

SURVEY OF PLANT DENSITY TOLERANCE IN U.S. MAIZE GERMPLASM

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

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Abstract

Global demand for cereal crops like maize is rising at a rapid pace as the world population expands beyond 7 billion people. To meet these needs, productivity (i.e. grain yield) per unit area must be increased. A survey of U.S. maize germplasm was conducted to identify sources of favorable alleles for plant density tolerance and better understanding the genetics involved. Hybrids created using a genetically diverse set of inbreds representing parentage of key heterotic sub-groups were evaluated at plant densities ranging from 19,000 plants per acre (ppA) to 54,000 ppA. Five categories of traits were hypothesized to be associated with plant density tolerance: photosynthetic capability, growth responses, source-sink relationship, general stress tolerance, and plant architecture. Fifty phenotypic traits from these five categories were evaluated in three environments that differed for levels of moisture availability. The relationship between plant density and grain yield was assessed for each hybrid, with a wide range of responses observed. Five hybrids showed substantial tolerance to plant densities $\geq 47,000$ ppA based on grain yield. Phenotypic trait correlations revealed a subset of traits associated with grain yield. Further analysis provided insight into relationships among traits that ultimately influence grain yield. All 5 categories of traits were found to have an association with grain yield directly and indirectly. Analysis of environments with differing moisture levels suggested that the 5 top-performing hybrids at high plant density have exceptional capacity for light utilization and translation of that energy into kernel mass. Estimates of heritability for grain yield at high plant densities were found to be similar to those at other plant densities, therefore requiring no alteration with breeding strategies used for new and improved maize lines. Results of this work will be used to

create plant materials for further characterization of the trait through QTL mapping and candidate gene approaches.

Acknowledgements

I would like to thank Dr. Rita Mumm for acting as my adviser and assistance during the research and preparation of my thesis. I would like to thank my committee members Dr. Fred Below, Dr. Bob Lambert, and Dr. Dick Johnson for their help and support. I would also like to thank Dr. Don Bullock, Dr. Don White, Dr. John Dudley, Dr. Andrew Hauck, and Dr. Christine Carbonero for their comments and support along the way. I am thankful for the contributions from my lab mates, with special thanks to Josh Macke and Dr. Xiaochun Sun, whose help has been invaluable over the years. Finally I wish to thank my family and friends, with special thanks to my parents and Kate for all the love and support.

Table of Contents

Introduction.....	1
Materials and Methods.....	12
Results and Discussion	20
Conclusions.....	33
References.....	34
Tables and Figures	43
Appendix.....	58

Introduction

Demand for cereal crops

Global demand for cereal crops is increasing at a rapid pace as world population expands beyond 7 billion people (Duvick and Cassman, 1999; Tilman et al., 2011; United States Census Bureau, 2012; USDA, 2011). Developing countries have more disposable income allowing for increased meat consumption, which requires more grain to feed livestock. At the same time, biofuel production is expected to increase (Edgerton, 2009) and will compete for available agricultural land. To meet these needs, productivity per unit area (i.e. grain yield) needs to be increased. This is particularly relevant to maize, which is one of the top three most important cereal crops in the world (Russell, 1991).

Grain yield gains resulting from plant breeding

Yield gains in the past 80 years have been attributed to plant breeding and improved cultural practices (Duvick, 2005a, b; Lee and Tollenaar, 2007; Tollenaar and Lee, 2002; Tollenaar and Wu, 1999). Yield and yield stability across a wide range of environments are some of the most important selection targets for a plant breeder (Duvick and Cassman, 1999; Fasoula and Fasoula, 2002; Moose and Mumm, 2008). Yield stability is defined as a hybrid's ability to maintain yield performance across diverse environments and multiple years (i.e. weather conditions). Because yield stability demands adaption to various growing conditions across a wide range of environments, hybrids with yield stability tend to have higher stress tolerance (Tollenaar and Lee, 2002) and demonstrate greater resource use efficiency (Ipsilandis and Vafias, 2005). Selection

of hybrids tolerant of high plant densities, for example, is one method to better improve resource use efficiency (Tokatlidis and Koutroubas, 2004).

Higher stress tolerance has allowed hybrids to reach more of their total yield potential (Duvick and Cassman, 1999; Fasoula and Fasoula, 2002). Total yield potential can be viewed as the maximum yield achieved under stress-free growing conditions and non-limiting resources (Tokatlidis and Koutroubas, 2004; Tollenaar and Lee, 2002). Various studies have suggested that breeders have not significantly changed yield potential, but they have increased stress tolerance which allows for more capture of the total yield potential (Duvick, 2005a, b; Tokatlidis and Koutroubas, 2004; Tollenaar and Lee, 2002; Tollenaar and Wu, 1999).

Several maize hybrid “era” studies have evaluated hybrids over a range of decades grown at different plant densities ranging from 4000 ppA up to 40,000 ppA (Duvick, 2005a; Echarte et al., 2000; Sangoi et al., 2002). Hybrids were evaluated under extremely low plant densities which represents a nearly stress-free environment and assumes that, under these conditions, all hybrids were allowed to express maximum yield potential. Considering that plants grown in the same field basically compete for sunlight, moisture, and nutrients from the soil, increasing the plant population would induce stress upon the hybrids as they compete for basic requirements for growth. Results indicated that older hybrids have as much yield potential as newer, more elite hybrids when grown under stress free conditions (Duvick, 2005b). However, recent hybrids grown under stressful conditions (e.g. higher plant density) yield significantly more per unit area, demonstrating that breeders have made progress in selecting for greater stress tolerance (Echarte et al., 2000; Russell, 1991; Sangoi et al., 2002). Greater stress tolerance allows

hybrids the opportunity to achieve total yield potential under a wider range of environmental conditions (Carlone and Russell, 1987; Duvick and Cassman, 1999; Ipsilandis and Vafias, 2005). With more plants per acre, productivity per unit area is increased under the inclination of maintaining yield per-plant.

Yield gains due to cultural practices: plant density

Increased plant density is an important cultural practice that has impacted grain yield in the Corn Belt (Duvick, 2005b; Lashkari et al., 2011; Sangoi, 2000). Plant density has increased from 12,000 ppA in the 1930s (Duvick, 2005a, b) to an estimated average for harvest plant stands in the U.S. Corn Belt of 28,500 ppA in 2010 (USDA-NASS 2010, 2011a, b) with a range of 25,000 to 42,000 ppA depending on the region (Coulter et al., 2010; Iowa State University Extension, 2009; Nafziger, 2008; Thomison, 2011; Van Roekel and Coulter, 2011, 2012).

At the same time grain yield per acre has increased from 20.5 bushels per acre (bu/A) in 1930 to 152.8 bu/A in 2010 (USDA-NASS, 2012). Plant density and grain yield per unit area have trended together in an increasing fashion over time since 1930 (Brekke et al., 2011a) (Figure 1). Based on this historic trend, one way to continue to increase grain yield per unit area is to increase the plant density (Brekke et al., 2011a).

There is substantial genetic variation for plant density tolerance (Sarlangue et al., 2007). Some hybrids yield more as plant density is increased while others exhibit no increase or even yield loss (Duvick and Cassman, 1999; Grassini et al., 2011; Hashemi et al., 2005; Monneveux et al., 2005). Plant breeding, particularly selection for high yield and yield stability, has indirectly improved stress tolerance which has led to higher optimum plant densities (Fasoula and Fasoula, 2002; Tokatlidis and Koutroubas, 2004).

Plant density tolerance

Sunlight, water, and nutrients from the soil are basic requirements for plant growth. Utilization of these requirements is critical to achieve maximum yield (Tollenaar and Wu, 1999). Increased plant density intensifies interplant competition for these resources (Lashkari et al., 2011; Sangoi, 2000; Tollenaar and Wu, 1999). Stress is defined as a condition when any resource is limiting (Tollenaar and Lee, 2002). If any resource is limiting, as it may be under higher plant densities, yield may be altered at some point of deprivation (Hashemi et al., 2005).

For this experiment, a top-down approach was considered to plant density tolerance. Five categories of traits were hypothesized to be involved: photosynthetic capacity, growth response, source-sink relationship, general stress tolerance, and plant architecture. Traits from each category thought to be associated with plant density tolerance were identified.

Photosynthetic capacity

The photosynthetic capacity of a maize plant can be interpreted as the amount of sunlight that is being intercepted for conversion into assimilates. Light interception is critical for determining crop yield (Stewart et al., 2003). Rate of leaf expansion which influences the time of canopy closure, the amount of leaf area, and stay green are important factors that influence the amount and duration of light intercepted by the canopy (Tollenaar and Wu, 1999; Valentinuz and Tollenaar, 2006). Competition for light can occur before canopy shading begins from neighboring plants (Sattin et al., 1994), thus the plant canopy needs to capture light as efficiently as possible. One method to capture more light per unit area is to increase the number of plants per unit area; however

this increases competition for resources thus reducing the efficiency per plant. Rate of leaf expansion can influence the timing of the light intercepted by the plant. Increased leaf area allows more surface area to intercept light. However, higher leaf area can decrease efficiency if shading effects from other plants alter yield (Westgate et al., 1997). Longer stay green (slower rate of senescence) allows for light to be intercepted over a longer period of time (Hammer et al., 2009). Westgate et al. (1997) showed that increasing plant density to intercept more light is not sufficient to offset yield loss when hybrids are not radiation use efficient. However, plant density tolerance can improve resource capture efficiency which leads to higher yields (Dwyer et al., 1991; Sangoi, 2000; Tokatlidis and Koutroubas, 2004).

Growth response

Intra-specific competition at higher plant densities induces a gradual stress upon the plant throughout the growing season that may alter plant growth (Borras et al., 2003). Typically as plant density increases, plant growth rate during reproductive stages may become reduced (Echarte et al., 2000; Gambin et al., 2006; Rossini et al., 2011) leading to delayed pollen shed and silking within a given hybrid (Tokatlidis and Koutroubas, 2004). As plants intercept red light, far-red light is reflected creating a far-red light enriched environment. This leads to shade avoidance response causing plants to partition more assimilates towards vegetative growth instead of reproductive growth (Kebrom and Brutnell, 2007). As a result, plant height increases and stem diameter decreases (Lashkari et al., 2011; Sangoi et al., 2002). Brekke et al. (2011b) compared an unselected base population to a population that had undergone 17 cycles of selection for plant density tolerance. The authors found that the unselected population did not respond to

increased plant density while the selected population had a significant height increase as plant density increased. Furthermore, when environmental conditions favor slower plant growth rates (e.g. high plant density) barrenness may result (Ipsilandis and Vafias, 2005). This is due to the ear being a weak sink because of its axillary position subject to apical dominance (Sangoi et al., 2002). Assimilates are partitioned to the shoot rather than the ear which results in a higher chance that the ear will not become functional (Sangoi, 2000).

Source-sink relationship

The source sink ratio can be defined as the supply and demand of assimilates within a plant during grain fill (Rajcan and Tollenaar, 1999). This relationship can be greatly influenced by plant density. Increasing plant density influences this ratio by altering total light intercepted by the plant and effecting the final kernel number and kernel characteristics (Borras et al., 2003). Several studies have investigated the impact of increased plant density upon yield and yield components. Lashkari et al. (2011) reported that kernels per row, kernels per plant, and ear diameter decreased as plant density increased. Sangoi et al. (2002) reported reduced kernels per plant and a 16% decrease in kernel weight as plant density increased from 10,000 to 40,000 ppA. Likewise, Hashemi et al. (2005) reported that kernels per row and kernel weight both decreased as plant density increased, suggesting a complex interaction between the sink and assimilate supply. Gambin et al. (2006) reported drought and plant density greatly influence kernel growth rates and final kernel weights. Maddonni et al. (2006) reported smaller kernels as plant density increased. Widdicombe and Thelen (2002) reported minimal, but significant, increases in test weight as well as significant increases in grain

yield as plant density increased from 22,000 to 36,000 ppA. Test weight increased from 57.7 to 58.0 pounds per bushel; grain yield increased from 167.6 to 185.8 bu/A. As such, yield component traits are affected by plant density tolerance and therefore can impact productivity per unit area.

General stress tolerance

In any given season, a corn crop can be subject to general abiotic and biotic stresses such as excess/insufficient moisture, temperature, disease, insect pressure, and weed competition creating increased competition for resources (Carcova and Otegui, 2001; Stanger and Lauer, 2007; Tollenaar and Wu, 1999). A common general stress response is increased anthesis-silking interval (ASI), the period between 50% pollen shed and 50% silking (Brekke et al., 2011b; Daynard and Muldoon, 1983; Edmeades and Daynard, 1979; Tokatlidis and Koutroubas, 2004). A longer ASI reduces the chance that all female spikelets will be pollinated and thus increases the potential for yield reduction (Carcova et al., 2000; Monneveux et al., 2005; Sangoi, 2000). Carcova and Otegui (2001) reported that under high plant density, improved kernel set lead to improved grain yield which was associated with a shorter ASI. Brekke et al. (2011b) found additional cycles of selection can reduce the ASI resulting in better stress tolerance.

As plant maturity is approached, the environment and plant density can influence lodging (Stanger and Lauer, 2007; Widdicombe and Thelen, 2002). Stanger and Lauer (2007) observed that as plant population increased from 26,000 to 50,000 ppA, stalk lodging increased from 6 to 18% which promotes the potential for yield loss at harvest. Improvement through selection is effective because there is considerable variation for

this trait present in maize (Brekke et al., 2011a; Sangoi et al., 2002; Sarlangue et al., 2007).

During early reproductive stages, general stresses which limit plant resources can influence yield by altering ear structure, kernel set, and grain fill (Hashemi et al., 2005; Iowa State University Extension, 2009; Sangoi et al., 2002; Seebauer et al., 2009; Westgate et al., 1997). For example, Lashkari et al. (2011) found ear length generally decreased as plant density increased, which was most likely a result of interplant competition for water and nutrients. Sangoi et al. (2002) reported that the inability to fill the ear also leads to yield loss under conditions where the environment has limited resources. Yield loss can also occur from abnormal ears (Nielsen, 2011) and incomplete kernel set (Carcova et al., 2000). An example would be paired spikelet collapse (referred to as the zipper effect), which results in an abnormal ear with incomplete kernel set, where entire rows on the underside of the ear failed to pollinate or were aborted. This stress response is generally associated with nitrogen deficiency, drought, or defoliation (Iowa State University Extension, 2009). All of these general stress responses can each contribute a small percentage to reduced grain yield under stress conditions, such as increased plant density (Borras et al., 2003).

Plant architecture

Plant architecture plays a role in how light is intercepted by the plant and can factor into assimilate partitioning. Leaf angle plays a key role in the amount of light that is intercepted by the canopy (Hammer et al., 2009; Lee and Tollenaar, 2007). Increased leaf angle increases the efficiency of intercepted light due to more even distribution of light within the canopy (Lee and Tollenaar, 2007; Tollenaar and Wu, 1999). However,

more vertical leaves require higher plant densities to maximize light interception (Lambert and Johnson, 1978). Brekke et al. (2011b) found that increasing plant densities does not affect leaf angle (i.e. a specific hybrid grown at 15,000 or 39,000 ppA in the same environment does not have significantly different leaf angles). However, their results from a long term breeding program for adaptation to high plant density found the unselected base population to have less vertical leaf angles in comparison to the 17th cycle of selection which had the most upright leaf angles. Leaf number has trended with leaf angle as the numbers of cycles for selections have increased. Brekke et al. (2011b) reported that leaf number has increased with cycles of selection. In contrast to leaf angle, leaf number does decrease slightly as plant density increases. The authors also compared plant height between the unselected base population and the 17th cycle of selection. They found mean plant height to be 240 cm for the base population and 200 cm for the 17th cycle for selection.

Grain yield is a product of the female inflorescence (ear) in maize. The tassel is the male inflorescence. Tassels and ears are initially bisexual (Cheng et al., 1983), this is to say that upon initiation they are physiologically the same. As they develop stamen primordia abort on the ear while gynoecium abort on the tassel causing the differentiation between the male and female inflorescence (Cheng et al., 1983). Due to the physiological relationship between male and female inflorescence, tassel characteristics were evaluated for this study.

Decreased tassel size is generally associated with increased yield potential (Duvick and Cassman, 1999). Decreased tassel size could influence yield in several ways. A smaller tassel size is suggestive of more assimilate partitioning towards the ear

(Sangoi, 2000). Sangoi (2000) reported that a smaller tassel produces less auxin, which decreases auxin derived apical dominance, thus minimizing the effect of apical dominance on the axillary bud (ear). Monneveux et al. (2005) also found a negative association between tassel size and grain yield. Sangoi (2002) found that modern hybrids allocate ~20% less dry matter to the tassel. Smaller tassels require less photoassimilates from the plant to support their development. Efficient assimilate partitioning is critical as plant density is increased (Monneveux et al., 2005). Monneveux et al. (2006) reported hybrids with a reduced tassel size and reduced root biomass in the upper 50 cm of rooting depth exhibited increased drought tolerance and greater rooting depth. This suggests plants with reduced biomass in organs (tassels and upper roots) that are capable of competing with the ear for assimilates during development have greater grain yield (Bolanos et al., 1993). Lambert and Johnson (1978) reported tassels with fewer branches was associated with increased grain yield under intense cultural practices such as increased plant density, perhaps due to reduced shading from the tassel.

Research justification

Numerous studies have investigated the influence of various traits on grain yield. Various studies focus on specific categories of traits or specific combination of traits from a few categories such as leaf area or leaf angle. This experiment investigates plant density tolerance by hypothesizing that traits from each category may play a role in plant density tolerance.

This study is a first step to identifying sources of favorable alleles for plant density tolerance and better understanding the genetics involved. The specific objectives were to (A) identify top performing germplasm (hybrids, inbred, heterotic subgroups)

which yield well at high plant densities, (B) evaluate changes in grain yield from low to high plant density in hybrids representing genetic diversity in the U.S. commercial germplasm base to investigate the genetic relationship between plant density and grain yield in various heterotic sub-groups, (C) investigate the relationship of various traits with grain yield as plant densities change, (D) evaluate the effect of environment on performance for plant density tolerance, and (E) estimate heritability of grain yield at high plant density for use in formulating breeding strategies for increased plant density tolerance. Results will be used to proceed with further characterization of the trait through QTL mapping and candidate gene approaches.

Materials and Methods

Germplasm

The hybrids evaluated in this study were created from a genetically diverse set of inbreds that represent important heterotic subgroups in the current US maize commercial germplasm base. Mikel and Dudley (2006) compiled a database of maize lines for which Plant Variety Protection had expired by 2004, assessing the number of times each line was used as an ancestor in developing new elite inbreds. From this work, 12 inbreds were identified as representing a vast amount of the genetic diversity in the current maize commercial germplasm pool (Johnson, 2008). Two inbred lines are public lines whereas the other 10 inbred lines were developed by Pioneer Hi-Bred International or Holden's Foundation Seeds (Table 1). Four of the inbreds are Stiff Stalk Synthetic (SSS) while the other eight are of Non-Stiff Stalk (NSS) background. With the aim to identify hybrids with potential for high yield under high plant density, all SSS were crossed to all NSS lines in a North Carolina Design II mating scheme to produce 32 SSS by NSS hybrid combinations. Hybrid combinations of SSS by SSS and NSS by NSS were not considered because these lines were not expected to yield well due to the genetic relationships between both inbred parents.

Experimental design

The 32 hybrids, along with 4 commercial checks (Table A1) and the 12 inbred parents, were grown in an incomplete block split plot design with three replications in three environments. Due to plant height and vigor differences between inbreds and hybrids, the inbreds were grouped separately from the hybrids in the field. Each

replication consisted of 16 incomplete blocks with 3 entries, the entries being either all hybrids or inbreds. With entries as whole plots, each entry was grown at plant densities of 19,000, 26,000, 33,000, 40,000, 47,000, and 54,000 ppA. The lower plant densities of 19,000 and 26,000 ppA represent the planting densities of the time frame that these lines were originally produced, and were considered in combination as ‘low’ density. The plant densities of 33,000 and 40,000 ppA represent the range of currently recommended plant densities used across the Corn Belt, and were considered in combination as ‘medium’ density. Plant densities of 47,000 and 54,000 ppA were chosen as upper bounds to push past the optimum plant density for all hybrid combinations, and were considered in combination as ‘high’ density. Plant densities were randomized within entries to maximize precision in evaluating response across densities for each entry. The experiment was grown in three different environments across two years: Environment 1 (Env1) in 2010 at Urbana, IL, was irrigated and early planted; Environment 2 (Env2) in 2010 at Urbana, IL, was non-irrigated and late planted; Environment 3 (Env3) in 2011 at Urbana, IL was grown under non-irrigated conditions. Rain gauges were placed at each location and monitored after every weather event that produced precipitation. Two rain gauges were placed at different locations in the irrigated location to ensure there was no irrigation gradient across the field. In the irrigated environment, the main pipe was laid on 30 foot centers with risers 8 feet tall to reach above the corn canopy. Sprinkler irrigation was used to supplement water as needed. The three environments differed for planting date and moisture availability (e.g. weekly rainfall or supplemented water) (Table 2). Env1 was not water stressed during flowering, whereas Env2 experienced limited water supply one week prior to flowering and Env3 experienced limited water

supply throughout most of the reproductive stages (Table 2). At all environments, the previous year's crop was soybean. Prior to planting, the seed bed was prepared by deep tillage in the fall, followed by one pass with a field cultivator in the spring.

Nitrogen (N) was applied pre-plant in the form of 28% urea-ammonium nitrate (UAN). A rate of 300lbs/acre of nitrogen was applied to the fields uniformly. Likewise, phosphorous (P) and potassium (K) were applied pre-plant at a rate of 100lb/acre over recommended levels as determined by soil testing done by the University of Illinois Crop Science Research and Education Center. N, P, and K levels were held at a high consistent rate across plots and environments to ensure that these nutrients were non-limiting across the trial and not confounded with plant density. This is essential because the focus of this experiment is to identify hybrids coping with high density stress aside from nutrient efficiency.

Preemergence herbicides were applied and incorporated 10 days ahead of planting. Guardsman Max/Aatrex mixture was used to control weeds to eliminate interspecific competition. In addition to herbicidal control, plots were kept weed free by means of hand hoeing for the remainder of the growing seasons. Force 3G insecticide was applied in the furrow at planting for rootworm control.

Plots were comprised of four rows, with observations recorded from only the center two rows to minimize potential effects due to height and maturity differences from neighboring plots. The plot length was 17.5' which is approximately 1/1000 of an acre. The 2010 trials were planted with a John Deere 7000 series cone planter with 30 inch row spacing. The 2011 trial was planted with an Almaco Seed Pro 360 planter with 30 inch row spacing. Plots were over planted at a higher density and then thinned at V1-V2 to

ensure consistent and accurate plant stands were achieved. The plots were thinned in order to prevent additional interplant competition above desired levels to minimize early plant growth responses to shading beyond the specified plant density for the plot. Stand counts were verified at V5 and recorded.

Trait collection

Entries were observed and evaluated for multiple traits from each of the five categories of traits thought to be associated with plant density tolerance (Table 3). Vigor, days to canopy closure, days to pollen shed, and days to silking were collected before/during R1. All other traits were collected post-flowering.

To estimate leaf area, three plants per plot were destructively sampled for leaf tissue collection post-pollination. All leaves were removed from the stalk and counted to obtain the leaf number count. The sheath was separated from the leaf tissue. Leaf tissue was divided into two parts: ear leaf and all leaves above the ear. All leaves above the ear were considered to be upper canopy leaf area. Leaf area was measured in square centimeters using a Li-Cor 3100 leaf area machine. The combined ear leaf and upper canopy was considered to be total leaf area. Due to a large number of plots, total leaf area and ear leaf area were collected from a subset of plots across the trials. In Env1, the 26,000, 40,000, and 54,000 density plots were evaluated for total leaf area and the ear leaf area was evaluated in the 33,000 and 47,000 plant densities. Ear leaf area was evaluated in all plots in Env2; total leaf area was evaluated in all plots in Env3. Nonetheless, consistency in competition and plant stand was maintained in keeping with other environments by removing three plants from all plots. A regression model for estimation of un-sampled leaf area was developed using the selection option in SAS 9.2

Proc Reg (SAS Institute, 2009). Ear leaf area, maturity, and plant height were used as independent variables to construct models for prediction of total leaf area. All traits previously mentioned were used independently and in combination to obtain multiple regression models for estimating total leaf area. The best model was selected based on a low Akaike information criterion (AIC) indicating goodness of fit and a r-square of 0.8514 indicating a large portion of the variation in the data set is accounted for by the model (Kutner et al., 2004). Ear leaf area alone was found to produce the best regression model of total leaf area:

$$\text{Total leaf area} = 0 + (5.2251 * \text{Ear Leaf Area})$$

The regression model was trained using Env3 data and then validated with data from Env1. This model was used to estimate total leaf area for unsampled plots. Total leaf area per plant was multiplied by plant stand to determine an estimate of leaf area per plot. Leaf area per plot was divided by pounds of grain produced to determine leaf area required to produce 1 pound of grain.

Lower stem diameter was measured above the second node with a Tresna digital caliper. Due to variations within the stalk, two measurements were taken. The first measurement was used as a base line with the second measurement recorded after a 90 degree turn of the caliper. Upper stem diameter was measured on the 3rd internode below the flag leaf with a Tresna digital caliper. Lower and upper measurements of the stem allow for assessment of stalk size at different stages of growth.

Statistical analysis

The experiment was designed as a randomized incomplete block split plot with 3 replications. The linear model used for statistical analysis of traits for hybrids was

$$y_{ijklm} = \mu + E_i + R_{(i)j} + B_{(ij)k} + G_l + GE_{il} + GB_{(ij)kl} + D_m + ED_{im} + RD_{(i)jm} + BD_{(ij)km} + GD_{lm} + EGD_{ilm} + GDB_{(ij)klm}$$

where

- y_{ijklm} = observation for the i^{th} environment within the j^{th} rep within the k^{th} block for the l^{th} hybrid with the m^{th} plant density
- μ = overall mean
- E_i = fixed effect of the i^{th} environment ($i=1,2,3$)
- $R_{(i)j}$ = random effect of the j^{th} replication nested in the i^{th} environment ($j=1,2,3$); pooled for Error 1
- $B_{(ij)k}$ = random effect of the k^{th} block nested within the j^{th} replication and i^{th} environment ($k=1,2,\dots,12$); pooled for Error 1
- G_l = fixed effect of the l^{th} hybrid ($l=1,2,\dots,36$)
- GE_{il} = fixed interaction between the i^{th} environment and the l^{th} hybrid
- $GB_{(ij)kl}$ = random interaction between the k^{th} block, nested in the j^{th} replication and i^{th} environment, and the l^{th} hybrid; Error 2
- D_m = fixed effect of the m^{th} plant density ($m=1,2,3,4,5,6$)
- ED_{im} = fixed interaction between the i^{th} environment and m^{th} plant density
- $RD_{(i)jm}$ = random interaction between the j^{th} rep nested in the i^{th} environment and the m^{th} plant density; pooled for Error 3
- $BD_{(ij)km}$ = random interaction between k^{th} block, nested in the j^{th} replication and i^{th} environment, and the m^{th} plant density; pooled for Error 3
- GD_{lm} = fixed interaction between l^{th} hybrid and m^{th} plant density
- EGD_{ilm} = fixed interaction between i^{th} environment, l^{th} hybrid, and m^{th} plant density
- $GDB_{(ij)klm}$ = random interaction between k^{th} block, nested in the j^{th} replication and i^{th} environment, and the l^{th} hybrid, and the m^{th} plant density; pooled for Error 3

The data was analyzed using the MIXED procedure featured in SAS 9.2 (SAS Institute, 2009). Individual environments were evaluated for homogeneous variance and upon confirmation; a combined analysis across environments was conducted. Simple linear regression of grain yield on plant density was performed for each hybrid combination to evaluate hybrid yield responses to increased plant density. Mean grain yield was calculated for each hybrid at high, medium, and low density and across all densities for each environment and across all environments. Hybrids were ranked by

mean yield using Tukey's method of multiple comparisons to evaluate differences between hybrids. Phenotypic Pearson correlation coefficients were calculated using the CORR procedure of SAS 9.2 for each trait with grain yield to evaluate the relationship as density was increased. Phenotypic correlations were calculated across all test hybrids, plant densities, and environments. Traits exhibiting some phenotypic correlation with grain yield were evaluated as well as phenotypic correlations between traits. Genetic correlations (r_A) were calculated as defined in Falconer (1989):

$$r_A = \frac{cov_{xy}}{\sqrt{var_x var_y}}$$

where

cov_{xy} = covariance between trait x and trait y

var_x = additive variance of trait x

var_y = additive variance of trait y

The cov_{xy} was calculated by first creating a dummy variable, $x + y$

where

x = observed value for trait x

y = observed value for trait y

Additive variance components were obtained for trait x, trait y, and (x+y) using the VARCOMP procedure of SAS 9.2. The $var(x+y) = var_x + var_y + 2cov_{xy}$ (Isik, 2009); thus the cov_{xy} can be derived by solving the equation. Genetic correlations are associated with large standard errors because variance components which have large standard errors are used to calculate them (Bernardo, 2010); thus, r values can be outside of theoretical values (Isik, 2009).

The linear model for estimation of genetic effects was

$$y_{ijkl} = \mu + E_i + R_{(i)j} + G_k + G_l + S_{kl} + EG_{ik} + EG_{il} + ES_{ikl} + e_{ijkl}$$

where

- μ = overall mean
- E_i = random effect of the i^{th} environment (i=1,2,3)
- $R_{(i)j}$ = random effect of the j^{th} replication nested within the i^{th} environment (j=1,2,3)
- G_k = random GCA effect of the k^{th} parent (k=1,2,3,4)
- G_l = random GCA effect of the l^{th} parent (l=1,2,...12)
- S_{kl} = random SCA effect of the cross between parents k and l
- EG_{ik} = random effect for the interaction between GCA of parent k and the i^{th} environment
- EG_{il} = random effect for the interaction between GCA of parent l and the i^{th} environment
- ES_{ikl} = random interaction effect between SCA of parents k and l and the i^{th} environment
- e_{ijkl} = random error of observation $ijkl$

The data were analyzed using the MIXED procedure of SAS 9.2. Heritability estimates for grain yield were calculated as outlined in Zare et al. (2011) in keeping with Teklewold and Becker (2005).

Broad sense heritability:

$$H^2 = \frac{2\sigma_{GCA}^2 + \sigma_{SCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + \left(\frac{2\sigma_{GCA \times E}^2}{Env}\right) + \left(\frac{\sigma_{SCA \times E}^2}{Env}\right) + \left(\frac{\sigma_{error}^2}{Env * Rep}\right)}$$

Narrow sense heritability:

$$h^2 = \frac{2\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + \left(\frac{2\sigma_{GCA \times E}^2}{Env}\right) + \left(\frac{\sigma_{SCA \times E}^2}{Env}\right) + \left(\frac{\sigma_{error}^2}{Env * Rep}\right)}$$

Results and Discussion

Analysis of variance for grain yield concluded the main effects genotype and plant density to be significant ($\alpha = 0.0005$) indicating variation within the germplasm and significantly different responses between plant densities (Table A2). The three way interaction between environment, genotype, and plant density was non-significant ($\alpha = 0.10$)(Table A2).

Top performing hybrids

The distribution of mean hybrid grain yields across all plant densities distinguished the performance of current commercial checks as expected (Figure 2), reflecting the progress of corn breeding in recent decades. The overall mean grain yield was 173.8 bu/A, with a range of 132.9 to 236.0 bu/A. Five test hybrids were set apart from the others (Figure 2) on the basis of mean grain yield by Tukey's multiple comparison test.

Specifically at high density, mean grain yield broadened with a range of 123.3 to 248.1 bu/A, suggesting wider variance of plant density tolerance. Nonetheless, the ordering by performance did not change greatly. The commercial checks remained in top rankings for mean grain yield (Figure 3). Looking specifically at test hybrids, 5 hybrids yielded significantly higher according to the Tukey test ($\alpha = 0.05$) than all other test hybrids. At high density, the 5 top yielding hybrids were PHG39xLH82, PHG39xPHG47, PHJ40xPHG84, B73xLH82, and B73xPHG47 (Table 4). Overall, the mean performance of test hybrids at high density was 167.4 bu/A with a range of 123.3 to 215.3 bu/A, indicating significant genetic variation for the trait among a diverse set of SSS by NSS hybrid crosses.

To assess the general contribution of particular inbreds which represent different heterotic subgroups, the mean grain yields at high density for hybrids can be compared to the overall hybrid mean grain yield by inbred (Table 5). The SSS inbreds contributing to the top hybrid grain yields at high density were PHG39, PHJ40, and B73, respectively. The NSS inbreds contributing to the top hybrid grain yields at high density were LH82, PHG47, and PHG84 (Table 5). These inbreds comprise the parents of the 5 top-performing hybrids at high density exclusively (Table 4). These inbred lines are representative of various heterotic subgroups (Lee and Tollenaar, 2007), thus suggesting specific heterotic subgroups with favorable alleles for high density tolerance.

Relationship of grain yield and plant density

For each of the 32 test hybrids, grain yield was regressed on plant density to evaluate each genotype's ability to produce more grain as the number of plants per acre was increased. A positive slope (b_1) expressing a linear relationship would signal the ability to achieve higher yields with higher plant densities. Twenty-one hybrids exhibited no significant response of grain yield across plant densities ($H_0: \beta=0$; $\alpha = 0.05$) (Table 6). Nine hybrids exhibited a highly significant positive change in grain yield as plant density increased ($H_a: \beta \neq 0$; $\alpha = 0.01$). In addition, two hybrids exhibited a significant negative change in grain yield as plant density increased ($H_a: \beta \neq 0$; $\alpha = 0.05$ or 0.01).

To further evaluate the relationship between grain yield and plant density for the top-performing hybrids at high density, quadratic responses as well as linear responses were graphed for each of the top 5 hybrids (Figure 4). Whereas a significant linear response estimates an increase in grain yield proportional to a given increase in plant

density, a quadratic response can provide insight as to where the optimum plant density is achieved for a specific hybrid and there is no longer yield gains per unit area due to increased plant density. Although the hybrid shows a gain up to a certain plant density, yield loss per unit area begins to occur after the optimum level of plant density is exceeded. Considering the top 5 hybrids (featured in order of ranking by mean grain yield at high density), only the first (PHG39xLH82) exhibits a statistically significant quadratic response (Figure 4). However, graphs suggest that PHG39xPHG47 and PHJ40xPHG84 have capacity to increase grain yield with higher plant densities beyond the scope of this study. PHG39xPHG47 just begins to plateau at high density while there is no plateauing observed for grain yield as plant density is increased for PHJ40xPHJ84. In contrast, B73xLH82 and B73xPHG47 both exhibit rather stable responses in general across all densities, and this is reflected in the lack of significance of both the linear and quadratic parameter estimates. This suggests that these hybrids have less capacity for increased grain yield as plant densities push higher.

Mean slopes were calculated across all hybrid combinations by inbred (Table 6). Three SSS inbreds exhibited mean slopes greater than zero, with PHJ40 exhibiting a mean slope >1 . Likewise, six NSS inbreds exhibited a mean slope >0 , with LH82 and PHG47 exhibiting mean slopes >1 . LH123HT, MO17, and LH1 exhibit negative mean slopes across hybrid combinations.

Of the top 6 inbreds contributing to high yield under high plant density (PHG39, PHJ40, B73, LH82, PHG47, and PHG84), not all exhibited a high mean slope across all hybrid combinations (Table 6). For example, B73 had a mean slope of 0.32 and PHG84 had a mean slope of 0.35. For these, specific combining ability for high yield under high

plant density was great whereas for inbreds like PHJ40 and LH82, general combining was more prominent. Based on indications of the greatest general combining ability, PHJ40 and PHG39 on the SSS side appear to provide the most promise of favorable alleles for high density tolerance, although clearly B73 possesses some. Likewise, among NSS lines, LH82 and PHG47 provide strong promise of favorable alleles for high density tolerance. Although PH207 exhibited a high slope as evidence for increased yield as plant density was increased, it did not display a comparable high level of overall performance for grain yield (Tables 5 and 6). Whereas PHG84 exhibited good performance, it was inconsistent in response to increased plant density in hybrid combination, suggesting that it may provide some favorable alleles but will likely not be foundational to developing improved plant density tolerance.

Traits associated with plant density tolerance

As outlined above, the five top-performing hybrids at high density exhibit a positive relationship between grain yield and plant density (i.e. as plant density increases, so does grain yield for each of these genotypes). Traits correlated with grain yield across plant densities would suggest traits and categories of traits that may underlie plant density tolerance. The third objective is to identify traits that are associated with grain yield performance across the span of plant densities. Genetic correlations were calculated for traits associated with grain yield (Table A6). Due to the large standard error associated with these estimates, phenotypic correlations were evaluated for the rest of this study.

Of 50 phenotypic traits measured in this work, most exhibited no phenotypic correlation with grain yield. However, 12 traits were found to be significantly correlated with grain yield ($\alpha = 0.0001$), 7 positively and 5 negatively (Table 7). Number of

rows per ear, number of kernels per plant, individual plant yield, number of days to canopy closure, stay green, number of kernels per row, and ear width were positively correlated with grain yield (i.e. as grain yield increased, these traits also increased). On the other hand, ASI, root lodging, zipper effect, the percentage of barren plants, and leaf area to produce one pound of grain were negatively correlated with grain yield (i.e. as grain yield increased, these traits decreased). Across the 12 traits significantly correlated with grain yield, several categories of traits are represented, supporting the theory that many factors underlie plant density tolerance.

Number of kernel rows per ear and grain yield are phenotypically correlated at $r = 0.45$ (Table 7). Rows per ear are determined early in the growing season, by approximately V7 (Abendroth et al., 2011). This is significant because it emphasizes that plant density tolerance comes into play early in plant development, even before intense shading from neighboring plants begins. While highly statistically significant, the correlation is not strong. However, when plotting values (Figure 5), there is an apparent trend which suggests there is a relationship with plant density tolerance, but further investigation is needed before firm conclusions can be made. However, to investigate this trait farther, phenotypic correlations were ran between the number of rows per ear and other traits (Table 8). Again, all 5 categories of traits appear to impact this trait. Nine traits showed highly significant association with number of kernels rows per ear; 6 positive and 3 negative. Kernels per plant, lower stem diameter, leaf angle, tassel branch number, ear leaf area, and kernels per row were positively correlated, while ASI, zipper effect, and kernel width were negatively correlated with rows per ear.

Kernels per plant and grain yield are phenotypically correlated at $r = 0.41$ (Table 7). Other studies have also found kernel number to be associated with final grain yield under high plant density and other stress conditions (Bolanos and Edmeades, 1996; Echarte et al., 2000; Sangoi et al., 2002). To understand factors influencing kernels per plant, traits correlated with kernels per plant were investigated (Table 9). Here again, all 5 categories of traits appear to be influencing this trait. Eight traits were positively correlated with kernels per plant while 4 traits were negatively correlated. Kernels per row, rows per ear, individual plant yield, fill length, lower stem diameter, tassel weight, upper stem diameter, and days to canopy closure were all positively correlated while barrenness, ASI, zipper effect, and leaf area to produce 1 pound of grain were negatively correlated. Kernels per row was phenotypically correlated at $r = 0.82$. Kernels per row are determined approximately one week prior to flowering (Abendroth et al., 2011). Kernel rows per ear was phenotypically correlated at $r = 0.72$, which was previously mentioned to be determined early in the growing season. This indicates that not only is early season plant density tolerance important, but mid-season plant density tolerance is also important to insure yield components are not reduced due to competition which would influence final grain yield. The combination of rows per ear and kernels per row may be a significant combination in plant density tolerance. These findings suggest that hybrids with high plant density tolerance may be tolerant of early and mid-season stress from high plant-to-plant competition that can trigger changes to ear structure. Thus unaltered kernel set (i.e. no significant reduction in rows per ear and/or kernels per row) would allow more kernels per plant, which would support high grain yield under high plant density.

Days to canopy closure and grain yield are phenotypically correlated at the $r = 0.38$ (Table 7). A component of grain yield is the ability of the leaves to intercept light and turn it into usable energy for the plant. How soon leaves appear and develop to intercept the light is important, thus one practice is to increase plant density to capture more light sooner (Nafziger, 2008). The findings here are in contrast to the notion of capturing light as early as possible through rapid canopy closure; however, these findings are of significance. Prior work has shown that early canopy closure is ideal to capture more light early in the season. These findings suggest that under high plant densities, a delay in canopy closure is more beneficial. More erect leaves early in the growing season would allow for approximately the same light interception, but considerably less shading from neighboring plants. This finding suggests that the delay of interplant competition is important. To understand the impact of delayed canopy closure on other traits, traits associations were evaluated (Table 10). Kernels per row, kernels per plant, and fill length were all positively correlated. This suggests that by delaying shading effects, there is less kernel abortion leading to more kernels per plant. At the same time barrenness and the zipper effect were negatively correlated. This suggests that delayed shading does not inhibit or alter ear formation. This notion is in agreement with Westgate et al. (1997) where the authors concluded that hybrids prone to barrenness and low photosynthetic capacity will not benefit from early canopy closure.

Stay green was phenotypically correlated with grain with at $r = 0.32$ (Table 7). Stay green is an important factor influencing final grain yield. Poor stay green (higher rate of leaf senescence) under high plant density is associated with poor assimilate supply (Borras et al., 2003). The results here indicate that a longer stay green is associated with

higher grain yield. A higher stay green rating (slower rate of leaf senescence) would allow a longer duration of light interception thus not limiting light dependent assimilates (Borras et al., 2003). Therefore, the results suggest a slower rate of senescence could be important to improve yield potential under high plant density. This is in agreement with Duvick and Cassman (1999) where the authors found a reduced rate of leaf senescence to be associated with increased grain yield potential.

ASI is phenotypically correlated with grain yield at $r = -0.23$ (Table 7). Brekke et al. (2011b) concluded a shorter ASI to be a good adaptation for plant density tolerance. Sangoi et al. (2002) reported a shorter ASI can help reduce barrenness due to parallel plant development and improve kernel set. Carcova and Otegui (2001) also found similar results in that a short ASI improves kernel set which can improve final grain yield. The results here are in agreement with the previous work. The negative correlation between ASI and grain yield indicate that a shorter ASI is associated with higher grain yield. This is important because under high plant density, ear growth rate can be reduced (Rossini et al., 2011) leading to an increased ASI. Therefore, hybrids exhibiting a short ASI under high plant density would be an indicator for hybrids with general stress tolerance.

Root lodging and grain yield are phenotypically correlated with an $r = -0.25$. Hammer et al. (2009) reported that the root system architecture greatly influences water capture. The authors attributed greater water capture to enhanced growth rate. The significance of the negative correlation between root lodging and grain yield is that as grain yield decreased, root lodging increased which would indicate the plant is unable to capture water efficiently. An insufficient water supply means the plant has assimilate limitations that can lead to a slower growth rate. Hammer et al. (2009) concluded that

plants with an insufficient root system are more prone to a slower growth rate leading to an enhanced ASI, which as mentioned previously can alter kernel set leading to yield reductions.

The percentage of barren plants is phenotypically correlated with grain yield at an $r = -0.41$, that is to say that as grain yield per unit area increases, the percentage of barrenness decreases. The negative correlation found from this study indicates that barrenness can significantly reduce final yield per unit area. This is significant under high plant density because as plant density increases, competition for resources also increases which can lead to barrenness (Edmeades and Daynard, 1979; Sangoi, 2000). This is an important trait influencing plant density tolerance because as plant density increases, each plant needs to produce an ear to contribute to the final grain yield per unit area (Brekke et al., 2011a).

Leaf area to produce one pound of grain can be used as a ratio to estimate the efficiency of the plant at converting light to kernels. A large ratio would indicate low efficiency due to the large amount of leaf area required to produce 1 pound of grain while a small ratio would indicate high efficiency. This study found it be negatively phenotypically correlated with grain yield at an $r = -0.57$ (Table 7). This indicates that high grain yield is associated with high efficiency while low grain yield is associated with low efficiency. Dwyer et al. (1991) concluded similar results in that hybrids with high photosynthetic efficiency have higher grain yields. The significance is that this trait is an estimation of efficiency and efficiency is key for plant density tolerance due to the increase in competition for resources. Further investigation of this trait was done by evaluating correlations with other traits to gain an understanding of what traits are

directly impacting overall efficiency (Table 11). Four traits were positively correlated (trait increases with a decrease in efficiency) while 5 traits were negatively correlated (trait decreases with a decrease in efficiency). Percent barren plants, ASI, zipper effect, and percent root lodging were positively correlated while rows per ear, days to canopy closure, kernels per plant, kernels per row, and individual plant yield were negatively correlated. These results are significant in that they indicate general stress traits decrease the efficiency while source-sink traits decrease as the efficiency decreases. These results show increased barrenness, ASI, zipper effect, and root lodging all decrease efficiency (decrease the amount of grain yield per amount of leaf area). This has been noted in various studies that the previous traits all influence reductions in final grain yield (Duvick and Cassman, 1999; Ipsilandis and Vafias, 2005; Westgate et al., 1997). When looking at the negatively correlated traits, source sink traits, rows per ear, kernels per plant, kernels per row, and individual plant yield all decrease as the efficiency decreases (ratio becomes larger). This suggests where yield loss is coming from under decreased efficiency. The correlation with days to canopy closure is interesting here. The results indicate that rapid canopy closure decreases the efficiency. This is to say that when the canopy closes early, hybrids lacking efficiency for resource capture may have reduced yields. This is consistent with Hammer et al. (2009) and Westgate et al. (1997).

Comparison of responses in different environments

The three environments represented a range of moisture availability as water received by the plots as rainfall or irrigation differed by approximately 7 inches across the set: Env1 received 27.00" precipitation and/or water supplement, Env2 received 20.60" precipitation, and Env3 received 12.25" precipitation. Plants in Env1 exhibited no

visual symptoms of moisture stress during the season due to supplemented water provided by irrigation. This created a unique environment whereby competition among plants was for light only, not water or soil nutrients. The 5 top-performing hybrids under high density and the 5 worst-performing hybrids under high density were compared in each environment to evaluate the change in mean yield from low to high plant density. Notably, the top 5 hybrids all significantly increased ($\alpha = 0.10$) in yield in Env1 as plant density went from low to high (Table 12) whereas the 5 worst-performing hybrids showed no significant yield gain ($\alpha = 0.10$) and 1 hybrid even exhibited significant yield loss despite the supplemented water. Looking at the performance of these hybrids in Env2 and Env3 revealed the top performing hybrids exhibited no significant yield reductions, whereas the worst performing hybrids all exhibited yield loss as plant density increased from low to high.

Looking specifically at the top five hybrids, all exhibited significant increases in grain yield as plant density increased in Env1 suggesting outstanding light capture and utilization. Thus, the germplasm represented in the five top hybrids appears to have genes related to light use efficiency in environments with high competition for light, which is essential for grain yield. This finding suggests that alleles identified from the parental inbreds of the top five hybrids could combine well and perhaps even synergistically, with transgenic events being developed to impart drought tolerance efficiency. Drought tolerance could lower the water requirement for the crop. Thus, environments with water limitations could become more favorable due to the lower amount of water needed by the crop. Under favorable conditions, optimal plant densities can be pushed even higher (Tokatlidis et al., 2011).

Heritability of grain yield

Broad sense and narrow sense heritability of grain yield was calculated at each of the plant densities 19,000, 26,000, 33,000, 40,000, 47,000, and 54,000 as well as all combined densities (Table 13). Narrow sense heritability represents additive effects while broad sense heritability represents both additive and non-additive effects including dominance and epistasis. The estimate of narrow sense heritability for overall densities compares to estimates commonly obtained for grain yield. Furthermore, estimates of heritability are generally increased as plant density decreased. This evidences the reduced level of stress with lower plant densities, leading to higher repeatability of performance. However, heritability changes little as plant density increases. Moreover, a high level of non-additive effects was observed, noting the differentials between broad sense and narrow sense estimates in each case (Table 13), again as is typical with grain yield in maize which is subject to heterosis. Results suggest that no alteration in breeding methods relative to those that would be utilized for improving grain yield in general is warranted. In new line development, hybrid performance would be the primary indicator, not inbred performance.

Likewise, broad sense and narrow sense heritability of grain yield was calculated for each environment (Table 13). Estimates of heritability for the irrigated environment (Env1) were considerably higher. Thus, a highly managed Env1 facilitated a relatively lesser degree of environmental variation leading to greater repeatability of performance and higher values of heritability. This resulted because highly managed environments tend to have reduced genotype by stress interaction thus reducing the variability within the environment (Banziger and Cooper, 2001). As the environments became more

stressed, environmental variation increased thus leading to a lower repeatability and lower values for heritability. This is due to larger genotype by stress interactions thus leading to greater variability (Banziger and Cooper, 2001). Thus, environment conditions can impact heritability estimates (Smalley et al., 2004). The results from this study are in agreement with other findings indicating that environmental conditions impact heritability estimates.

Conclusions

The survey showed there is variation in the corn germplasm base to exploit for selection to improve plant density tolerance. In particular, 5 hybrids with substantial plant density tolerance were identified: PHG39xLH82, PHG39xPHG47, PHJ40xPHG84, B73xLH82, and B73xPHG47. As a result of this work, three female inbreds (PHG39, PHJ40, and B73) and three male inbreds (LH82, PHG47, and PHG84) were chosen to create a population to map QTLs for plant density tolerance. Given that all 5 categories of traits were found to have important associations with performance for grain yield as plant density is increased, it is anticipated that QTLs which represent genes associated with diverse metabolic pathways associated with photosynthetic capacity, plant growth and development, source-sink relationship, and adaptation and stress response will be identified. In particular, identified QTLs/genes will increase competitiveness for energy capture and translation of energy to kernel mass (yield), rather than competitiveness for water or soil nutrients. When water is non-limiting, the top 5 hybrids continued to exhibit increased grain yield as plant density increased indicating these materials perform well under high competition for light. Given that heritability of grain yield at high plant density is similar to that for grain yield under other circumstances, use of breeding strategies commonly employed in the development of new and improved maize lines should be effective for improving this ability. With the identification of QTLs/chromosomal regions that can be tracked with molecular markers through the breeding process, strategies for genomics-assisted selection would have a place in this future work.

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Tables and Figures

Table 1. Genetic background of the 12 inbred lines used as parents to create hybrids for evaluation. (SSS = Iowa Stiff Stalk Synthetic; NSS = Non Stiff Stalk Synthetic)

Line	Group	Assignee	Background
B73	SSS	None (Public)	Iowa Stiff Stalk Synthetic
PHG39	SSS	Pioneer Hi-Bred International	Maize Amargo/Iowa Stiff Stalk Synthetic; B37/B14 type
PHJ40	SSS	Pioneer Hi-Bred International	Iowa Stiff Stalk Synthetic
LH1	SSS	Holden Foundation Seeds	Iowa Stiff Stalk Synthetic; B37 type
Mo17	NSS	None (Public)	Lancaster
PH207	NSS	Pioneer Hi-Bred International	Iodent/Long Ear OPV/MINN13
LH82	NSS	Holden Foundation Seeds	Krug derived/W153 derived
PHG35	NSS	Pioneer Hi-Bred International	Oh07-Midland/Iodent/Linstrom Long Ear/Minn13
LH123HT	NSS	Holden Foundation Seeds	Pioneer Hybrid 3535
PHG84	NSS	Pioneer Hi-Bred International	Oh07-Midland/Minn13/Iodent/ReidYD/OsterlandYD/Lancaster/ Pioneer Female Composite OPV
PHG47	NSS	Pioneer Hi-Bred International	Oh43/Iodent*WF9/MKSDTA C10 Synthetic
PHZ51	NSS	Pioneer Hi-Bred International	Minn13/Iodent/ReidYD/OsterlandYD/Lancaster/ South US Land Race Synthetic/FunksG4949/Midland

Table 2. Field conditions of the 3 testing environments: location, planting date, harvest date, precipitation (inches), water supplement (inches; denoted by *), and flowering period (highlighted).

		Env1	Env2	Env3
Location		Urbana, IL	Urbana, IL	Urbana, IL
Plant Date		4/22/2010	5/6/2010	5/20/2011
Harvest Date		9/14-16/2010	10/7-8/2010	10/12,14/2011
Week	Week Number	Precipitation	Precipitation	Precipitation
April 4-10	1	-	-	-
April 11-17	2	-	-	-
April 18-24	3	0.2	-	-
April 25 - May 1	4	1.2	-	-
May 2-8	5	0.8	0	-
May 9-15	6	0.9	0.9	-
May 16-22	7	1.2	1.2	0
May 23-29	8	1*	0	3.55
May 30 - June 5	9	0.8	0.8	0.9
June 6-12	10	3.6	3.6	0.2
June 13-19	11	3.6	3.6	0.9
June 20-26	12	1.4	1.4	1.3
June 27 - July 3	13	1*	0	1.2
July 4-10	14	1*	0.7	0
July 11-17	15	1*	0.6	0.3
July 18-24	16	2.3	2.3	0.5
July 25-31	17	1*	0.2	0
August 1-7	18	1*	0.3	0
August 8-14	19	1.1*	0.6	0.2
August 15-21	20	1	1	0
August 22-28	21	1*	0	0.6
August 29 - Sept.4	22	1.7	1.7	0
Sept. 5 - 11	23	0.2	0.2	0.7
Sept. 12 - 18	24	-	0	0.4
Sept. 19 - 25	25	-	1.1	0.3
Sept. 26 - Oct. 2	26	-	0.4	1.2
Oct. 3 - 9	27	-	-	-
Oct. 10-16	28	-	-	-
Oct. 17-23	29	-	-	-
Total Water		27"	20.6"	12.25"

Table 3. Description, basis, and unit of measurement for the phenotypic traits collected.

Category	Trait	Basis	Description	Unit
Photosynthetic capacity	Seedling vigor	whole plot	V3-4 rating with 1 being the worst 10 being the best	rating
Photosynthetic capacity	Days to canopy closure	whole plot	Days to max light interception	days
Photosynthetic capacity	Ear leaf area	3 plants	Area of ear leaf	cm ²
Photosynthetic capacity	Upper leaf area	3 plants	Area of all leaves above the ear	cm ²
Photosynthetic capacity	Total leaf area	3 plants	Ear leaf area + all leaves above the ear	cm ²
Photosynthetic capacity	Est. total leaf area	derived	Estimate of leaf area for unsampled plots	cm ²
Photosynthetic capacity	Leaf area to prod. 1 pound of grain	derived	(Leaf area per plot) / Pounds of grain per plot	cm ²
Photosynthetic capacity	Stay green	whole plot	Rated 1 being the worst 10 being the best	rating
Growth response	Days to pollination	whole plot	Days to 50% plants shedding 10% pollen	days
Growth response	Days to silk	whole plot	Days to 50% plants with visible silks	days
Growth response	Plant height	whole plot	Distance from ground to node with flag leaf	cm
Growth response	Ear height	whole plot	Distance from ground to node with ear	cm
Growth response	Lower stem diameter	5 plants	Measured above 2nd node; 2 angles	mm
Growth response	Upper stem diameter	3 plants	Measured on 3rd internode below flag leaf	mm
Growth response	Barren plants	whole plot	Count of plants producing no kernels	count
Growth response	Percent barren	whole plot	Plants producing no kernels / plant stand	percent
Source-sink relationship	Bu/A	whole plot	Grain yield per plot at 15.5% moisture	bushel
Source-sink relationship	Test weight	whole plot	Sample adjusted to 15.5% moisture	lbs/bu
Source-sink relationship	Individual plant yield	whole plot	Pounds of grain per plot / plant stand	lbs
Source-sink relationship	Rows/ear	5 ears	Number of kernel rows	count
Source-sink relationship	Kernels/row	5 ears	Number of kernels per row	count
Source-sink relationship	Kernels/plant	5 ears	Rows/ear*kernels/row	count
Source-sink relationship	Ear width	5 ears	Width from the middle of the ear	mm
Source-sink relationship	Cob diameter	5 ears	Width from the middle of the cob	mm
Source-sink relationship	Cob weight	5 ears	Ear weight - shelled grain weight	g
Source-sink relationship	100K/volume	5 ears	100 kernels measured in graduated cylinder	mm ³
Source-sink relationship	100k/weight	5 ears	Weight of 100 kernels	g
Source-sink relationship	Kernel length	10 kernels 2x	Length of 10 kernels arranged tip to crown	mm

Table 3. Continued

Category	Trait	Basis	Description	Unit
Source-sink relationship	Kernel width	10 kernels 2x	Length of 10 kernels arranged side by side	mm
Source-sink relationship	Kernel depth	10 kernels 2x	Length of 10 kernels stacked on top of each other	mm
Source-sink relationship	Kernel size	10 kernels 2x	Length*Width*Depth	mm ³
General stress tolerance	Anthesis-silking interval	derived	Days to pollen - Days to silk	days
General stress tolerance	Stalk lodging	whole plot	Count of broken stalks below the ear	count
General stress tolerance	Percent stalk lodging	whole plot	Number of broken stalks/plant stand	percent
General stress tolerance	Root lodging	whole plot	Number of plants leaning more than 45 degree	count
General stress tolerance	Percent root lodging	whole plot	Leaning plants / plant stand*100	percent
General stress tolerance	Percent regular ears	5 ears	Percentage of ears altering row structure	percent
General stress tolerance	Zipper effect	5 ears	Percentage of aborted rows	percent
General stress tolerance	Ear length	5 ears	Length of whole ear including cob	mm
General stress tolerance	Fill length	5 ears	Length of ear with just kernels present	mm
Plant architecture	Leaf number	3 plants	Number of leaves in upper canopy including ear leaf	count
Plant architecture	Leaf angle	3 plants	Measured on 3rd leaf below flag leaf	degree
Plant architecture	Plant height	whole plot	Distance from ground to node with flag leaf	cm
Plant architecture	Ear height	whole plot	Distance from ground to node with ear	cm
Plant architecture	Tassel weight	5 plants	Weight of tassel	g
Plant architecture	Total spike length	5 plants	Length from lowest branch to tip of spike	cm
Plant architecture	Central spike length	5 plants	Length from highest branch to tip of spike	cm
Plant architecture	Tassel branch number	5 plants	Number of branch numbers	count
Plant architecture	Branching region	5 plants	Length from lowest branch to highest branch	cm
Plant architecture	Branch length	5 plants	Length of lowest, highest, and middle branch	cm
Plant architecture	Tassel size	5 plants	Total spike length + (branch number*Branch length)	score
Plant architecture	Tassel density	5 plants	Tassel Size/(1 + branch number)	score

Table 4. Top 5 hybrids for grain yield at high density.

Hybrid combination	Yield bu/A	Rank based on yield
PHG39xLH82	215.3	1
PHG39xPHG47	213.0	2
PHJ40xPHG84	200.5	3
B73xLH82	199.1	4
B73xPHG47	196.6	5

32 Hybrid Mean: 167.4 bu/A

32 Hybrid Range 123.3-215.3 bu/A

LSD: 23.1 bu/A; Alpha = 0.05

Table 5. Hybrid combinations mean grain yield at high plant density (bu/A).

Genotype	LH123HT	LH82	Mo17	PH207	PHG35	PHG47	PHG84	PHZ51	Mean
B73	166.8	199.1	143.1	167.7	155.8	196.6	163.5	161.3	169.2
LH1	144.6	174.8	155.4	130.4	123.3	156.0	165.7	160.5	151.3
PHG39	164.3	215.3	144.6	178.9	171.1	213.0	184.5	163.8	179.4
PHJ40	146.0	183.8	145.4	173.8	162.5	190.9	200.5	154.8	169.7
Mean	155.4	193.3	147.1	162.7	153.2	189.1	178.5	160.1	

Table 6. Slopes (b_1) reflecting the linear relationship between grain yield and plant density for hybrid combination and mean slopes across all hybrid combinations for each inbred. Slopes were tested for differences from 0.

Genotype	LH123HT	LH82	Mo17	PH207	PHG35	PHG47	PHG84	PHZ51	Mean
B73	-0.07	0.97	-0.85	0.79	0.67	1.09	-0.01	-0.05	0.32
LH1	-1.16**	0.68	-0.75	0.12	-0.78*	-0.20	-0.06	-0.45	-0.33
PHG39	-0.39	1.48**	-0.65	1.19**	1.03**	1.81**	-0.52	-0.10	0.48
PHJ40	0.57	1.45**	0.47	1.70**	1.25**	1.96**	2.00**	0.62	1.25
Mean	-0.26	1.14	-0.45	0.95	0.54	1.17	0.35	0.01	

Significance

*0.05

**0.01

Table 7. Phenotypic correlations between select traits and grain yield across plant densities and environments.

Trait	r value	Trait category
Rows/ear	0.45*	Source-sink relationship
Kernels/plant	0.41*	Source-sink relationship
Individual plant yield	0.39*	Source-sink relationship
Days to canopy closure	0.38*	Photosynthetic capacity
Stay green	0.32*	Photosynthetic capacity
Kernels/row	0.24*	Source-sink relationship
Ear width	0.22*	Source-sink relationship
Anthesis-silking interval	-0.23*	General stress tolerance
Root lodging	-0.25*	General stress tolerance
Zipper effect	-0.26*	General stress tolerance
Percent barren plants	-0.41*	Growth response
Leaf area for 1 lb. of grain	-0.57*	Photosynthetic capacity

Significance

*0.0001

Table 8. Phenotypic correlations between select traits and rows per ear across plant densities and environments.

Trait	r value	Trait category
Kernels/plant	0.72*	Source-sink relationship
Lower stem diameter	0.42*	Growth response
Leaf angle	0.37*	Plant architecture
Tassel branch number	0.34*	Plant architecture
Ear leaf area	0.31*	Photosynthetic capacity
Kernels/row	0.22*	Source-sink relationship
Anthesis-silking interval	-0.16*	General stress tolerance
Zipper effect	-0.24*	General stress tolerance
Kernel width	-0.65*	Source-sink relationship

Significance

*0.0001

Table 9. Phenotypic correlations between select traits and kernels per plant across plant densities and environments.

Trait	r value	Trait category
Kernels/row	0.82**	Source-sink relationship
Rows/ear	0.72**	Source-sink relationship
Individual plant yield	0.71**	Source-sink relationship
Fill length	0.63**	General stress tolerance
Lower stem diameter	0.59**	Growth response
Tassel weight	0.49*	Plant architecture
Upper stem diameter	0.37**	Growth response
Days to canopy closure	0.36**	Photosynthetic capacity
Percent barren plants	-0.21**	Growth response
Anthesis-silking interval	-0.28**	General stress tolerance
Zipper effect	-0.43**	General stress tolerance
Leaf area to produce 1lb of grain	-0.54**	Photosynthetic capacity

Significance

*0.0001

Table 10. Phenotypic correlations between traits and days to canopy closure across plant densities and environments.

Trait	r value	Trait category
Kernels/row	0.41	Source-sink relationship
Kernels/plant	0.36	Source-sink relationship
Fill length	0.33	General stress tolerance
Percent barren plants	-0.25	Growth response
Zipper effect	-0.31	General stress tolerance

Significance

*0.0001

Table 11. Phenotypic correlations between select traits and leaf area to produce 1lb of grain across plant densities and environments.

Trait	r value	Trait category
Percent barren	0.50*	Growth response
Anthesis-silking interval	0.49*	General stress tolerance
Zipper effect	0.40*	General stress tolerance
Percent root lodging	0.33*	General stress tolerance
Rows/ear	-0.33*	Source-sink relationship
Days to canopy closure	-0.34*	Photosynthetic capacity
Kernels/plant	-0.54*	Source-sink relationship
Kernels/row	-0.54*	Source-sink relationship
Individual plant yield	-0.74*	Source-sink relationship

Significance

*0.0001

Table 12. Yield change in bu/acre from low to high plant density by hybrid ranking (Hybrids ranked by grain yield at high density.)

Hybrid combination	Hybrid yield ranking	Env1	Env2	Env3
All 32 test hybrids		21.4*	-19.8*	-19.4*
PHG39xLH82	1	36.1*	1.6	8.5
PHG39xPHG47	2	56.7*	3.1	28.5*
PHJ40xPHG84	3	63.4*	38.4*	0.8
B73xLH82	4	23.5*	-14.2	-12.4
B73xPHG47	5	31.3*	-13.1	-21.3
PHG39xMo17	28	-17.1	-66.1*	-33.1*
LH1xLH123HT	29	-23.6*	-45.6*	-63.4*
B73xMo17	30	0.8	-57.7*	-68.2*
LH1xPH207	31	3.5	-18.7	-34.2*
LH1xPHG35	32	9.1	-43.2*	-50.3*

Significance

*0.10

Table 13. Variances for general combining ability (GCA), specific combining ability (SCA) and their interactions with the environment as well as narrow and broad sense heritabilities of grain yield at a) specific plant densities and b) across all plant densities by environment.

a) Plant density	GCA	SCA	GCA*E	SCA*E	Residual	Narrow sense	Broad sense
						h^2	H^2
19	158.25**	38.53	.	.	262.94	0.71	0.80
26	105.45***	31.71***	18.58*	31.34	253.26	0.70	0.82
33	109.38***	34.64**	38.94*	0.09*	407.50	0.66	0.77
40	153.25**	63.20**	34.17*	33.10	616.53	0.64	0.78
47	194.62*	97.87*	56.97*	1.24*	641.45	0.64	0.80
54	171.64*	172.40	112.29*	105.33*	207.77	0.48	0.73
All	106.64***	64.61***	42.56**	71.57	532.74	0.55	0.71
b) Plant density	GCA	SCA			Residual	h^2	H^2
Env1	168.42***	98.30***	.	.	267.57	0.64	0.83
Env2	167.23***	145.79***	.	.	411.08	0.48	0.70
Env3	119.58*	153.53***	.	.	868.86	0.35	0.58

Significance

*0.1

**0.01

***0.001

Figure 1. Plant density and yield have trended together over the past 80 years.

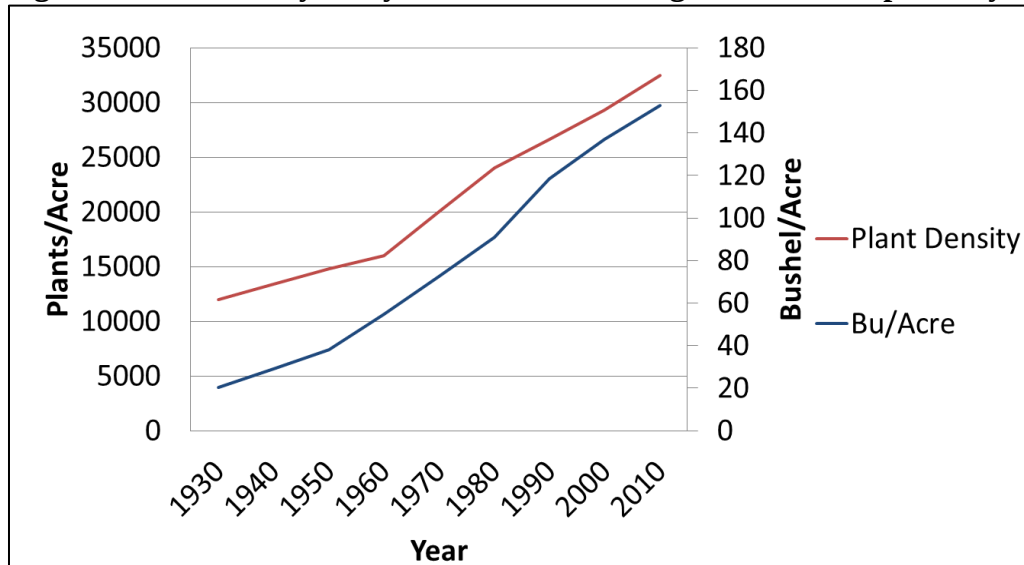
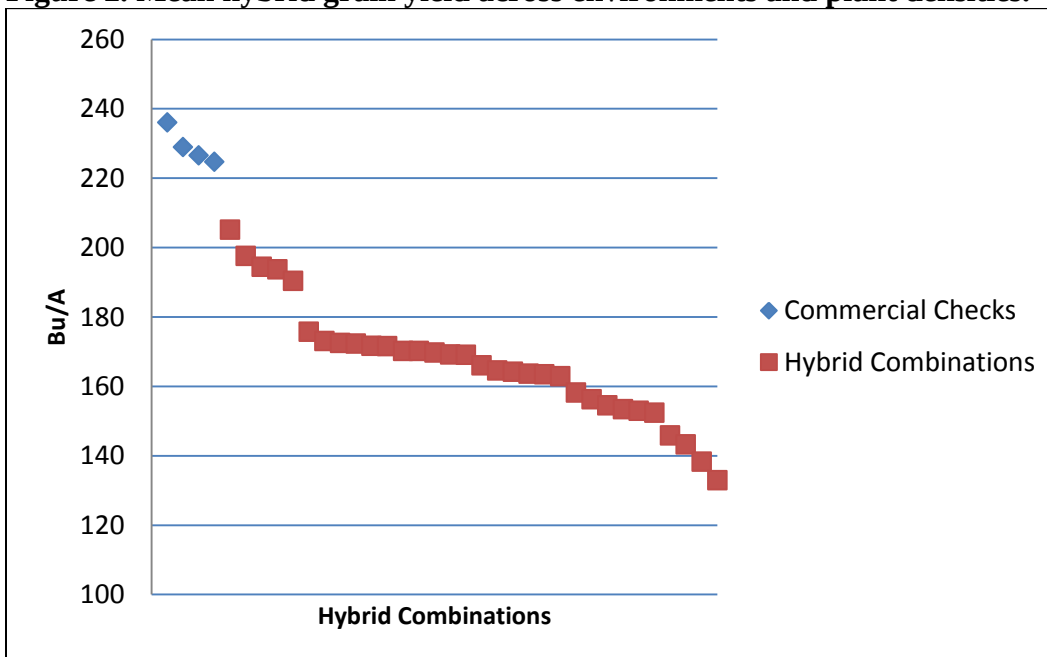


Figure 2. Mean hybrid grain yield across environments and plant densities.

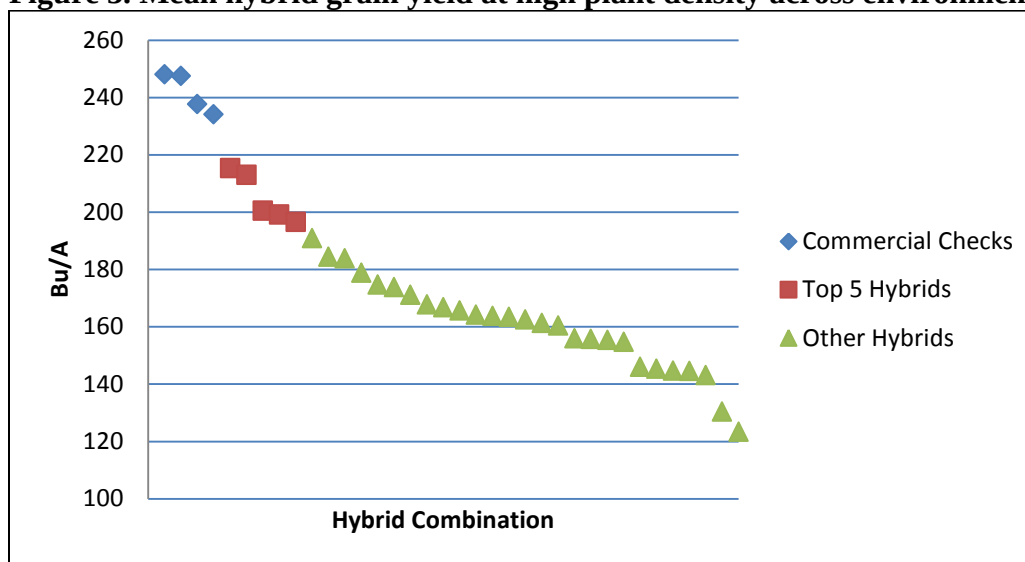


Hybrid mean grain yield: 173.8 bu/A

Hybrid range: 132.9 – 236.0 bu/A

LSD: 18.2 bu/A; Alpha = 0.05

Figure 3. Mean hybrid grain yield at high plant density across environments.



Hybrid mean grain yield: 175.5 bu/A

Hybrid range: 123.3 – 248.1 bu/A

LSD: 24.8 bu/A; Alpha = 0.05

Figure 4. Linear (solid lines) and quadratic (dotted lines) responses for grain yield of top 5 performing hybrids across plant density.

A: PHG39xLH82

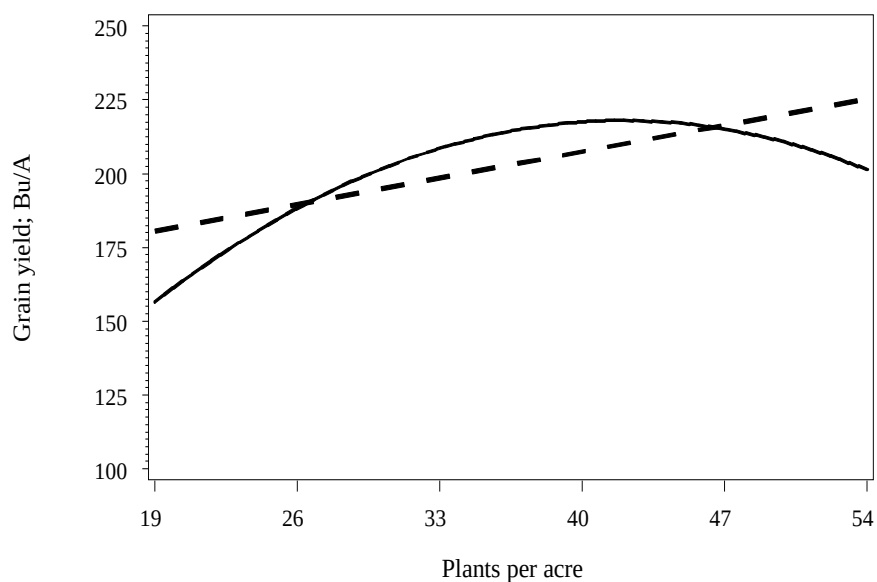


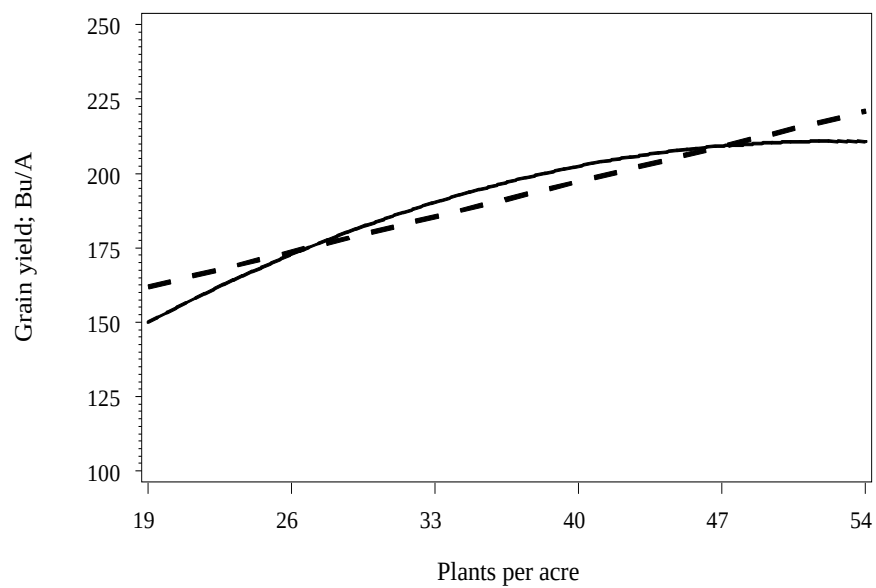
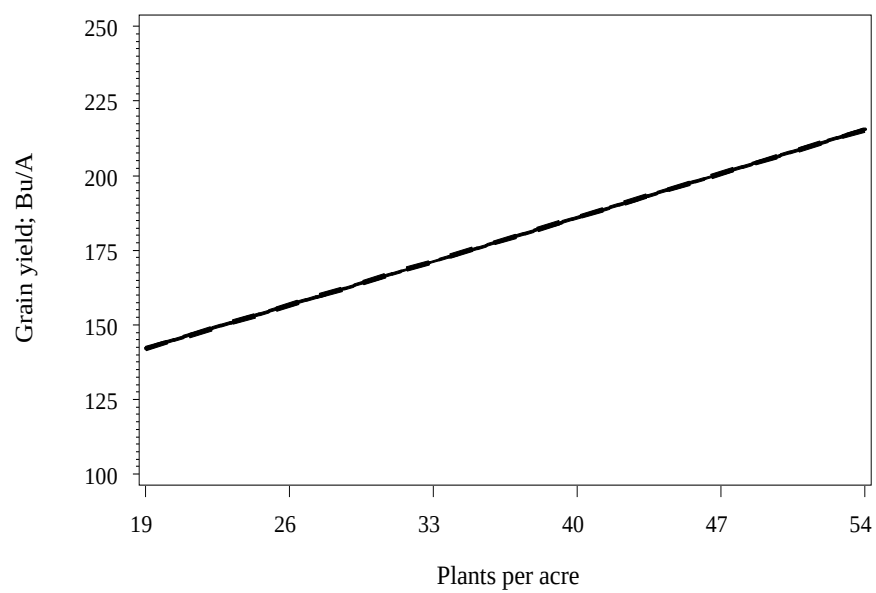
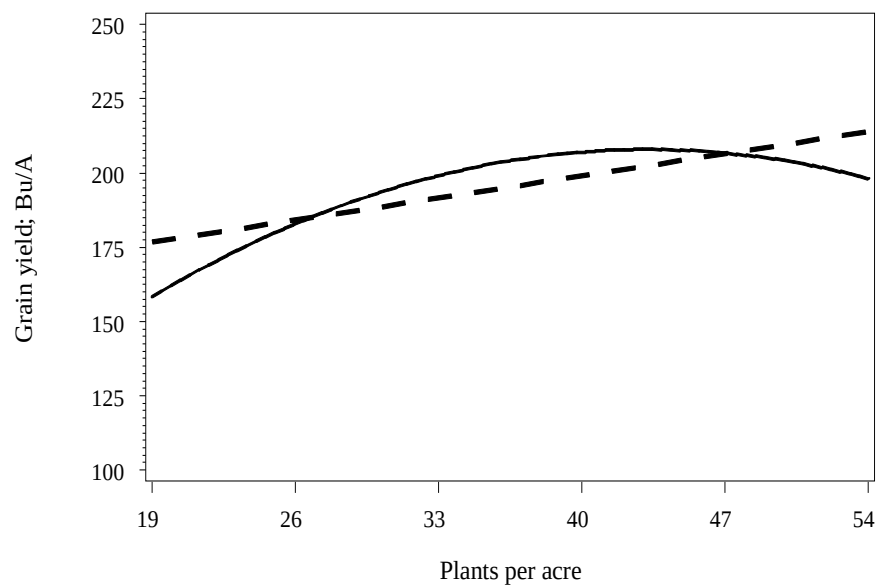
Figure 4. Continued**B: PHG39xPHG47****C: PHJ40xPHG84**

Figure 4. Continued

D: B73xLH82



E: B73xPHG47

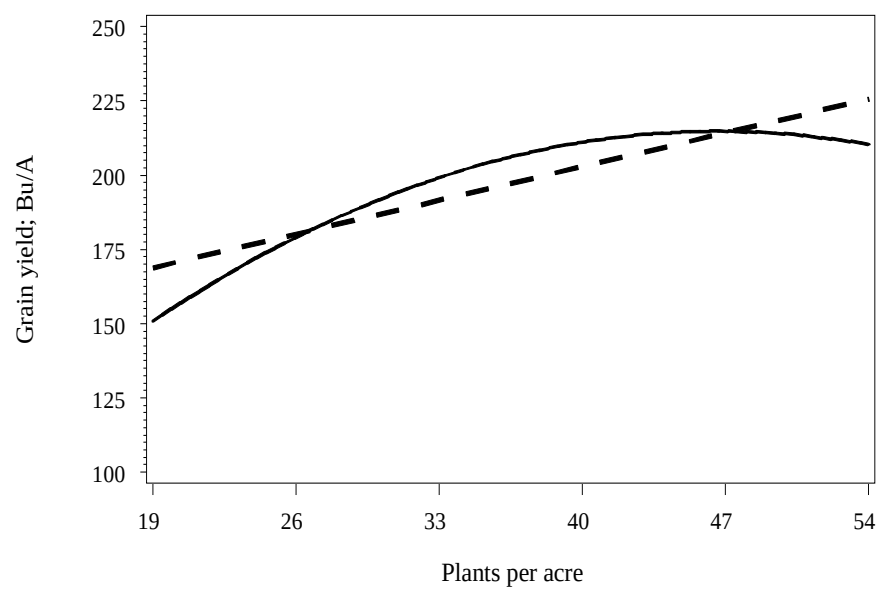
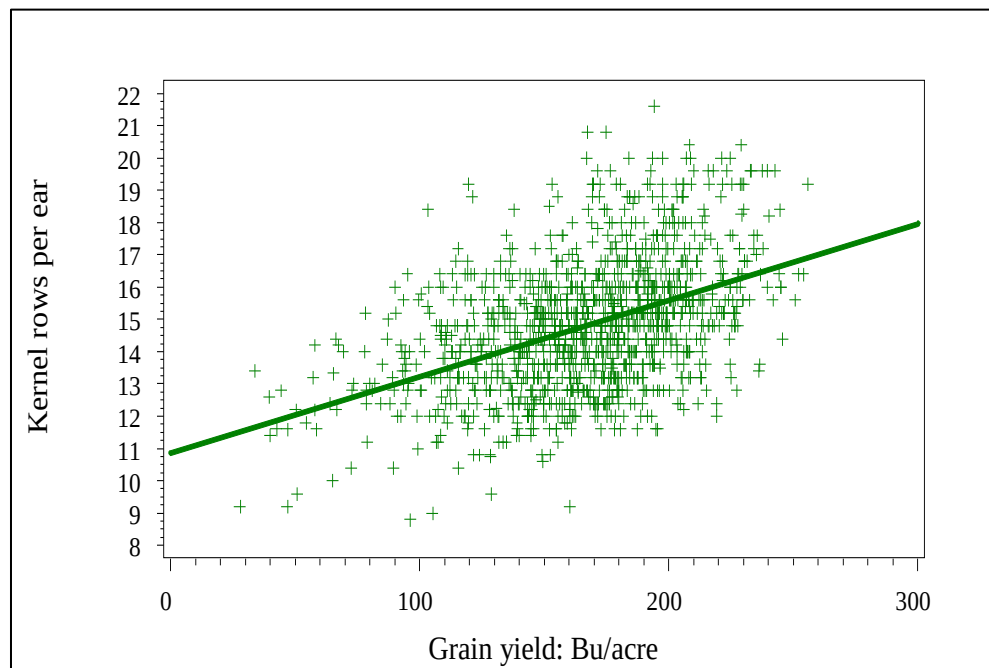


Figure 5. Rows per ear plotted against grain yield across environments and plant densities.



Appendix

Table A1. Brand, number, and transgenic trait resistance components of commercial checks.

Identification	Brand	Number	Transgenic Trait Resistances
Check 1	Pioneer	P1395XR	Corn borer, Corn rootworm, glufosinate, glyphosate
Check 2	Golden Harvest	H-853C	Corn borer, Corn rootworm, glufosinate
Check 3	Golden Harvest	H-8937	Corn borer, glufosinate
Check 4	DEKALB	DKC65-63	Corn borer, Corn rootworm, glyphosate

Table A2. Analysis of variance for the effects of environment, genotype, and plant density on grain yield across 3 environments.

Source of variation	df	Grain yield
Environment	2	0.0213
Rep	5	<.0001
Block	88	<.0001
Genotype	35	<.0001
Genotype x Environment	70	0.0001
Genotype x Block	85	<.0001
Density	5	0.0002
Environment x Density	7	<.0001
Rep x Density	20	<.0001
Block x Density	352	0.5557
Genotype x Density	175	<.0001
Environment x Genotype x Density	245	0.9027
Residual	329	.
Total	1419	

Table A3. Grain yield of 32 test hybrids at low density across environments.

Low Density		
Pedigree	Rank	Yield
PHG39xPHG84	1	197.0
PHG39xLH82	2	180.8
PHG39xLH123HT	3	180.5
B73xLH82	4	179.4
LH1xMo17	5	178.1
LH1xLH123HT	6	177.0
LH1xPHZ51	7	176.8
B73xPHG47	8	173.6
B73xLH123HT	9	173.0
B73xMo17	10	169.7
B73xPHG84	11	167.6
LH1xPHG84	12	167.3
PHG39xPHZ51	13	167.1
PHG39xPHG47	14	165.7
PHG39xMo17	15	163.7
B73xPHZ51	16	162.8
LH1xPHG47	17	161.8
LH1xLH82	18	160.2
PHJ40xPHG84	19	152.2
PHG39xPH207	20	151.6
B73xPH207	21	148.3
PHJ40xLH82	22	148.2
PHG39xPHG35	23	146.6
LH1xPHG35	24	145.1
B73xPHG35	25	144.0
PHJ40xPHZ51	26	142.7
PHJ40xPHG47	27	140.6
PHJ40xMo17	28	136.8
PHJ40xLH123HT	29	136.0
PHJ40xPH207	30	131.9
LH1xPH207	31	131.3
PHJ40xPHG35	32	131.1

Mean: 159.1 bu/A

LSD: 27.4; alpha = 0.05

Table A4. Grain yield of 32 test hybrids at medium density across environments.

Medium Density		
Pedigree	Rank	Yield
PHG39xLH82	1	214.1
PHG39xPHG84	2	208.7
B73xLH82	3	200.9
PHG39xPHG47	4	197.2
B73xPHG47	5	197.2
LH1xPHZ51	6	180.6
LH1xLH82	7	177.4
LH1xPHG47	8	176.7
B73xLH123HT	9	176.3
B73xPHG84	10	176.2
LH1xPHG84	11	175.8
PHG39xPH207	12	175.8
PHJ40xPHG47	13	175.3
PHJ40xPHG84	14	174.7
PHG39xPHZ51	15	174.6
PHG39xLH123HT	16	173.2
PHJ40xLH82	17	171.7
B73xPH207	18	170.8
PHG39xPHG35	19	168.7
B73xPHZ51	20	168.3
LH1xLH123HT	21	168.0
PHJ40xPH207	22	163.6
LH1xMo17	23	161.6
PHJ40xPHG35	24	159.2
PHJ40xPHZ51	25	158.6
B73xPHG35	26	158.3
B73xMo17	27	157.7
PHG39xMo17	28	156.4
PHJ40xLH123HT	29	152.4
PHJ40xMo17	30	145.9
LH1xPHG35	31	145.8
LH1xPH207	32	135.9

Mean: 171.9 bu/A

LSD: 23.3; alpha=0.05

Table A5. Grain yield of 32 test hybrids at high density across environments.

High Density		
Pedigree	Rank	Yield
PHG39xLH82	1	215.3
PHG39xPHG47	2	213.0
PHJ40xPHG84	3	200.5
B73xLH82	4	199.1
B73xPHG47	5	196.6
PHJ40xPHG47	6	190.9
PHG39xPHG84	7	184.5
PHJ40xLH82	8	183.8
PHG39xPH207	9	178.9
LH1xLH82	10	174.8
PHJ40xPH207	11	173.8
PHG39xPHG35	12	171.1
B73xPH207	13	167.7
B73xLH123HT	14	166.8
LH1xPHG84	15	165.7
PHG39xLH123HT	16	164.3
PHG39xPHZ51	17	163.8
B73xPHG84	18	163.5
PHJ40xPHG35	19	162.5
B73xPHZ51	20	161.3
LH1xPHZ51	21	160.5
LH1xPHG47	22	156.0
B73xPHG35	23	155.7
LH1xMo17	24	155.4
PHJ40xPHZ51	25	154.7
PHJ40xLH123HT	26	146.0
PHJ40xMo17	27	145.4
PHG39xMo17	28	144.6
LH1xLH123HT	29	144.6
B73xMo17	30	143.1
LH1xPH207	31	130.4
LH1xPHG35	32	123.3

Mean: 167.4 bu/A

LSD: 23.1; alpha=0.05

Table A6. Genetic correlations between selected traits and grain yield.

Trait	r value	Trait category
Rows/ear	0.46	Source-sink relationship
Kernels/plant	0.46	Source-sink relationship
Individual plant yield	1.98 [¶]	Source-sink relationship
Days to canopy closure	-0.10	Photosynthetic capacity
Stay green	0.52	Photosynthetic capacity
Kernels/row	-0.06	Source-sink relationship
Ear width	0.72	Source-sink relationship
Anthesis-silking interval	-0.53	General stress tolerance
Root lodging	0.00	General stress tolerance
Zipper effect	-0.24	General stress tolerance
Percent barren plants	0.00	Growth response
Leaf area for 1 lb. of grain	-0.69	Photosynthetic capacity

[¶] = Calculation produced a value outside of range; due to large standard errors.

Figure A1. Linear (solid line) and quadratic (dotted line) response for grain yield across plant density for the 32 test hybrids.

