rate (gN m^{-2} d^{-1})
		2015	2016	2017	3-year mean	2015	2016	2017	2016	2017	2015	2016	2017	2015	2016	2017	2016	2017	2-year mean	2016
Genotypes	**LSD**	**0.25**	**0.24**	**0.23**	**0.23**	**3.25**	**1.48**	**1.54**	**9.78**	**16.45**	**4.51**	**5.95**	**3.25**	**13.11**	**23.42**	**7.38**	**0.32**	**0.36**	**0.33**	**0.29**
	MG																			
LD00-3309	4	3.29	3.18	3.32	3.26	60.35	33.26	18.85	73.42	55.64	28.71	21.03	22.29	-10.69	144.25	59.05	-29.23	-29.24	-29.23	0.33
LG05-4292	4	2.97	2.97	3.39	3.11	49.68	28.09	19.81	73.70	73.48	34.88	21.56	28.14	-1.69	118.67	55.52	-28.93	-29.50	-29.13	0.19
LG05-4317	4	3.11	3.16	3.58	3.28	56.88	28.93	26.83	77.66	57.40	28.57	23.06	27.48	-1.94	122.81	59.46	-29.15	-28.90	-29.21	0.30
Magellan	4	3.26	3.01	3.18	3.15	64.06	35.87	20.96	74.92	52.06	34.38	26.88	27.92	-1.97	132.82	55.75	-29.15	-29.04	-29.09	0.37
S06-13640	4	2.95	2.72	3.24	2.97	44.84	30.01	23.23	74.19	64.14	24.60	13.30	20.53	3.26	140.11	63.85	-29.26	-29.20	-29.23	0.27
TN05-3027	5	3.21	3.13	3.45	3.26	67.75	31.70	26.11	75.56	57.13	30.24	18.28	26.84	-12.13	111.69	62.69	-29.24	-29.03	-29.13	0.33
Non-nodulating																				
Harsoy Non-Nod	2	-	1.40	1.44	1.42	-	5.50	2.87	-6.93	4.90	-	-	-	-	189.61	80.11	-29.59	-29.82	-	-0.02
Clark Non-Nod	4	-	1.46	-	1.46	-	6.45	-	6.93	-	29.63	-	-	11.67	136.90	-	-30.15	-	-	0.02
Lee Non-Nod	6	-	-	1.54	1.54	-	-	3.48	-	-4.90	-	-	-	-	-	71.39	-	-29.64	-	-

Table 3.5 Environmental conditions during measurement dates for canopy temperature.

Year	Date	Tmax (°C)	Tmin (°C)	Rainfall (mm)	Irrigation (mm)	Cum. water deficit (mm)	Solar radiation (MJ m^{-2} d^{-1})
2015	7/29	35.0	23.4	0.0	35.0	5.0	21.8
	8/11	30.8	20.7	0.0	0.0	21.0	19.5
2016	8/2	34.9	24.1	0.0	0.0	29.4	20.8
	8/11	36.0	23.2	0.0	0.0	40.0	21.9
2017	7/18	33.1	20.8	0.0	0.0	25.2	23.0
	8/2	30.5	19.0	7.6	0.0	15.4	21.4
	8/18	31.7	21.1	17.3	0.0	0.0	19.4
	8/20	34.3	20.1	0.0	0.0	12.6	22.2
	8/22	31.3	23.3	0.8	0.0	21.4	16.5
	8/28	27.5	18.4	0.0	37.0	3.0	17.1

Table 3.6 Environmental conditions during measurement dates for canopy wilting.

Year	Date	Tmax (°C)	Tmin (°C)	Rainfall (mm)	Irrigation (mm)	Cum. water deficit (mm)	Solar radiation (MJ m^{-2} d^{-1})
2015	8/3	33.0	20.8	0.0	0.0	35.0	22.0
	8/14	29.5	17.2	0.0	0.0	37.4	21.2
2016	8/10	34.1	21.0	2.0	0.0	38.0	22.3
	8/24	33.2	23.7	0.0	0.0	38.1	17.8
2017	7/19	34.1	22.9	0.0	0.0	31.1	21.7
	7/25	34.6	22.3	0.0	0.0	33.0	19.8
	8/4	29.5	18.7	0.0	0.0	34.0	17.5
	8/25	29.1	15.0	0.0	0.0	36.1	21.6

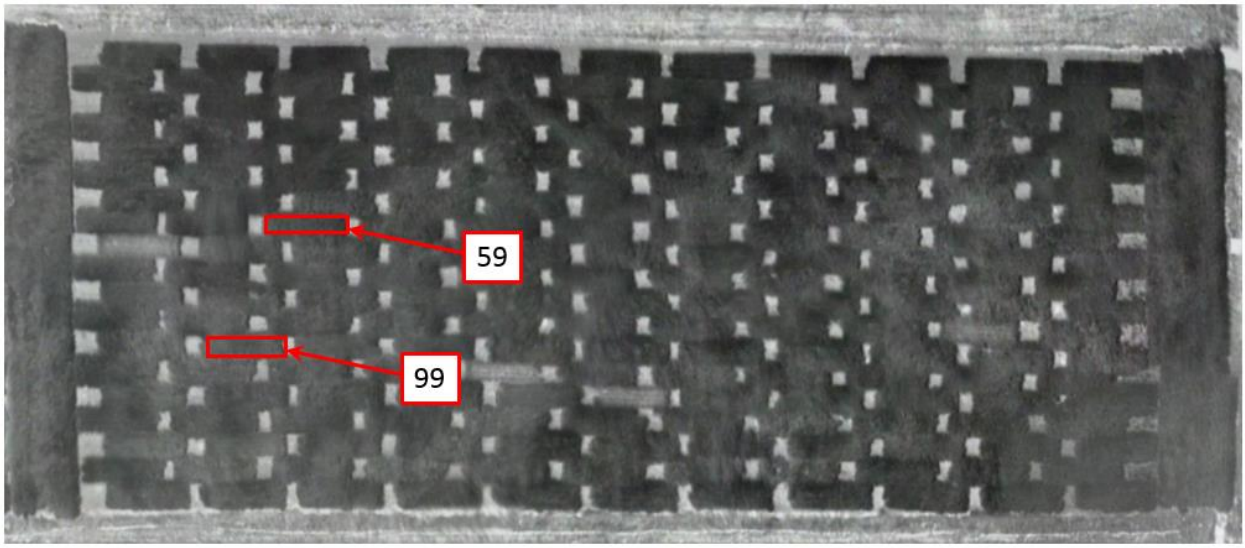

Figure 3.1 The aerial infrared image was taken from drone to access canopy temperature and analyzed using Field Analyzer to extract relative canopy temperature values for each plot. A plot with a darker gray shade had a lower relative canopy temperature (59), while a plot with a lighter gray shade was found to have a higher canopy temperature (99).

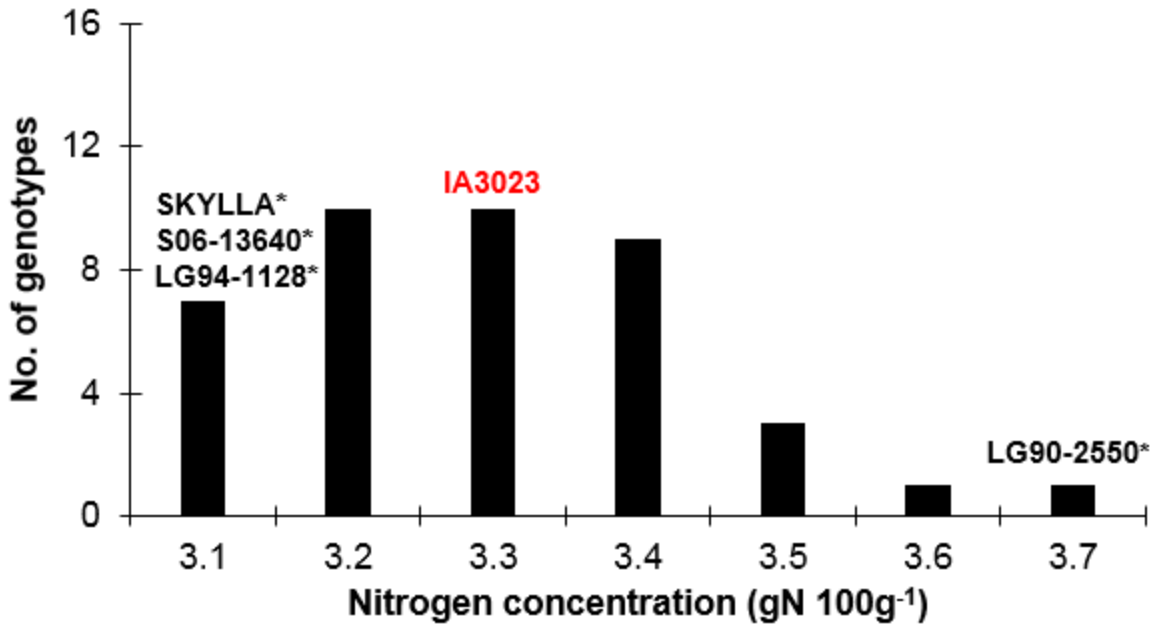

Figure 3.2 Frequency distribution graphs of SoyNAM parental genotypes for nitrogen concentration averaged over three years. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

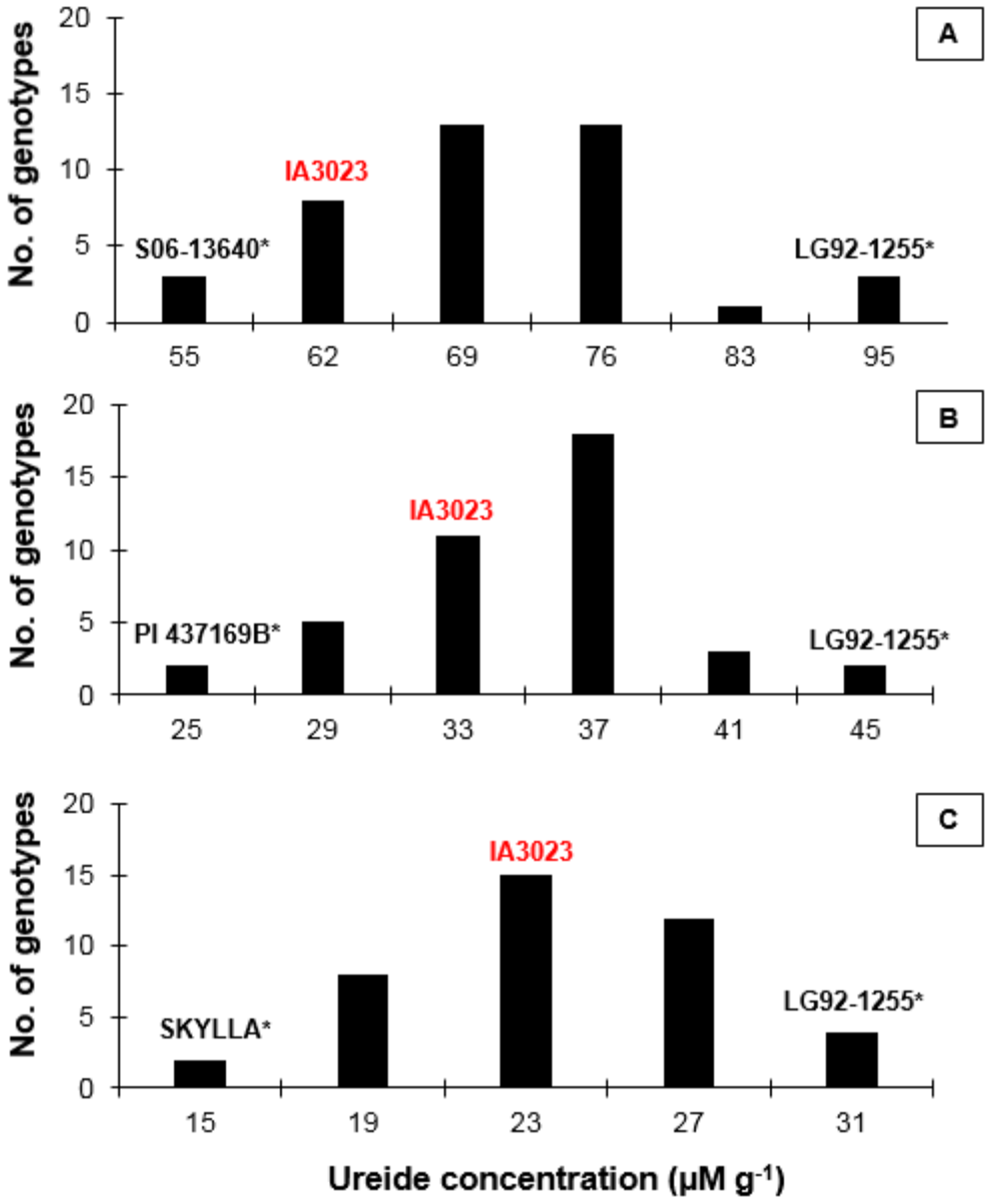

Figure 3.3 Frequency distribution graphs of SoyNAM parental genotypes for ureide concentration in years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

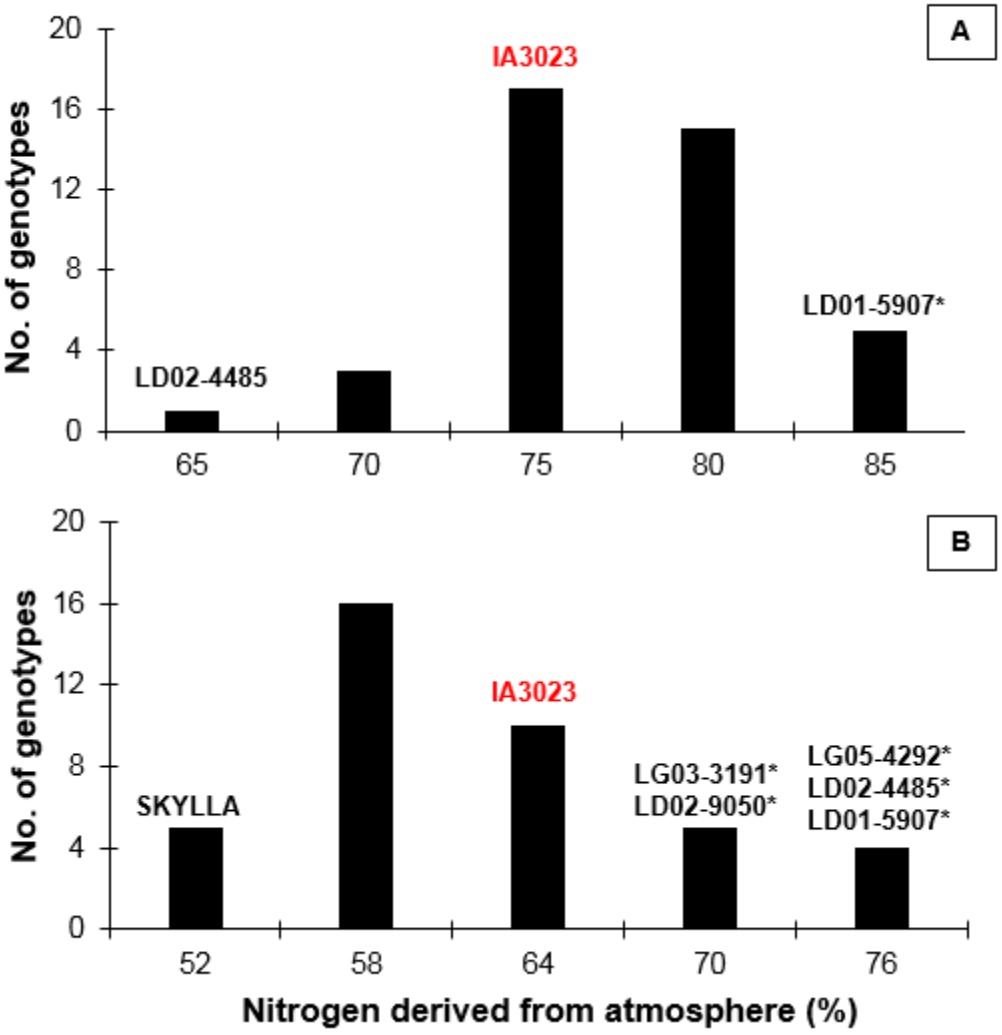

Figure 3.4 Frequency distribution graphs of SoyNAM parental genotypes for nitrogen derived from atmosphere in the years (A) 2016, and (B) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

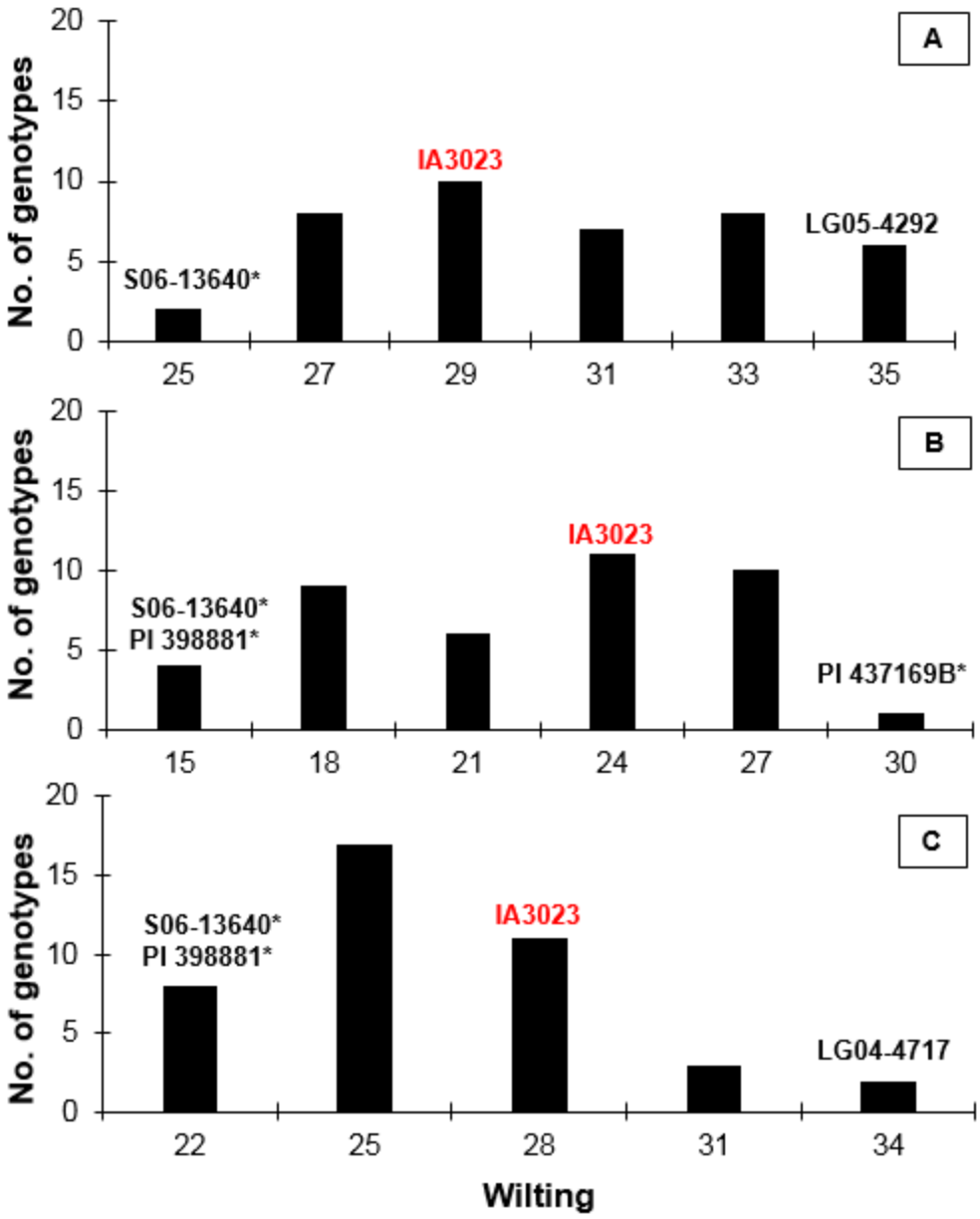

Figure 3.5 Frequency distribution graphs of SoyNAM parental genotypes for wilting in years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

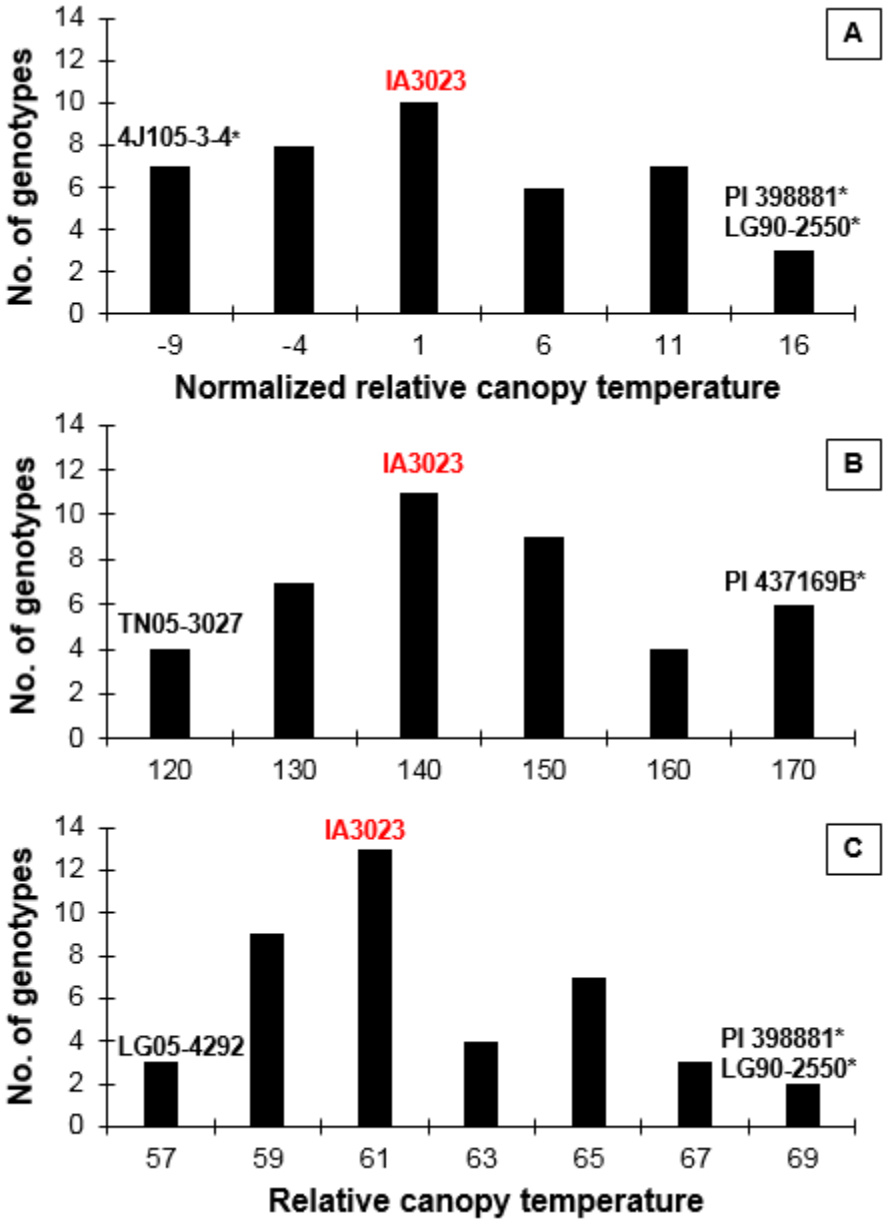

Figure 3.6 Frequency distribution graphs of SoyNAM parental genotypes for relative canopy temperature in the years (A) 2015, (B) 2016, and (C) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*. The data from 2015 was normalized to decrease variability. During 2015 and 2017, all the dates of data collection had sufficient moisture availability (water deficit less than 20mm). In 2016 the measurement dates had cumulative water deficit of 30mm and above.

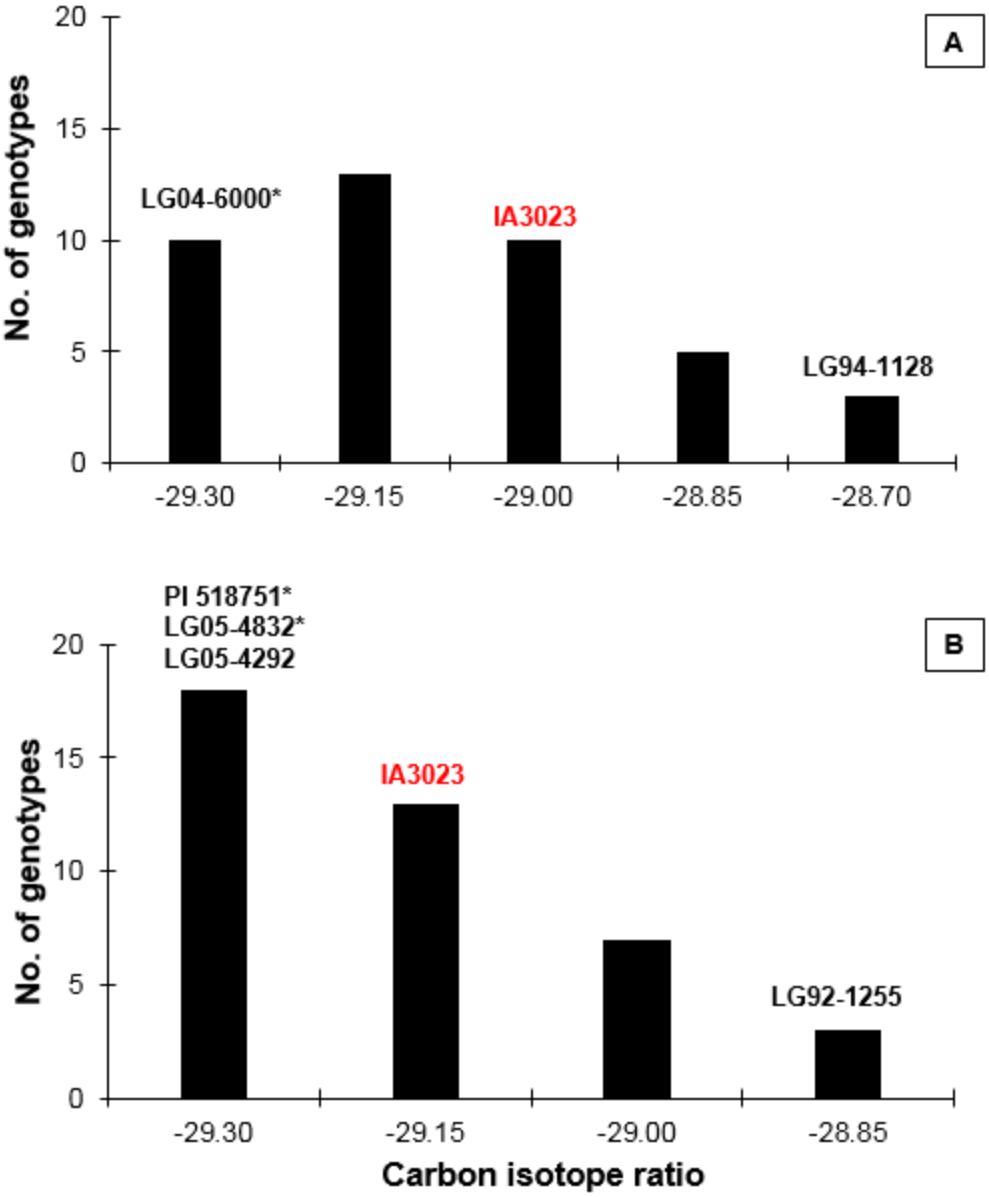

Figure 3.7 Frequency distribution graphs of SoyNAM parental genotypes for carbon isotope ratio in the years (A) 2016, and (B) 2017. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

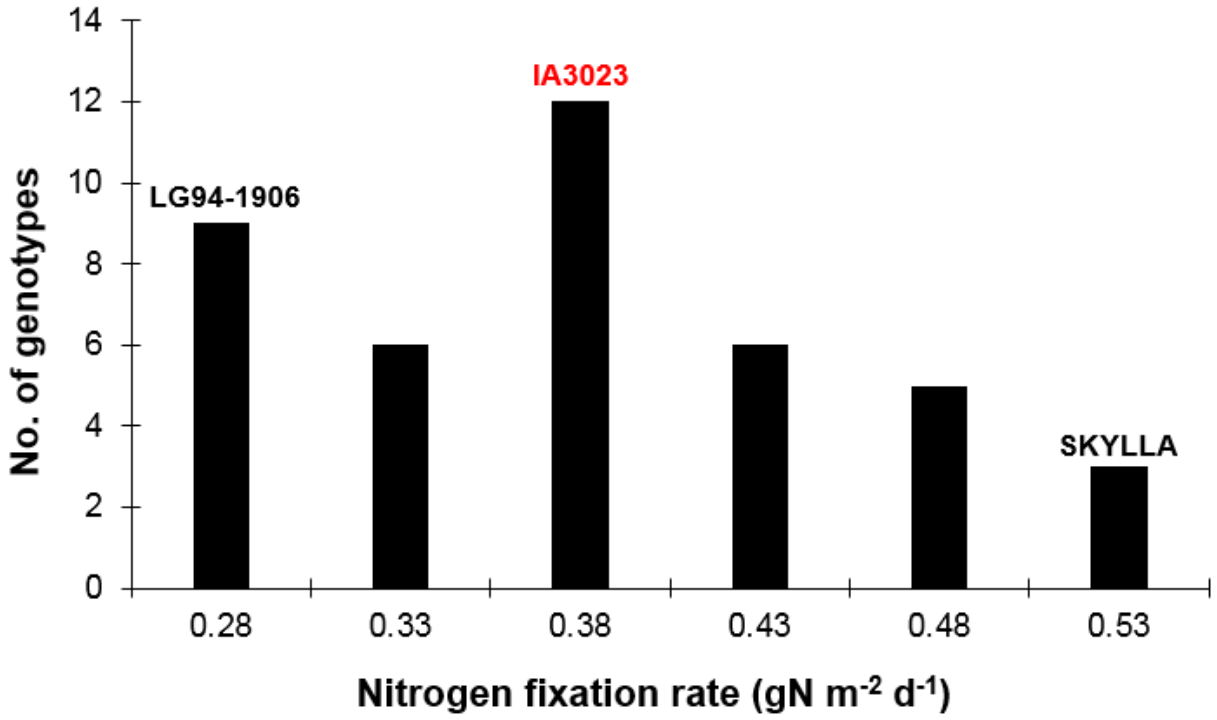

Figure 3.8 Frequency distribution graphs of SoyNAM parental genotypes for nitrogen fixation rate in 2016. Extreme genotypes of both the ends are shown, and genotypes significantly different from the hub parent IA3023 (shown in red) are represented by*.

References

Amani, I., R.A. Fischer, and M.P. Reynolds. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. Journal of Agronomy and Crop Science. 176(2):119-129.

Bai, H, and L.C. Purcell. 2018a. Aerial canopy temperature differences between fast- and slow-wilting soybean genotypes. J Agro Crop Sci. 00:1–9. https://doi.org/10.1111/jac.12259.

Bai, H., and L.C. Purcell .2018b. Response of carbon isotope discrimination and oxygen isotope composition to mild drought in slow- and fast-wilting soybean genotypes. Journal of Crop Improvement. 32:2, 239-253, DOI: 10.1080/15427528.2017.1407856.

Bergersen, F.J., J. Brockwell, R.R. Gault, L. Morthorpe, M.B. Peoples, and G.L. Turner. 1989. Effects of available soil nitrogen and rates of inoculation on nitrogen fixation by irrigated soybeans and evaluation of ^{15}N methods for measurement. Aust. J. Agric. Res. 40:763–780. doi:10.1071/AR9890763.

Board, J.E. 1987. Yield Components Related to Seed Yield in Determinate Soybean 1. Crop Sci. 27(6):1296-1297.

Boyer, J. S., P. Byrne, K.G. Cassman, M. Cooper, D. Delmer, T. Greene, F. Gruis, J. Habben, N. Hausmann, et al. 2013. The US drought of 2012 in perspective: A call to action. Global Food Security 2:139–43.

Bremner, J.M. 1965. Total Nitrogen 1. Methods of soil analysis. Part 2. Chemical and microbiological properties: 1149-1178.

Carter, T.E., Jr., S.M. Todd, and A.M. Gillen. 2016. Registration of 'USDA-N8002′ soybean cultivar with high yield and abiotic stress resistance traits. Journal Plant Registrations 10:238–45.

Condon, A., G. Farquhar, and R. Richards. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. Functional Plant Biol. 17:9-22.

Condon A.G., G.D. Farquhar, G.J. Rebetzke, R.A Richards. 2004. Breeding for high water-use efficiency. J Exp Bot. 55:2447–2460.

Charlson, D.V., S. Bhatnagar, C.A. King, J.D. Ray, C.H. Sneller, T.E. Carter, and L.C. Purcell. 2009. Polygenic inheritance of canopy wilting in soybean [Glycine max (L.) Merr.]. Theoretical and Applied Genetics. 119: 587-594.

Dhanapal, A.P., J.D. Ray, S.K. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, and F.B. Fritschi. 2015a. Genome-wide association analysis of diverse soybean genotypes reveals novel markers for nitrogen traits. The Plant Genome 8.

Dhanapal, A.P., J.D. Ray, S.K. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, P.B. Cregan, Q. Song, and F.B. Fritschi. 2015b. Genome-wide association study (GWAS) of carbon isotope ratio ($\delta^{13}C$) in diverse soybean [Glycine max (L.) Merr.] genotypes. Theoretical and Applied Genetics 128:73-91.

De Bruin, J.L., and P. Pedersen. 2008. Soybean Seed Yield Response to Planting Date and Seeding Rate in the Upper Midwest All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Agron. J. 100(3):696-703.

Egli, D.B. 1993. Cultivar maturity and potential yield of soybean. Field Crops Res. 32(1-2):147-158.

Egli, D.B. 1997. Cultivar maturity and response of soybean to shade stress during seed filling. Field Crops Res. 52(1-2):1-8.

Egli, D.B., J.H. Orf, and T.W. Pfeiffer. 1984. Genotypic variation for duration of seedfill in soybean. Crop Sci. 24:587–592.

Egli, D.B. and I.F. Wardlaw. 1980. Temperature Response of Seed Growth Characteristics of Soybeans1. Agron. J. 72(3):560-564.

FAOSTATS. 2011. Food and Agriculture Organization of the United Nations Statistics Division. http://faostat3.fao.org/home/ (accessed 30 Jun. 2018).

FAO. 2018. Irrigation through underground water. http://www.fao.org/nr/water/aquastat/didyouknow/index3.stm. (Last updated, December 2014. Visited June 6, 2018).

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa.

Foley, J.A., N. Ramankutty, K.A. Brauman, E.S. Cassidy, J.S. Gerber, M. Johnston et al. 2011. Solutions for a cultivated planet. Nature 478:337–342. doi: 10.1038/nature10452.

Feng, X., A. Porporato, and I. Rodriguez-Iturbe. 2013. Changes in rainfall seasonality in the tropics. Nature Climate Change 3(9):811.

Hanway, J., and C. Weber. 1971. Dry matter accumulation in eight soybean (Glycine max (L.) Merrill) varieties. Agron. J. 63:227-230.

Heatherly, L.G., and R.W. Elmore. 2004. Managing inputs for peak production. In: Boerma, H.R., Specht, J.E. (Eds.), Soybeans: Improvement, Production and Uses. ASA, CSSA, SSSA, Madison, WI 451–536.

Hwang, S., C.A. King, M.K. Davies, J.D. Ray, P.B. Cregan, and L.C. Purcell. 2013. QTL analysis of shoot ureide and nitrogen concentrations in soybean [Glycine max (L.) Merr.]. Crop Science, *53*(6):2421-2433.

Hwang, S., C.A. King, J.D. Ray, P.B. Cregan, P. Chen, T.E. Carter, Z. Li, H. Abdel-Haleem, K.W. Matson, W. Schapaugh, and L.C. Purcell. 2015. Confirmation of delayed canopy wilting QTLs from multiple soybean mapping populations. Theoretical and applied genetics. 128(10):2047-2065.

IPCC. 2012. "Chapter 3," in Changes in Climate Extremes and their Impacts on the Natural Physical Environment, eds C. B. Field, V. Barros, T. F. Stocker, D. Qin, D. J. Dokken, K. L. Ebi, et al. (Cambridge: Cambridge University Press). 109–230.

Jones, H.G., Serraj, R., Loveys, B.R., Xiong, L., Wheaton, A. and Price, A.H. 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Functional Plant Biology.36(11):978-989.

Kaler, A.S., J.D. Ray, W.T. Schapaugh, , A.R. Asebedo, C.A. King, E.E. Gbur, and L.C. Purcell. 2018. Association mapping identifies loci for canopy temperature under drought in diverse soybean genotypes. Euphytica. 214(8):135.

King, C.A., and L.C. Purcell. 2001. Soybean nodule size and relationship to nitrogen fixation response to water deficit. Crop science 41:1099-1107.

King, C.A., and L.C. Purcell. 2005. Inhibition of N_2 fixation in soybean is associated with elevated ureides and amino acids. Plant Physiol. 137:1389–1396. doi:10.1104/pp.104.056317.

King, C.A., and L.C. Purcell. 2006. Genotypic variation for shoot N concentration and response to water deficits in soybean. Crop science 46 (6):2396-2402.

King, C.A., L.C. Purcell, and K.R. Brye. 2009. Differential wilting among soybean genotypes in response to water deficit. Crop Sci. 49:290-298.

King, C.A., L.C. Purcell, A. Bolton, and J.E. Specht. 2014. A possible relationship between shoot N concentration and the sensitivity of N 2 fixation to drought in soybean. Crop Science. 54(2):746-756.

Kohl, D.H., and G.B. Shearer. 1981. The use of soils lightly enriched in 15N to screen for N2–fixing activity. Plant Soil 60:487–489. doi:10.1007/BF02149646

Liebisch F, N. Kirchgessner, D. Schneider, A.Walter, A. Hund .2015. Remote, aerial phenotyping of maize traits with a mobile multi-sensor approach. Plant Methods 11:9.

Lenssen, A. 2012. Soybean response to drought. http://crops.extension.iastate.edu/cropnews/2012/06/soybean-response-drought.

Lobell, D. B., M. Bänziger, C. Magorokosho, and B. Vivek. 2011. Nonlinear heat effects on African maize as evidenced by historical yield trials. Nat. Clim. Change 1:42–45. doi:10.1038/nclimate1043.

Lugg, D.G., and T.R. Sinclair. 1981. Seasonal changes in photosynthesis of field-grown soybean leaflets II. Relation to nitrogen content. Photosynthetica. 15:138–144.

Muchow, R., and T. Sinclair. 1986. Water and nitrogen limitations in soybean grain production II. Field and model analyses. Field Crops Res. 15:143-156.

Passioura, J. 2007. The drought environment: physical, biological and agricultural perspectives. J. Exp. Bot. 58(2): 113-117.

Purcell, L.C., and C.A. King. 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. Journal of Plant Nutrition 19:969-993.

Purcell, L.C., R. Serraj, M. de Silva, T.R. Sinclair, and S. Bona. 1998. Ureide concentration of field-grown soybean in response to drought and the relationship to nitrogen fixation. J. Plant Nutr. 21:949–966. doi:10.1080/01904169809365456.

Purcell, L.C., C.A. King, and R.A. Ball. 2000. Soybean cultivar differences in ureides and the relationship to drought tolerant nitrogen fixation and manganese nutrition. Crop Sci. 40:1062–1070. doi:10.2135/cropsci2000.4041062x.

Purcell, L.C., and J.E. Specht. 2004. Physiological traits for ameliorating drought stress. p. 569-620. In: (eds. J.E. Specht and H.R. Boerma) Soybeans: Improvement, production, and uses. Agronomy Monographs, 3rd edition. 16 ASA CSSA SSSA, Madison, WI.

Purcell, L.C., J.T. Edwards, and K.R Brye. 2007. Soybean yield and biomass responses to cumulative transpiration: Questioning widely held beliefs. Field Crops Res. 101(1):10-18.

Ray, J.D., A.P. Dhanapal, S.K. Singh, V. Hoyos-Villegas, J.R. Smith, L.C. Purcell, C.A. King, D. Boykin, P.B. Cregan, Q. Song, and F.B. Fritschi. 2015. Genome-wide association study of ureide concentration in diverse maturity group IV soybean [Glycine max (L.) Merr.] accessions. G3: Genes, Genomes, Genetics. 3-115.

Ries, L. L., L.C. Purcell, T.E. Carter, J.T. Edwards, and C.A. King. 2012. Physiological traits contributing to differential canopy wilting in soybean under drought. Crop science. 52(1): 272-281.

Rotundo, J.L., L. Borrás, J. De Bruin, and P. Pedersen. 2014. Soybean nitrogen uptake and utilization in Argentina and United States cultivars. Crop Sci. 54(3):1153-1165.

Serraj, R., and T.R. Sinclair. 1997. Variation among soybean cultivars in dinitrogen fixation response to drought. Agron. J. 89:963–969. doi:10.2134/agronj1997.00021962008900060019x.

Serraj, R., T.R. Sinclair, and L.C. Purcell. 1999a. Symbiotic N_2 fixation response to drought. Journal of Experimental Botany 50:143-155.

Shevah, Y. 2015. Water Resources, Water Scarcity Challenges, and Perspectives. Water Challenges and Solutions on a Global Scale, American Chemical Society p.185.

Sharma, K., W. Neelu, K. Sharma, and N. Walia. 1996. Growth and Yield of Soybean Glycine max (L.) Merill as Influenced by Light Intensity and Cytokinin. Indian Agric. 39: 253–257.

Shearer, G.B., D.H. Kohl, and J.E. Harper. 1980. Distribution of ^{15}N among plant parts of nodulating and non-nodulating isolines of soybeans. Plant Physiol. 66:57–60. doi:10.1104/pp.66.1.57

Sinclair, T.R., C.T. de Wit. 1976. Analysis of the carbon and nitrogen limitations to soybean yield. Agron. J. 68, 319–324.

Sinclair TR. 2012. Is transpiration efficiency a viable plant trait in breeding for crop improvement? Funct Plant Biol. 39:359–365.

Singer, J.W., R.W. Malone, D.W. Meek, and D. Drake. 2004. Predicting yield loss in indeterminate soybean from pod density using simulated damage studies. Agron. J. 96(2):584-589.

Soystasts. 2018.U.S. export history. http://soystats.com/wp-content/uploads/SoyStats2018.pdf

Tuberosa, R. 2013. Phenotyping for drought tolerance of crops in the genomics era. Frontiers in physiology 3:347.

Tuberosa, R., and S. Salvi. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends in plant Sci. 11(8):405-412.

USDA. 2013. Economic Research Service: U.S. drought 2012: farm and food impacts. United States Department of Agriculture. http://ers.usda.gov/topics/in-the-news/us-drought-2012-farm-and-food-impacts.aspx#.VDMwNvldVrU (Last updated 20 Sept. 2017. Accessed Sept. 6, 2018).

USDA, ERS, 2017. Soybean-acreage- USA. United States Department of Agriculture, Economic research service data. Mark Ash. https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/related-data-statistics/ (Last updated October 6, 2017. Accessed Sept. 6, 2018).

USDA, ERS, 2018. Soybean-irrigated, crop acreage, yield, and production, by county, 2013-2014. United States Department of Agriculture, Economic research service data. Marcel Aillery. https://www.ers.usda.gov/topics/farm-practices-management/irrigation-water-use/ (Last updated April 30, 2018. Accessed June 6, 2018).

Van Roekel, R.J., L.C. Purcell, and M. Salmerón. 2015. Physiological and management factors contributing to soybean potential yield. Field Crops Research. 182:86-97.

Waseem, M., A. Ali, M. Tahir, M.A. Nadeem, M. Ayub, A. Tanveer et al. 2011. Mechanism of drought tolerance in plant and its management through different methods. Continental J. of Agricultural Sci. 5(1):10-25.

Westcott, P.C. and M. Jewison. 2013. Weather effects on expected corn and soybean yields. Washington DC: USDA Economic Research Service FDS-13g-01.

Young, E.G., and C.F. Conway. 1942. On the estimation of allantoin by the Rimini-Schryver reaction. Journal of Biological Chemistry. 142(2):839-853.

Yousfi, S., N. Kellas, L. Saidi, Z. Benlakehal, L. Chaou, D. Siad, F. Herda, M. Karrou, O. Vergara, A. Gracia, and J.L. Araus, 2016. Comparative performance of remote sensing methods in assessing wheat performance under Mediterranean conditions. Agricultural Water Management. 164:137-147.

Zipper, S.C., J. Qiu, and C.J. Kucharik. 2016. Drought effects on US maize and soybean production: spatiotemporal patterns and historical changes. Environmental Research Letters 11(9):094021.

4. CHAPTER: Conclusions

Currently, the US is the world's largest soybean producer *(Glycine max* L. Merr.), contributing 34% to the world's soybean supply. Soybean is also the second largest crop grown in the US and is among the four primary staple crops worldwide. However, there is still potential for soybean yield improvement. For example, Van Roekel et al. (2015) pointed out that the highest reported soybean yield is about three times greater than the highest reported U.S. average. The gene pool of soybean in North America, however, is narrow with only 17 accessions contributing to 86% of the parentage of modern soybean cultivars (Carter et al., 2004; Gizlice et al., 1994). This narrow genetic base may limit the future yield gains in soybean.

Another important limitation with respect to maintenance of soybean yield is the increasing annual temperatures around the world and the sensitivity of soybean yield to drought (Heatherly and Elmore, 2004). At present, there are only a few differences in tolerance to drought among commercial cultivars, because of the narrow gene pool of soybean in North America. Traditional breeding programs aiming to improve drought tolerance based strictly on yield have not met success because of insufficient diversity among genotypes used in such programs and also because of low heritability, epistasis, polygenic control, and genotype by environment interactions of yield (Khan et al., 2016).

Therefore, for the diversification of the soybean gene pool and identification and mapping of useful traits, the soybean nested association mapping (SoyNAM) population was developed by crossing 40 diverse soybean genotypes from maturity groups (MG) 1 through 5 with a common MG 3 parent (IA3023), resulting in 40 recombinant inbred populations of 140 recombinant inbred lines (RILs) per population. All these recombinant inbred populations have been genotyped with molecular markers. Thus, the SoyNAM population is a tremendous resource for identifying and mapping traits of interest in soybean. Physiological traits important

for yield can serve as sources of novel alleles that can be incorporated into elite germplasm to improve yield. The current research characterized the SoyNAM parental genotypes for important yield-associated physiological traits that have not been mapped previously or extensively, as a step in using SoyNAM as a source of physiological traits for future studies.

The traits for which we characterized SoyNAM include: canopy coverage, radiation use efficiency, seed growth rate, effective filling period, harvest index, seed weight, canopy temperature, canopy wilting, shoot ureide concentration, shoot N concentration, N_2 fixation rate, nitrogen derived from atmosphere (NDFA), and carbon isotopic ratio ($\delta^{13}C$). The experiment was conducted for three consecutive years (2015, 2016, and 2017) at Fayetteville, Arkansas under irrigated conditions, and evaluated the 41 SoyNAM parental genotypes along with non-nodulating genotype each year for the above-mentioned physiological traits. Identifying the parental genotypes that differ from the hub parent for specific traits would be important in choosing a particular biparental population from the 40 SoyNAM populations for mapping purposes.

Several extreme genotypes differing statistically from the hub parent were identified. A total of 16 genotypes were identified as extreme genotypes for different traits, including genotypes on both the extremes for all the 14 traits studied. Some genotypes were identified as common extremes for more than one trait.

The current study was also useful for understanding the role and significance of these 14 traits with regards to yield and development of drought tolerance. Our findings and a review by Van Roekel and Purcell (2014) indicate that yield increases can be obtained through a number of ways including: (1) An increase in light interception through early canopy coverage (Rowntree et al., 2014; Salmeron et al., 2014), (2) lengthening of the reproductive period (Cooper, 2003), (3)

increase in RUE (Van Roekel and Purcell, 2014; Sinclair et al., 2003), and (4) increase in N accumulation rates (Rotundo et al., 2014; Van Roekel and Purcell, 2014). However, there are possible trade-offs among these traits. For example, genotype PI 398881 which was identified as a slow wilting genotype under drought conditions, but it also had a high canopy temperature under sufficient moisture conditions. This likely means that although PI 39881 is predicted to perform well under drought (slow wilting) it also had limited transpiration under optimum conditions that would limit, photosynthesis and yield potential under optimum conditions. Similarly, a low N concentration under optimum conditions (as identified in SKYLLA) is associated with drought-tolerant N_2 fixation, however, a low N concentration under optimum conditions would likely limit photosynthesis and the amount of N available to translocate to seed. Finally, an understanding of genetics and the genotype by environment interactions of such yield-related traits is also essential to completely understand the contribution of these traits to yield (Van Roekel et al., 2015). Future studies will be needed to confirm these associations, and/or verify if the quantitative trait loci (QTLs) associated with these traits are also associated with yield.

Difficulties in using physiological traits in breeding include that they are quantitative with many genes having relatively small effect on phenotype, a large genotype by environment interaction that it is often difficult to phenotype or measure for many of these traits. Identification of specific molecular markers helps to overcome the limitation of low heritability due to quantitative nature, and it is also a solution for the complications due to genotype by environment interactions. Once, specific QTLs for these quantitative traits have been identified in specific genotypes, such genotypes can be crossed with stable high yielding cultivars already being grown commercially. Then, Marker Assisted selection (MAS) can be performed to verify

the transfer of desirable genes. Pyramiding of these additive traits can lead to cumulative additive effect of each desirable allele which can result in an overall increase in yield. Employing high throughput phenotyping (Araus and Cairns, 2014) is the key technology in improving our phenotypic ability and overcoming the limitation to dissect the genetics of quantitative traits. The methodologies for determining canopy temperature and wilting are still in their initial stage of development and can be improved to decrease environmental variation. Ongoing studies in our lab have begun addressing these issues and future studies will also be useful in making an attempt in this direction.

Extensive identification of QTLs associated with yield-associated physiological traits will thus lay the path for a targeted approach for breeding using physiological traits and associated molecular markers. Based on the results from our phenotyping, specific mapping populations can now be identified that will likely have the most segregation for the traits and can be used in future mapping studies.

References

Araus, J.L., and J.E. Cairns. 2014. Field high-throughput phenotyping: The new crop breeding frontier. Trends Plant Sci. 19:52–61. https://doi.org/10.1016/j.tplants.2013.09.008.

Carter, T.E., R. Nelson, C. Sneller, and Z. Cui. 2004. Soybeans: Improvement Production and Uses. Agron. J. 16:303-416.

Cooper, R.L. 2003. A delayed flowering barrier to higher soybean yields. Field Crops Res. 82: 27–35.

Gizlice, Z., T. Carter, and J. Burton. 1994. Genetic base for North American public soybean cultivars released between 1947 and 1988. Crop Sci. 34:1143-1151.

Heatherly, L.G., and RW. Elmore. 2004. Managing inputs for peak production. In: Boerma, H.R., Specht, J.E. (Eds.), Soybeans: Improvement, Production and Uses. 3rd ed. ASA, CSSA, SSSA, Madison, WI p. 451–536.

Khan, A., V. Sovero, , and D. Gemenet. 2016. Genome-assisted Breeding for Drought Resistance. Current Genomics 17(4):330–342. http://doi.org/10.2174/1389202917999160211101417

Rotundo, J.L., L. Borrás, J. De Bruin, and P. Pedersen. 2014. Soybean nitrogen uptake and utilization in Argentina and United States cultivars. Crop Sci. 54(3):1153-1165.

Rowntree, S.C., J.J. Suhre, N.H. Weidenbenner, E.W. Wilson, V.M. Davis, S.L. Naeve, S.N. Casteel, B.W. Diers, P.D. Esker, and S.P. Conley. 2014. Physiological and phenological responses of historical soybean cultivar releases to earlier planting. Crop Sci. 54: 804–813.

Salmeron, M., E.E. Gbur, F.M. Bourland, N.W. Buehring, L. Earnest, F.B. Fritschi, B.R. Golden, D. Hathcoat, J. Lofton, T.D. Miller, and C. Neely. 2014. Soybean maturity group choices for early and late plantings in the Midsouth. Agronomy Journal 106(5):1893-1901.

Sinclair, T.R., R. Farias, N. Neumaier, A.L. Nepomuceno. 2003. Modeling nitrogen accumulation and use by soybean. Field Crops Res. 81:149–158.

Van Roekel, R.J. and L.C. Purcell. 2014. Soybean biomass and nitrogen accumulation rates and radiation use efficiency in a maximum yield environment. Crop Science 54(3):1189-1196.

Van Roekel, R.J., L.C. Purcell, and M. Salmerón. 2015. Physiological and management factors contributing to soybean potential yield. Field Crops Research 182:86-97.

Appendix

Table A.1 Phenotypic characteristics of the parental genotypes for maturity group, stem termination, flower color, pubescence color, pod color, seed coat luster, seed coat color, and hilum color.

Genotype	MG	Stem type	Flower color	Pub. color	Pod color	Seed coat luster	Seed coat color	Hilum color
U03-100612	1	Indeterminate	Purple	Light	Brown	Dull	Yellow	Black
LG92-1255	2	Indeterminate	Purple	Tawny	Tan	Dull	Yellow	Black
LG94-1128	2	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Imperfect black
LG94-1906	2	Indeterminate	White	Gray	Brown	Shiny	Yellow	Buff
PI 404.188A	2	Indeterminate	White	Tawny	Brown	Shiny	Yellow	Black
PI 437.169B	2	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Imperfect black
PI 507.681B	2	Indeterminate	White	Gray	Brown	Intermediate	Yellow	Yellow
PI 518.751	2	Indeterminate	Purple	Gray	Brown	Intermediate	Yellow	Imperfect black
PI 574.486	2	Indeterminate	White	Tawny	Brown	Shiny	Yellow	Black
SKYLLA	2	Indeterminate	Purple	Tawny	Tan	Dull	Yellow	Black
LD02-4485	2	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Buff
IA3023	3	Indeterminate	White	Light	Tan	Dull	Yellow	Black
4J105-3-4	3	Indeterminate	White	Light	Brown	Dull	Yellow	Black
5M20-2-5-2	3	Indeterminate	Purple	Light	Brown	Dull	Yellow	Black
CL0J095-4-6	3	Indeterminate	Purple	Tawny	Tan	Dull	Yellow	Green
CL0J173-6-8	3	Indeterminate	White	Light	Tan	Dull	Yellow	Black
HS6-3976	3	Indeterminate	White	Light	Tan	Dull	Yellow	Black
NE3001	3	Semi-indeterminate	White	Gray	Tan	Shiny	Yellow	Buff
PROHIO	3	Indeterminate	Purple	Tawny	Tan	Shiny	Yellow	Black
LG05-4832	3	Indeterminate	White	Light	Brown	Shiny	Yellow	Brown
LG90-2550	3	Semi-determinate	Purple	Tawny	Tan	Shiny	Yellow	Black
LG97-7012	3	Indeterminate	White	Gray	Tan	Dull	Yellow	Buff
LG98-1605	3	Indeterminate	White	Tawny	Brown	Dull	Yellow	Yellow
LG00-3372	3	Indeterminate	Purple	Light	Brown	Dull	Yellow	Black
LG03-2979	3	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Imperfect black
LG04-4717	3	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Buff
LG05-4464	3	Indeterminate	Purple	Gray	Tan	Dull	Yellow	Imperfect black
PI 398.881	3	Indeterminate	Purple	Tawny	Brown	Dull	Yellow	Black
PI 427.136	3	Indeterminate	White	Tawny	Brown	Shiny	Yellow	Black
PI 561.370	3	Indeterminate	Purple	Tawny	Brown+Tan	Shiny	Yellow	Black

Table A.1 (Cont.)

Genotype	MG	Stem type	Flower color	Pub. color	Pod color	Seed coat luster	Seed coat color	Hilum color
LD01-5907	3	Indeterminate	Purple	Gray	Tan	Dull	Yellow	Buff
MAVRCK	3	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Buff
LD02-9050	4	Indeterminate	Purple	Tawny	Brown+Tan	Dull	Yellow	Black
LG03-3191	4	Indeterminate	Purple	Tawny	Brown	Dull	Yellow	Gray/Yellow/Brown/ Buff/Black
LG04-6000	4	Indeterminate	White	Light	Brown	Dull	Yellow	Black
LG05-4292	4	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Imperfect black
LG05-4317	4	Indeterminate	White	Tawny	Brown	Dull	Yellow	Black
MAGELLAN	4	Indeterminate	Purple	Gray	Brown+Tan	Shiny	Yellow	Buff
LD00-3309	4	Indeterminate	Purple	Tawny	Brown	Dull	Yellow	Black
S06-13640	4	Indeterminate	Purple	Tawny	Brown	Dull	Yellow	Black
TN05-3027	5	Indeterminate	White	Gray	Brown	Intermediate	Yellow	Buff
Non-nodulating Genotypes								
Harosoy (PI547728)	2	Indeterminate	Purple	Gray	Brown	Dull	Yellow	Yellow
Clark (PI547655)	4	Indeterminate	Purple	Tawny	Brown	Dull	Yellow	Black
Lee (PI573285)	6	Determinate	Purple	Tawny	Tan	Intermediate	Yellow	Black

Table A.2 List of all physiological traits studied.

Physiological Traits	
Drought-related	**Yield-related**
Canopy coverage	Nitrogen concentration
Radiation use efficiency (RUE)	Ureide concentration
Seed growth rate (SGR)	Nitrogen derived from atmosphere (NDFA)
Effective filling period (EFP)	Canopy wilting (WLT)
Seed yield	Canopy temperature
Seed weight	Carbon isotope ratio ($\delta^{13}C$)
Harvest index (HI)	Nitrogen fixation rate

Table A.3 Environment data for 2015.

Date	Average T_{max} (°C)	Average T_{min} (°C)	Average Rainfall (mm)	Average Irrigation (mm)	Average Water deficit (mm)	Average Solar Radiation (MJ m^{-2} d^{-1})
6/1-6/7	N.A.	N.A.	0.00	0.00	5.83	0.00
6/8-6/14	N.A.	N.A.	0.00	0.00	7.17	0.00
6/15-6/21	N.A.	N.A.	0.00	5.71	0.50	0.00
6/22-6/28	38.00	20.00	0.00	5.71	0.78	4.08
6/29-7/5	30.93	21.33	4.43	0.00	3.45	14.80
7/6-7/12	28.65	20.56	13.48	0.00	7.51	18.52
7/13-7/19	35.24	23.34	0.00	3.57	27.54	22.67
7/20-7/26	32.64	23.06	14.43	5.00	14.79	20.01
7/27-8/2	33.11	22.00	0.00	5.00	21.02	21.14
8/3-8/9	33.36	23.04	1.57	5.00	27.75	20.00
8/10-8/16	30.63	19.01	0.00	5.00	25.34	20.60
8/17-8/23	27.84	17.69	11.33	0.00	7.63	18.70
8/24-8/30	29.33	16.76	0.00	0.00	21.09	20.10
8/31-9/6	32.39	21.21	0.00	8.71	20.61	18.28
9/7-9/13	28.67	17.29	6.21	0.00	5.10	17.67
9/14-9/20	27.10	18.13	0.29	0.00	24.77	14.87
9/21-9/27	29.04	14.93	0.00	0.00	39.71	17.81
9/28-10/1	26.33	15.40	0.00	0.00	40.00	15.02
Overall Average	**30.62**	**19.70**	**2.97**	**2.51**	**15.94**	**14.67**

Table A.4 Environment data for 2016.

Date	Average T_{max} (°C)	Average T_{min} (°C)	Average Rainfall (mm)	Average Irrigation (mm)	Average Water deficit (mm)	Average Solar Radiation (MJ m^{-2} d^{-1})
6/8-6/14	30.44	19.67	0.00	0.00	10.23	15.72
6/15-6/21	32.38	21.67	0.00	0.00	15.50	22.02
6/22-6/28	32.30	21.75	2.71	1.20	15.12	21.79
6/29-7/5	30.51	20.85	10.06	0.00	7.19	20.74
7/6-7/12	30.61	22.53	3.00	0.00	10.40	18.82
7/13-7/19	31.50	19.84	4.86	0.00	14.39	22.14
7/20-7/26	33.97	23.70	7.23	5.36	23.48	20.71
7/27-8/2	32.37	22.40	1.36	0.00	16.24	20.09
8/3-8/9	33.84	23.44	0.26	3.57	29.20	20.05
8/10-8/16	29.70	21.14	1.50	3.57	23.84	17.25
8/17-8/23	28.23	18.10	3.24	0.00	24.47	18.50
8/24-8/30	31.30	21.20	6.71	4.29	9.80	18.03
8/31-9/6	28.17	17.60	0.00	3.57	14.19	16.84
9/7-9/13	30.61	18.47	0.71	3.57	17.41	18.12
9/14-9/20	30.54	18.11	5.49	0.00	6.29	17.57
9/21-9/27	31.10	16.70	0.00	0.00	25.57	5.24
Overall Average	**31.11**	**20.64**	**2.70**	**1.44**	**16.12**	**16.85**

Table A.5 Environment data for 2017.

Date	Average T_{max} (°C)	Average T_{min} (°C)	Average Rainfall (mm)	Average Irrigation (mm)	Average Water deficit (mm)	Average Solar Radiation (MJ m^{-2} d^{-1})
6/8-6/14	29.03	18.46	0.00	0.00	11.65	21.68
6/15-6/21	29.93	18.87	5.26	0.00	10.88	21.92
6/22-6/28	29.11	18.37	1.23	0.00	17.23	22.02
6/29-7/5	28.96	20.31	7.84	0.00	8.64	19.60
7/6-7/12	32.07	21.41	4.03	0.00	10.12	21.67
7/13-7/19	32.60	22.21	0.00	3.14	23.85	21.09
7/20-7/26	34.46	23.14	0.00	11.43	14.96	21.79
7/27-8/2	29.23	20.09	2.98	0.00	9.96	18.89
8/3-8/9	28.50	18.75	4.75	0.00	15.43	19.34
8/10-8/16	27.95	20.44	13.21	0.00	4.06	16.31
8/17-8/23	31.45	20.86	6.42	0.00	11.93	19.09
8/24-8/30	28.56	16.92	0.00	5.29	24.23	19.40
8/31-9/6	27.82	15.78	0.00	5.29	25.11	18.97
9/7-9/13	26.91	11.94	0.00	5.29	17.77	20.27
9/14-9/20	30.20	17.67	3.59	0.00	8.51	17.55
9/21-9/27	30.52	19.16	0.87	0.00	29.86	15.90
9/28-10/1	25.39	12.56	0.00	0.00	40.00	16.16
Overall Average	**29.72**	**18.86**	**2.88**	**1.75**	**15.11**	**18.50**

Table A.6 Pearson correlations among canopy coverage (CC), seed growth rate (SGR), effective filling period (EFP), seed yield (YIELD), nitrogen concentration (NC), ureide concentration (UC), canopy wilting (WLT), canopy temperature (CT), seed weight (SDWT), and harvest index (HI) evaluated on the SoyNAM parental genotypes for the year 2015, N=41.

Traits	CC	SGR	EFP	YIELD	NC	UC	WLT	CT wet†	SDWT	HI
CC	1									
SGR	-0.09	1								
EFP	0.16	-0.79**	1							
YIELD	-0.03	-0.61**	0.47**	1						
NC	-0.07	-0.05	0.11	-0.04	1					
UC	-0.24	0.32*	-0.20	-0.42**	0.61**	1				
WLT	-0.24	0.16	-0.18	-0.07	-0.01	0.003	1			
CT wet†	0.20	0.41**	-0.23	-0.72**	0.08	0.18	0.24	1		
SDWT	0.25	0.54**	-0.11	-0.42**	-0.06	0.25	-0.16	0.39*	1	
HI	-0.13	-0.59**	0.32*	0.68**	0.25	-0.13	-0.003	-0.45**	-0.47**	1

*, ** indicate significance at $P = 0.05$ and 0.01 levels, respectively.

† For all measurement dates there were no visible drought symptoms.

Table A.7 Pearson correlations among canopy coverage (CC), radiation use efficiency (RUE), seed growth rate (SGR), effective filling period (EFP), seed weight (SDWT), harvest index (HI), seed yield (YIELD), nitrogen concentration (NC), ureide concentration (UC), nitrogen derived from atmosphere (NDFA), canopy wilting (WLT), canopy temperature (CT), carbon isotope ratio ($\delta^{13}C$), and nitrogen fixation rate (NFR) evaluated on the SoyNAM parental genotypes for the year 2016, N=41.

Traits	CC	RUE	SGR	EFP	SDWT	HI	YIELD	NC	UC	NDFA	WLT	CT	$\delta^{13}C$	NFR
CC	1													
RUE	0.20	1												
SGR	0.20	0.27	1											
EFP	-0.15	-0.11	-0.79**	1										
SDWT	0.36*	0.08	0.23	0.20	1									
HI	-0.23	0.03	-0.07	0.22	-0.12	1								
YIELD	-0.17	0.003	-0.05	-0.002	-0.40**	0.56**	1							
NC	-0.20	-0.30*	-0.14	0.07	-0.18	0.31*	-0.03	1						
UC	-0.21	-0.04	-0.02	0.22	0.21	0.39**	-0.10	0.22	1					
NDFA	0.10	0.13	0.08	-0.05	-0.05	-0.26	-0.30*	-0.15	0.04	1				
WLT	-0.13	0.16	-0.004	0.03	0.005	-0.11	0.09	0.11	-0.10	-0.05	1			
CT	0.22	-0.13	-0.13	0.23	0.39**	-0.34*	-0.70**	0.17	0.12	0.18	0.12	1		
$\delta^{13}C$	0.006	-0.08	0.06	-0.014	-0.06	0.13	0.015	-0.02	0.02	0.10	-0.25	0.07	1	
NFR	0.20	0.49**	0.08	-0.07	-0.09	0.20	0.016	0.42**	-0.09	0.09	0.10	0.04	-0.23	1

*, ** indicate significance at $P = 0.05$ and 0.01 levels, respective

Table A.8 Pearson correlations among canopy coverage (CC), seed growth rate (SGR), effective filling period (EFP), seed weight (SDWT), harvest index (HI), seed yield (YIELD), nitrogen concentration (NC), ureide concentration (UC), nitrogen derived from atmosphere (NDFA), canopy wilting (WLT), canopy temperature (CT), and carbon isotope ratio ($\delta^{13}C$) evaluated on the SoyNAM parental genotypes for the year 2017, N=41.

Traits	CC	SGR	EFP	SDWT	HI	YIELD	NC	UC	NDFA	WLT	CT wet†	^{13}C:^{12}C
CC	1											
SGR	-0.10	1										
EFP	0.33*	-0.84**	1									
SDWT	0.20	0.28	0.16	1								
HI	-0.21	-0.10	-0.05	0.01	1							
YIELD	0.38*	-0.50**	0.39*	-0.24	0.14	1						
NC	-0.13	0.07	-0.04	0.16	0.43**	-0.01	1					
UC	0.43**	0.003	0.04	0.13	0.37*	0.36*	0.44**	1				
NDFA	0.54**	-0.10	0.09	-0.07	0.12	0.26	0.11	0.28	1			
WLT	0.17	-0.09	0.03	-0.07	0.21	0.36*	0.15	0.27	0.19	1		
CT wet†	-0.28	0.19	-0.23	-0.09	0.16	-0.42**	0.05	-0.11	-0.13	-0.50**	1	
$\delta^{13}C$	0.39**	-0.006	0.11	0.20	0.08	0.31*	0.05	0.38*	-0.03	0.22	-0.19	1

*, ** indicate significance at $P = 0.05$ and 0.01 levels, respectively.

† For all the measurement dates there were no visible drought symptoms

Table A.9 Growth stage R1 reaching dates for genotypes in 2015 averaged over replications.

Genotype	MG	R1 date
4J105-3-4	3	1-Jul
5M20-2-5-2	3	30-Jun
CL0J095-4-6	3	30-Jun
CL0J173-6-8	3	1-Jul
Clark Non-nod	4	4-Jul
HS6-3976	3	2-Jul
IA3023	3	30-Jun
LD00-3309	4	1-Jul
LD01-5907	3	1-Jul
LD02-4485	4	30-Jun
LD02-9050	2	30-Jun
LG00-3372	3	3-Jul
LG03-2979	3	30-Jun
LG03-3191	4	1-Jul
LG04-4717	3	30-Jun
LG04-6000	4	1-Jul
LG05-4292	3	30-Jun
LG05-4317	4	1-Jul
LG05-4464	4	30-Jun
LG05-4832	3	3-Jul
LG90-2550	3	1-Jul
LG92-1255	3	30-Jun
LG94-1128	2	30-Jun
LG94-1906	2	30-Jun
LG97-7012	2	30-Jun
LG98-1605	3	1-Jul
Magellan	4	30-Jun
Maverick	3	30-Jun
NE3001	3	30-Jun
PI 398.881	3	4-Jul
PI 404.188A	2	1-Jul
PI 427.136	3	4-Jul
PI 437.169B	2	2-Jul
PI 507.681B	3	4-Jul
PI 518.751	2	1-Jul
PI 561.370	2	30-Jun
PI 574.486	2	2-Jul
Prohio	3	4-Jul
S06-13640	4	1-Jul
Skylla	2	30-Jun
TN05-3027	5	30-Jun
U03-100612	1	30-Jun
4J105-3-4	3	1-Jul

Table A.10 Growth stage R1 and R5 reaching dates for genotypes in 2016 averaged over replications.

Genotype	MG	R1 date	R5 date
4J105-3-4	3	6-Jul	9-Aug
5M20-2-5-2	3	7-Jul	9-Aug
CL0J095-4-6	3	4-Jul	8-Aug
CL0J173-6-8	3	5-Jul	7-Aug
Clark Non-nod	4	6-Jul	-
Harsoy Non-Nod	2	4-Jul	2-Aug
HS6-3976	3	5-Jul	7-Aug
IA3023	3	6-Jul	6-Aug
LD00-3309	4	8-Jul	15-Aug
LD01-5907	3	6-Jul	8-Aug
LD02-4485	2	4-Jul	3-Aug
LD02-9050	4	6-Jul	11-Aug
LG00-3372	3	10-Jul	10-Aug
LG03-2979	3	4-Jul	6-Aug
LG03-3191	4	8-Jul	14-Aug
LG04-4717	3	6-Jul	5-Aug
LG04-6000	4	9-Jul	13-Aug
LG05-4292	4	7-Jul	15-Aug
LG05-4317	4	6-Jul	15-Aug
LG05-4464	3	5-Jul	6-Aug
LG05-4832	3	10-Jul	10-Aug
LG90-2550	3	7-Jul	5-Aug
LG92-1255	2	3-Jul	3-Aug
LG94-1128	2	5-Jul	5-Aug
LG94-1906	2	4-Jul	2-Aug
LG97-7012	3	8-Jul	4-Aug
LG98-1605	3	7-Jul	3-Aug
Magellan	4	6-Jul	12-Aug
Maverick	3	6-Jul	11-Aug
NE3001	3	6-Jul	1-Aug
PI 398.881	3	11-Jul	10-Aug
PI 404.188A	2	9-Jul	7-Aug
PI 427.136	3	9-Jul	7-Aug
PI 437.169B	2	7-Jul	2-Aug
PI 507.681B	2	10-Jul	3-Aug
PI 518.751	2	8-Jul	4-Aug
PI 561.370	3	7-Jul	3-Aug
PI 574.486	2	8-Jul	11-Aug
Prohio	3	9-Jul	9-Aug
S06-13640	4	14-Jul	20-Aug
Skylla	2	4-Jul	2-Aug
TN05-3027	5	6-Jul	11-Aug
U03-100612	1	4-Jul	1-Aug

Table A.11 Growth stage R1 and R5 reaching dates for genotypes in 2017 averaged over replications.

Genotype	MG	R1 date	R5 date
4J105-3-4	3	14-Jul	12-Aug
5M20-2-5-2	3	15-Jul	13-Aug
CL0J095-4-6	3	11-Jul	12-Aug
CL0J173-6-8	3	13-Jul	13-Aug
Harsoy Non-Nod	2	11-Jul	9-Aug
HS6-3976	3	15-Jul	13-Aug
IA3023	3	14-Jul	11-Aug
LD00-3309	4	16-Jul	13-Aug
LD01-5907	3	13-Jul	12-Aug
LD02-4485	2	11-Jul	8-Aug
LD02-9050	4	13-Jul	12-Aug
Lee Non-nod	6	-	-
LG00-3372	3	16-Jul	13-Aug
LG03-2979	3	11-Jul	11-Aug
LG03-3191	4	14-Jul	16-Aug
LG04-4717	3	13-Jul	10-Aug
LG04-6000	4	16-Jul	14-Aug
LG05-4292	4	14-Jul	14-Aug
LG05-4317	4	13-Jul	13-Aug
LG05-4464	3	12-Jul	12-Aug
LG05-4832	3	15-Jul	14-Aug
LG90-2550	3	13-Jul	11-Aug
LG92-1255	2	11-Jul	9-Aug
LG94-1128	2	13-Jul	11-Aug
LG94-1906	2	12-Jul	10-Aug
LG97-7012	3	13-Jul	7-Aug
LG98-1605	3	12-Jul	8-Aug
Magellan	4	11-Jul	12-Aug
Maverick	3	11-Jul	13-Aug
NE3001	3	13-Jul	8-Aug
PI 398.881	3	15-Jul	11-Aug
PI 404.188A	2	15-Jul	12-Aug
PI 427.136	3	16-Jul	13-Aug
PI 437.169B	2	13-Jul	6-Aug
PI 507.681B	2	16-Jul	11-Aug
PI 518.751	2	14-Jul	12-Aug
PI 561.370	3	13-Jul	12-Aug
PI 574.486	2	15-Jul	13-Aug
Prohio	3	15-Jul	14-Aug
S06-13640	4	15-Jul	17-Aug
Skylla	2	11-Jul	8-Aug
TN05-3027	5	15-Jul	13-Aug
U03-100612	1	12-Jul	6-Aug