AGRONOMIC AND MARKER-ASSISTED BREEDING STRATEGIES FOR IMPROVING NITROGEN USE EFFICIENCY IN MAIZE

BY

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DISSERTATION

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ABSTRACT

Nitrogen (N) is an essential mineral nutrient required for maize (*Zea mays* L.) development. Increased maize yields will be necessary as the world demand for food increases. However, with the growing concern for food security, maize yield increases must be obtained using more sustainable agricultural practices with less N fertilizer inputs. Improved nitrogen use efficiency (NUE) can maximize maize yield with minimal inputs of N fertilizer, but requires coordinated progress in several areas of the crop's production (e.g. genetic improvement, genetic characterization, and crop management). The objectives of this PhD research were i) evaluate the genetic variation of N-use traits and their interaction with the environment, ii) identify the genomic prediction accuracy of different N-use traits and their application in maize NUE breeding programs, iii) understand the relative merits of important agronomic factors (e.g. N stress tolerance, yield response to N fertilizer, crowding stress tolerance, and yield response to narrower row spacing) to maize yield, and iv) evaluate the importance of hybrid characterization for agronomic traits to obtain maximum maize yield potential. The research objectives were addressed using a large set of maize hybrids derived from expired plant variety protection- certified (named ex-PVP) and commercial germplasms and evaluating them in state-wide field experiments for different agronomic traits. In addition, all ex-PVP inbred lines were genotyped with 26,769 single-nucleotide polymorphism.

Field experiments evaluated the NUE performance of 522 maize hybrids derived from the genotyped ex-PVP inbreds. Genomic prediction accuracy for yield ranged from 0.17 to 0.53 and 0.17 to 0.72 under low and high N conditions (0 and 252 kg N ha^{-1}), respectively. The two major agronomic factors influencing a hybrid's NUE performance are the tolerance to N stress (yield under unfertilized N conditions) and the yield response to additional N fertilizer (yield change between unfertilized and N fertilized plots). However, yield under N stress conditions and yield response to N fertilizer are negatively correlated traits. As a result, less than 10% of all hybrids evaluated in this study combined above average performance for N stress tolerance and yield response to N fertilizer. Harvest index under low N was the secondary trait that was highly correlated (+0.63, $P \leq 0.001$) to yield under low N and provided the highest genomic prediction accuracy (ranged from 0.26 to 0.78) under low N conditions. Similarly, the yield response to N fertilizer (e.g. NUE) was highly correlated (+0.74, $P \leq 0.001$) to yield under high N and provided the highest genomic prediction accuracy (ranged from 0.05 to 0.51) under high N conditions. These traits could be integrated into maize breeding programs targeting for improved hybrid performance under N stress and high N conditions, respectively.

Maize yield stability and performance were influenced by N fertilizer and plant density conditions. Hybrids with above average yield performance under low N environments exhibited high yield stability under high N environments. On the other hand, hybrids with an above average yield response to N fertilizer and increased plant density exhibited greater yield in high N environments. Commercial hybrids showed a large variation in their yield response to different crop management conditions. In addition the level of N stress tolerance and the yield response to N fertilizer, the yield response to narrower row spacing was another important agronomic factor influencing maize yield. Maize genotypes grown under high N conditions, high plant density, and narrow row spacing (312 kg N ha^{-1}, 108,000 plant ha^{-1}, at 50 cm row spacing) combined high broad-sense heritability and yield performance. Therefore, breeding programs evaluating maize genotypes under intensive agronomic management conditions can obtain greater genetic gain. Future maize yield increases will rely on genotypes that combine improved yield response to N fertilizer and tolerance to high plant densities at narrower row spacing conditions. Accordingly, the characterization of maize hybrid’s responses to different agronomic factors gives farmers the knowledge to better match their hybrids with the recommended management to obtain the hybrid’s maximum yield potential.

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CHAPTER 1

GENETIC VARIATION OF NITROGEN USE TRAITS USING EXPIRED PLANT VARIETY PROTECTION GERMPLASM

ABSTRACT

Nitrogen use efficiency (NUE) in maize (*Zea mays* L.) is an important trait to maximize yield with minimal input of N fertilizer. Expired Plant Variety Protection (ex-PVP) Act- certified germplasm may be an important genetic resource for public breeding sectors. The objectives of this research were to evaluate the genetic variation of N-use traits and to characterize maize ex-PVP inbreds adapted to the U.S. Corn Belt for NUE performance. Eighty-nine ex-PVP and two public inbreds (37 stiff stalk synthetic, SSS and 54 non-stiff stalk synthetic, NSSS) were genotyped using 26,769 single-nucleotide polymorphisms, then 263 single-cross maize hybrids derived from these inbreds were grown in eight environments from 2011 to 2015 at two N fertilizer rates (0 and 252 kg N ha^{-1}) and three replications. Genetic utilization and the yield response to N fertilizer were stable across environments and were highly correlated with yield under low and high N conditions, respectively. Cluster analysis identified inbreds with desirable NUE performance. However, only one inbred (PHK56) was ranked in the top 10% for yield under both N-stress and optimal N conditions. Simultaneous genetic improvement for N-stress tolerance and the yield response to N fertilizer may be challenging since these traits are negatively correlated. Broad-sense heritability across 12 different N-use traits ranged from 0.11 to 0.77, but was not associated with breeding value accuracy. Breeding for improved NUE performance will require an integration of accurate field phenotypic evaluation and novel marker-assisted breeding techniques.

INTRODUCTION

World-wide, producers use approximately 84 million tons of nitrogen (N) fertilizer per year (FAOSTAT, 2002). Of that amount, more than 5 million tons are used for maize (*Zea mays* L.) production in the U.S. (USDA-NASS, 2013). Nitrogen is the macronutrient required in the greatest amount by the maize crop with uptake values measured at 280 kg N ha^{-1} for a crop producing 14.4 Mg ha^{-1} of grain (Bender et al. 2013). Although supplemental N fertilizer is often necessary to increase maize grain yield, N fertilizer consumption has remained constant in the U.S. for the last

20 years (FAOSTAT, 2002). The maize yield increases observed, despite the constant N fertilizer consumption in the U.S. during the last two decades, were a result of both genetic improvement and better agronomic practices (Duvick, 2005). In contrast, N fertilizer consumption in other agricultural regions, such as Brazil and China have greatly increased (+282 and +207%, respectively) during the last 20 years (FAOSTAT, 2002). The world population growth will require increased grain production and therefore more N fertilizer efficiency will be necessary to meet the world's demand (Cassman et al., 2002). Innovative agricultural technologies such as new N fertilizer sources, precision agriculture, and crop genetic improvement will be important to increase nitrogen use efficiency in maize production (Raun and Johnson, 1999).

Nitrogen use efficiency (NUE) is defined as the ratio of grain yield to N fertilizer supplied. Moll et al. (1982) defined NUE as the product of nitrogen uptake efficiency (NUpE, the ratio of the additional plant N content due to fertilizer N to the amount of fertilizer-applied N) and nitrogen utilization efficiency (NUtE, the ratio of yield increase to the difference in plant N content compared to those of an unfertilized crop). In addition, NUE is a complex phenotypic trait influenced by several plant physiological mechanisms (Moose and Below, 2009). Since most maize breeding programs developed their germplasm under high soil N conditions (Bertin and Gallais, 2001), genetic selection for improved NUE is often ignored (Kamprath et al, 1982). The genetic improvement of NUE in maize up to now was mainly achieved through indirect selection for increased hybrid yield performance (Moose and Below, 2009). Nonetheless, large genotypic differences in maize NUE have been reported (Bertin and Gallais 2001; Uribelarrea et al., 2007; Haegele et al., 2013).

Over the past few decades, maize hybrids in North America have increased yield performance under both low and high N availability conditions (Tollenaar et al., 1997), but the genetic gain of maize performance when grown under low N was almost twice the genetic gain found when hybrids were grown with high N fertility (Haegele et al., 2013). Genetic variation of NUE in maize has been attributed to hybrids expressing NUpE and NUtE at different levels (Presterl et al., 2002; Haegele et al., 2013). These N-responsive traits will contribute differently to NUE depending on the germplasm (Gallais and Coque 2005), the soil N status (Moll et al., 1982; Kamprath et al., 1982), and the progeny seed quality composition (Uribelarrea et al., 2007). Using the Illinois Protein Strain collection, Uribelarrea et al. (2007) reported that strain-hybrids with high seed protein concentration exhibited greater NUpE and lesser NUtE than strain-hybrids with low seed protein concentration. Phenotypic evaluation of NUpE and NUtE in a breeding population may be an important method to characterize and identify maize genotypes with desirable NUE performance (Lafitte and Edmeades 1995; Uribelarrea et al., 2007). Genetic improvement of NUE in U.S. germplasm using conventional or molecular breeding will require simultaneous enhancement of both NUpE and NUtE. As a result, more research is needed to evaluate the genetic characteristics underlying NUE in the U.S. Corn Belt germplasm.

Since the U.S. Plant Variety Protection (PVP) Act was passed in 1970, which protects seed-bearing varieties for 20 years, plant breeders have been generating new genetic combinations using only the most elite material available, thereby decreasing the genetic diversity of commercial breeding programs in the U.S. (Mikel and Dudley, 2006). Expired PVP Act- certified germplasm, named ex-PVP, are publically available and may represent an important genetic resource for both public and private breeding programs. Nelson et al. (2008) suggested that current U.S. germplasm has reduced allelic diversity and that most of the current germplasm originated from only seven progenitor lines B73, Mo17, PH207, PHG39, LH123Ht, LH82, and PH595. However, elite ex-PVP inbreds may be genetically diverse and an important genetic resource for maize breeding programs (Hauck et al., 2014). Although ex-PVP germplasm may not be integrated directly into a commercial breeding program, these genotypes can be used to originate new genetic combinations with desirable traits (Bari and Carena, 2016). Up to now, only a few agronomic and quantitative breeding research studies have been done using a representative number of maize ex-PVP parental lines and hybrid combinations.

The objectives of this research were to characterize ex-PVP maize hybrids for N-use traits, evaluate the genetic variation and the phenotypic correlation of different N-responsive traits across different maize heterotic groups, and identify parental lines and hybrid combinations with desirable NUE performance. General combining ability (additive gene effects) and specific combining ability (dominance and epistatic gene effects) were evaluated for different N-use traits on elite ex-PVP germplasm adapted to the U.S. Corn Belt. The identification of ex-PVP maize genotypes with high NUE performance could be incorporated into pre-breeding efforts for maize breeding population improvement and integrated into marker-assisted strategies for accelerating NUE improvement in maize breeding programs.

MATERIALS AND METHODS

Germplasm and genomic data

A collection of 89 ex-PVP and two public maize inbreds B73 and Mo17 were selected for this study (Table 1.1). All germplasm seed was obtained from the North Central Regional Plant Introduction Station (http://www.ars-grin.gov/npgs, verified 24 Aug. 2016). Twelve ex-PVP inbreds were selected that contain the majority of allelic diversity encountered in current U.S. maize germplasm (Hauck et al. 2014). In addition, a random set of inbreds adapted to the U.S. Corn Belt with more recently expired PVP certificates from a selection of seed companies were included. Findings from these most recently- released ex-PVP lines may reveal the genetic diversity shifts observed during the past 20 yr in germplasm usage by different breeding programs (Smith et al., 2004). Overall, the ex-PVP collection used for this study contains genotypes released from 1972 to 2011 developed by six different seed companies.

Leaf samples from all inbreds (14-day old seedlings) were collected for DNA extraction by Cole Hendrix in 2012. Inbreds were genotyped using the genotype by sequencing method (Elshire et al., 2011) and two enzyme combinations were used to reduce genomic complexity: PstI-HF,Bfal and PstI-HF, HinP1I. Sequenced data were obtained from Illumina HiSeq2000 (W.M. Keck Center for Comparative and Functional Genomics, Urbana, IL) and single-nucleotide-polymorphism (SNP) data were called using the GBS pipeline in TASSEL 3.0 (Bradbury et al., 2007). Minor allele frequency cutoff was set to 10%, and SNPs with more than 50% missing data were removed. A total of 26,769 SNPs were used for the analyses.

Discriminant analysis of principal components (DAPC) was performed for all inbred lines using the Adegenet package (Jombart et al., 2010) in R Studio (R Development Core Team, 2015). Since pedigrees from ex-PVP's are often vague (Nelson et al., 2008), DAPC is well suited to define genetic clusters in these situations (Jombart et al., 2010). Genotyping revealed that the ex-PVP germplasm used in this study was composed of 36 stiff-stalk synthetic (SSS) lines and 53 non-SSS (NSSS) lines, in which 19 lines were from the Iodent sub-heterotic group, and 34 lines were from the Lancaster sub-heterotic group (Figure 1.1). Knowledge of genetic relatedness between parental inbreds is fundamental for hybrid heterosis, due to dominance and epistatic effects (Bernardo, 2008). Therefore, all single cross maize hybrids evaluated in this study were generated between SSS and NSSS parental lines.

Hybrid seed were created in an incomplete factorial design between SSS and NSSS inbred lines in nursery trials from 2011 to 2014 at the University of Illinois, Department of Crop Sciences Research and Education Center in Champaign, IL. A total of 263 single cross maize hybrids derived from a random combination between SSS and NSSS parental lines were evaluated. On average, each SSS line was combined in 20 (range 3-57) and each NSSS line was combined in 13 (range 3-38) different hybrid combinations. A heatmap view of the incomplete factorial hybrid combination evaluated is shown in Figure 1.2.

Research sites and crop management

Maize hybrids were grown in eight field environments from 2011 through 2015. Data from 2012 of the original experiment was excluded from the analysis due to severe drought stress. Research sites were planted in one environment at DeKalb, IL (41°47′ N, 88°50′ W; 19 May 2014), five environments at Champaign, IL (40°3′ N, 88°14′ W; 17 May 2011, 20 May 2013, 22 April 2014, 24 April 2015, and 19 May 2015), and two environments at Harrisburg, IL (37°43′ N, 88°27′ W; 29 May 2013, and 23 May 2014). Field trials from 2011 and 2013 were conducted by Cole Hendrix. Soil types at the research sites were typically Flanagan silt loam at DeKalb, IL, Drummer silty clay loam at Champaign, IL, and Patton silty clay loam at Harrisburg, IL. The previous crop planted in each environment was soybean [*Glycine max* (L.) Merr.]. The experiment was planted using a precision plot planter (SeedPro 360, ALMACO, Nevada, IA) and plots were 5.6 m in length with 0.76 m row spacing and two rows in width. The target plant density was 79,000 plants ha^{-1}. All seeds were treated with Maxim® XL fungicide (Fludioxonil and Mefenoxam at 0.07 mg active ingredient kernel^{-1}; Syngenta Crop Protection, Greensburo, NC) and Cruiser® 5FS insecticide (Thiamethoxam at 0.80 mg active ingredient kernel^{-1}; Syngenta Crop Protection, Greensburo, NC) to prevent early season disease and insect damage, respectively. In addition, Force 3G® insecticide [Tefluthrin 2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1α,3α)-(Z)-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; Syngenta Crop Protection, Greensburo, NC] was applied at planting in-furrow (0.15 kg active ingredient ha^{-1}) to control soil pests. Pre-emergence herbicide Lumax® EZ (mixture of S-Metolachlor, Atrazine, and Mesotrione; Syngenta Crop Protection, Greensburo, NC) was applied at a rate of 7 L ha^{-1} to control early season weeds.

At maturity, plots were harvested with a two-row plot combine (SPC40, ALMACO, Nevada, IA). Grain yield is reported as Mg ha^{-1} at 15.5% grain moisture. Grain protein concentrations were estimated using a representative grain subsample from each plot collected during harvest by using near infrared transmittance (NIT) spectroscopy (Infratec 1241, FOSS, Eden Prairie, MN).

Experimental treatments and design

The 263 single-cross maize hybrids were grown in a randomized complete block design with three replications and two N fertilizer rates (0 and 252 kg N ha^{-1}; designated low and high N, or -N and +N, respectively) in a split-plot arrangement. The main-plot was hybrid and the split-plot was N fertilizer rate. On average, 83 hybrids were tested in each environment. Nitrogen stress tolerance was measured by yield of the check plot (0 kg N ha^{-1}), while 252 kg N ha^{-1} was chosen to obtain the maximum yield response to N from all hybrids regardless of their yield potential. Nitrogen fertilizer was hand applied in a diffuse band as urea (46-0-0) during the V2 to V3 growth stages (Ritchie et al., 1997). Nitrogen application dates were 17 June 2014 at DeKalb, IL, 02 June 2011, 04 June 2013, 04 June 2014, 18 May 2015, and 10 June 2015 at Champaign, IL, and 25 June 2013, and 13 June 2014 at Harrisburg, IL.

Phenotype measurements

Aboveground plant biomass from each plot was sampled at the R6 growth stage (physiological maturity), when the maximum biomass accumulation for maize is achieved (Ritchie et al., 1997). Six representative plants (visual assessment) from each plot were sampled and separated into stover (leaf, stem, and husks) and ear (grain and cob). The sampling criteria established consisted of selecting two adjacent plants near one end of the plot (1.2 m along the length of the first row), two adjacent plants at the center of the plot (approximately 2.7 m from the origin), and two adjacent plants at the other end of the plot (approximately 4.1 m along the length of the second row). Whole stover fresh weight was determined before shredding in a brush chipper (Vermeer BC600XL; Vermeer Midwest, Goodfield, IL). A representative subsample of the fresh shredded material was weighed and dried in a forced-draft oven (75°C) for approximately five days. Total stover dry weight was calculated using the fresh stover weight and the moisture level of the shredded material. Individual plant dry total biomass (g plant^{-1}) was the sum of the dry stover, cob, and grain weights (adjusted to 0% moisture). Dried stover samples were ground in a

Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 20-mesh screen, and N concentration (g kg^{-1}) was analyzed using a combustion technique (EA1112 N-Protein analyzer; CE Elantech, Inc., Lakewood, NJ). Grain N concentration was estimated by multiplying protein concentration by a factor of 6.25, and abbreviated as Protein$_{-N}$ or Protein$_{+N}$, from plants grown at 0 or 252 N ha^{-1}, respectively). Stover N content (g N plant^{-1}) was calculated by multiplying stover dry weight (g plant^{-1}) by stover N concentration. Similarly, grain N content (g N plant^{-1}) was calculated by multiplying grain dry weight (g plant^{-1}) by grain N concentration. Individual plant N content (g N plant^{-1}) was calculated as the sum of stover and grain N contents. Shelled grain weights from the ears sampled at R6 were combined with the remaining plot grain weight for yield determination.

In combination with grain yield and plant N content, NUE, N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), and N-harvest index (NHI) were calculated according to Eq. [1–5]:

$$\text{NUE} = (\text{Yield}_{+N} - \text{Yield}_{-N})/\text{NR} = (\text{kg yield kgN}^{-1}), \quad [1]$$

$$\text{NUpE} = (\text{PN}_{+N} - \text{PN}_{-N})/\text{NR} = (\text{kg}_{\text{plantN}}\ \text{kgN}^{-1}), \quad [2]$$

$$\text{NUtE} = (\text{Yield}_{+N} - \text{Yield}_{-N})/(\text{PN}_{+N} - \text{PN}_{-N}) = (\text{kg kg}_{\text{plantN}}{}^{-1}), \quad [3]$$

$$\text{NHI}_{+N} = \text{kg}_{\text{grainN}\ +N} / \text{PN}_{+N} = (\text{kg}_{\text{grainN}}\ \text{kg}_{\text{plantN}}{}^{-1}), \quad [4]$$

$$\text{NHI}_{-N} = \text{kg}_{\text{grainN}} / \text{PN}_{-N} = (\text{kg}_{\text{grainN}}\ \text{kg}_{\text{plantN}}{}^{-1}), \quad [5]$$

in which Yield$_{+N}$ corresponds to grain yield (kg ha^{-1}) at 252 kg N ha^{-1}, Yield$_{-N}$ corresponds to grain yield at 0 kg N ha^{-1}, NR is the N fertilizer rate (kgN, 252 kg N ha^{-1}), PN represents the total plant N content (kg plant N ha^{-1}) at 252 kg N ha^{-1} (PN$_{+N}$) and 0 kg N ha^{-1} (PN$_{-N}$), and PG is the individual plant grain mass (kg plant^{-1}) at 252 kg N ha^{-1} (PG$_{+N}$) and 0 kg N ha^{-1} (PG$_{-N}$). In addition, genetic utilization (GU) (kg yield kg$_{\text{plantN}}{}^{-1}$), which measures the physiological efficiency of plants to produce grain utilizing the plant N accumulated when grown without N fertilizer was calculated according to Eq. [6]:

$$\text{GU} = \text{PG}_{-N} / \text{PN}_{-N} \quad [6]$$

Statistical analysis

Since there is a weak correlation between the performances of inbred parents and their hybrid progeny's performance for NUE (Bertrán et al., 2003), the effects of general combining ability (GCA) and specific combining ability (SCA) of inbreds were evaluated using a random combination of ex-PVP hybrids. Moreover, the genetic variance and covariances between hybrids were calculated separately for each heterotic group (Stuber and Cockerham, 1966). Best linear unbiased predictions (BLUPs) were calculated for each phenotypic trait using the restricted maximum likelihood method to account for unbalanced data. In addition, year-location combinations were considered environments. General and specific combining abilities were obtained in PROC MIXED SAS version 9.4 (SAS Institute, 2013). A linear model for an incomplete factorial design according to Eq. [7] was used:

$$Y_{ijklm} = \mu + E_i + B_{j(i)} + S_k + N_l + SN_{kl} + ES_{ik} + EN_{il} + ESN_{ikl} + \varepsilon_{ijklm} \quad [7]$$

where Y_{ijklm} is the *m*th observation of the *kl*th hybrid in the *j*th block in the *i*th environment; μ is the grand mean, E_i is the random effect of *i*th environment (*i*=1 to 8); $B_{j(i)}$ is the random effect of *j*th block nested within the *i*th environment (*j*=1 to 3); S_k is the GCA effect of *k*th SSS inbred (*k*=1 to 36); N_l is the random GCA effect of *l*th NSSS inbred (*l*=1 to 53); SN_{kl} is the SCA effect of *kl*th hybrid (*kl*=1 to 522).; ES_{ik} is the random environment by SSS interaction; EN_{il} is the random environment by NSSS interaction; ESN_{ikl} is the random environment by hybrid interaction; and ε_{ijklm} is the random error term. Genotypic variance was calculated by multiplying the sum of the genetic variance components (SSS, NSSS, and hybrid) by two. Phenotypic variance was calculated as the sum of all variance components, except the variance component for block effect (Holland et al., 2003). Broad-sense heritability was calculated as the ratio of genotypic and phenotypic variance. The estimated breeding value of each hybrid was calculated according to Eq. [8]:

$$EBV_{kl} = \mu + GCA_k + GCA_l + SCA_{kl} \quad [8]$$

where EBV_{kl} is the estimated breeding value of *kl*th hybrid; μ is the grand mean; GCA_k is the GCA effect of *k*th inbred; GCA_l is the GCA effect of *l*th inbred; and ; SCA_{kl} is the SCA effect of *kl*th hybrid. Estimated breeding value (EBV) measures the average effect of an individual's genotypic value on the mean performance of its progeny (Falconer and Mackay, 1996) and it is a widely used measurement in maize breeding programs for the selection of superior genotypes.

Pearson's correlation coefficients were calculated in SAS version 9.4 (SAS Institute, 2013) between the GCA's of different N-use traits. Hierarchical cluster analysis was conducted on each heterotic group across different N-use traits using the Euclidean method in R Studio (R Development Core Team, 2015). The estimated breeding value (EBV) accuracy of the phenotypic traits was calculated according to Eq. [9], (Gilmour et al., 2004):

$$EBV_{\text{Accuracy}} = \sqrt{1 - \frac{SE}{(1+F)\sigma_A^2}} \quad [9]$$

where SE is the standard error of the inbred GCA, F is the inbreeding coefficient of the individual (assumed to be zero), and σ_A^2 is the additive variance component of the heterotic group (SSS or NSSS).

RESULTS AND DISCUSSION

Phenotypic variation of N-use traits

Yield under low N conditions ($\text{Yield}_{\text{-N}}$) accounted for 54% of the yield produced by the hybrids under high N conditions ($\text{Yield}_{\text{+N}}$) (Table 1.2). In addition, N fertilizer increased the mean harvest index (HI), the nitrogen harvest index (NHI), and the grain protein concentration. Average NUE, NUpE, NUtE, and GU values of 16.7 kg $\text{kg}_{\text{Nfert.}}^{-1}$, 0.43 $\text{kg}_{\text{plantN}}$ $\text{kg}_{\text{Nfert}}^{-1}$, 41.8 kg $\text{kg}_{\text{plantN}}^{-1}$, and 59.0 kg $\text{kg}_{\text{plantN}}^{-1}$, respectively are similar to other reports using U.S. Corn Belt germplasm (Uribelarrea et al., 2007; Haegele et al., 2013). Moreover, the additive effect distribution (range in GCA) of the two maize heterotic groups were similar for most N-use traits. In contrast, the NSSS group exhibited a greater additive effect range for NUE than the SSS group. The large additive effect variation observed among different N-use traits indicates that an opportunity exists for selecting maize genotypes with improved NUE.

The relative importance of the genotypic and phenotypic variation to broad-sense heritability was dependent on the N-use trait and the N fertilizer rate (Table 1.2). Yield at high N exhibited greater genetic variance (within heterotic groups and hybrids), and environmental variance, and lower residual variance than $\text{Yield}_{\text{-N}}$. Greater genetic variance under high N compared to low N has also been documented previously (Brun and Dudley, 1989; Trachsel et al., 2016). Conversely, genetic and environmental variance for harvest index at low N ($\text{HI}_{\text{-N}}$) was greater than harvest index at high N ($\text{HI}_{\text{+N}}$). Additionally, the genotype by environment interaction was greater under high N for yield and grain protein concentration, but greater at low N for HI and NHI.

Broad-sense heritability (H^2) ranged from 0.11 to 0.77 across phenotypic traits (Table 1.2), indicating a difference in additive and dominant effects among N-use traits (Table 1.2). Relatively large residual variances for $\text{Yield}_{\text{-N}}$, $\text{HI}_{\text{-N}}$, NHI at low N ($\text{NHI}_{\text{-N}}$), NUpE, and NUtE resulted in low

H^2 of these traits. However, heritability was higher for GU than NUpE or NUtE. The large genotypic variance of GU found is consistent with previous studies (Haegele et al., 2013).

Pearson's pairwise correlations between the GCA effects of different N-use traits are presented in Table 1.3. Yield at high N is generally positively correlated with Yield_{-N}, but the correlation tends to be lower under greater N stress (Banzinger et al., 1997; Presterl et al., 2003). Similarly, in this study, the correlation between Yield_{+N} and Yield_{-N} was +0.31. Hybrid correlation coefficients between Yield_{+N} and NUE, NUpE, and NUtE were +0.74, +0.64, and +0.44, respectively, in agreement with reports that these traits are frequently positively correlated (Abe et al., 2012; Haegele et al., 2013). On the other hand, Yield_{-N} was positively correlated with HI_{-N}, HI_{+N}, NHI_{+N}, and GU.

While significant genetic gains in maize yield have been documented over the past 60 years, grain protein concentration has consistently decreased during the same period (Duvick and Cassman, 1999). When averaged over hybrids and environments, grain protein concentration was negatively correlated to yield within each N fertilizer rate (Table 1.3). In addition, NUpE was positively correlated with grain protein concentration at low N ($r = 0.22$, $P \leq 0.05$) and NUtE was negatively correlated with grain protein concentration at high N (Protein_{+N}), ($r = -0.47$, $P \leq 0.001$). This finding reinforces the concept of the inverse relationship of starch and protein in maize grain, with greater N utilization underlying a greater proportion of starch than protein accumulation in the grain. Under high N fertility conditions, NHI was positively correlated to Protein_{+N}. Using the Illinois Protein-Strains germplasm, Uribelarrea et al. (2007) demonstrated that while all the strain-hybrids (low or high grain protein concentration) exhibited the same overall NUE, hybrids with high grain protein concentration exhibited high NUpE and NHI, and hybrids with low grain protein concentration exhibited high NUtE. Therefore, maize hybrids with high NUpE may exhibit greater root development and N uptake, while hybrids with high NUtE will show more ability to utilize N for starch production.

Genetic improvements have increased maize yield under low and high N conditions, yet plant N uptake levels have only increased under high N (Haegele et al., 2013). As such, the genotypic correlations between N-use traits indicate that traits related to N fertilizer response (NUE, NUpE, and NUtE) are associated with yield performance under high N conditions, and traits related to the efficiency of nutrient or biomass partitioning to the grain (HI_{-N}, HI_{+N}, NHI_{+N}, and GU) are associated with yield performance under N stress conditions. Although Yield$_{-N}$ and Yield$_{+N}$ are positively correlated, developing maize genotypes with high yield performance under high and low N conditions may be challenging, since the desirable traits for each of these N conditions are negatively correlated (HI, NHI, and GU vs. NUE, NUpE, and NUtE).

Genotype × environment interaction of N-use traits

In addition to the genotypic correlation between traits, another major challenge for breeding programs is to model the effect of the genotype × environment interaction (G × E) on desirable phenotypic traits (van Eeuwijk et al., 2016). While the genetic correlation of some N-use traits may be correlated to yield at low or high N conditions, their relationship might differ depending on other environmental conditions influencing yield. A way to compare the effect of an environment on yield is by measuring the average yield of multiple hybrids in each environment receiving similar crop management, termed the ‘environmental index’. Several studies have investigated the genetic variability of N-use traits across different N soil conditions (Moll et al., 1982; Brun and Dudley, 1989; Smiciklas and Below, 1990; Haegele et al., 2013), but few studies have investigated the effect of G × E on N-use traits. Therefore, regression analysis between an inbreds’ EBV at each environment (GCA + GCA×E + E) and the environmental index (E) was performed using the phenotypic traits that correlated to yield at low and high N conditions, respectively (Figure 1.3). Under low N conditions, GU was stable across environmental indices, and HI$_{-N}$ (0.04 kg kg^{-1}/ Mg ha^{-1}), HI$_{+N}$ (0.02 kg kg^{-1}/ Mg ha^{-1}), and NHI$_{+N}$ (0.02 kg kg^{-1}/ Mg ha^{-1}) increased as the environmental index increased (Figure 1.3A). Under high N conditions, NUE was stable across environmental indices, while NUtE decreased (-3.60 kg kg$_{plantN}^{-1}$ / Mg ha^{-1}) and NUpE increased (+0.03 kg$_{plantN}$ kg$_{Nfert}^{-1}$ / Mg ha^{-}1) as the environmental index increased (Figure 1.3B). The relationship between the G × E effect on N-use traits and the environmental index indicates the degree of trait dominance effects across different environmental yield conditions. A stable additive effect of NUE and GU across environmental indices is desirable for breeding selection in a wide range of environments.

Identification of maize genotypes with improved NUE

Hybrid NUE performance is determined by the plant’s ability to take up nitrogen from the soil (NUpE), the physiological capacity to generate and partition N to the grain (HI and NHI), and the sink strength to set kernels and accumulate starch under high or low N conditions (NUtE and

GU, respectively). Consequently, the aim of NUE breeding should be to integrate multiple desirable N-use traits into the same maize genotype. Hierarchical cluster analysis using the GCA effect of different phenotypic traits have categorized SSS (Group 1) and NSSS lines (Group 2) based on their NUE performance (Figure 1.4). Clusters within heterotic groups consisted of inbreds exhibiting correlated N-use traits (Table 1.4).

In the SSS cluster, groups 1A and 1B exhibited very unique characteristics with the lowest Yield$_{-N}$ and the lowest Yield$_{+N}$, respectively (Table 1.4). Group 1A also exhibited high grain protein concentration (under low and high N conditions), NUE, and NUpE, but the lowest GU within cluster group 1. In contrast, groups 1B and 1C exhibited high Yield$_{-N}$, but group 1B had the highest GU. Lastly, group 1D exhibited high Yield$_{+N}$, NUE, and NUtE, while group 1E had average performance for most N-use traits. In the NSSS cluster, groups 2A, 2B, and 2C exhibited higher HI and GU than groups 2D and 2E, but groups 2A and 2B had the lowest grain protein concentrations. Group 2A exhibited high Yield$_{-N}$ and Yield$_{+N}$ and the highest GU from group 2. In contrast, group 2D presented high Yield$_{+N}$ and the lowest GU.

Across heterotic groups, only seven inbreds (78551S, B73, LH128, ICI740, PHK56, W8304, and W8555) ranked in the top 25% GCA for Yield$_{-N}$ and Yield$_{+N}$, and only one inbred (PHK56) ranked in the top 10% for high yield performance under both N conditions (data not shown). Inbred PHK56 was one of the most referenced lines in the U.S. Patent database and was derived from PHG35 (from recombination of PHG47 and Oh07-Midland) from the Oh43 background (Mikel and Dudley, 2006). In addition, inbreds that are genetically related also exhibited similar NUE performance. As such, inbreds Mo17 and LH51 (97% identical by descent from Mo17), which are important progenitors of the Lancaster germplasm (Mikel and Dudley, 2006), were categorized in the same cluster (group 2E). Likewise, inbred PH207 is the main founder of the Iodent heterotic group and is an ancestor of several Pioneer Hi-Bred inbreds such as PHG29 and PHG50 (Mikel and Dudley, 2006). These inbreds exhibited high tolerance to N deficiency and high GU (Group 2C).

One breeding strategy for NUE improvement could be to utilize new inbred or hybrid combinations from the cluster groups with desirable N-use traits. Interestingly, group 2A was the only group exhibiting the combination of high Yield$_{-N}$ and Yield$_{+N}$. Group 2A represents approximately 5% of all NSSS lines tested in this study and could be used as a potential genetic resource for the development of maize genotypes with improved performance under high N or N-stress conditions. Inbred combinations between groups 1C × 2A and 1D × 2D, in theory would produce single cross hybrids with high NUE performance under low and high N conditions, respectively.

The identification of maize genotypes with high N-deficiency tolerance and/or high yield performance under optimal soil N conditions is important for better hybrid placement and agronomic management positioning for optimum yields. Among the 263 hybrids evaluated, only 22 produced yields ranked in the top 25% for both Yield_{-N} and Yield_{+N}, and only 5 hybrids obtained yields ranked in the top 10% for both N conditions. Moreover, hybrid ICI740×PHK56 (combination between groups 1C × 2A) exhibited high yield performance under low and high N conditions (Figure 1.5). This hybrid exhibited the highest average EBV for Yield_{-N} (6.2 Mg ha^{-1}) and the 9th highest EBV for Yield_{+N} (10.3 Mg ha^{-1}). Hybrid LH145×83IBI3 (groups 1B × 2C) exhibited high tolerance to N deficiency (Yield_{-N} = 5.2 Mg ha^{-1}), but low EBV for Yield_{+N} (8.2 Mg ha^{-1}). This hybrid combined above average EBV for HI and GU, and below average EBV for NUE and NUtE. In contrast, hybrid F118×LH214 (groups 1A × 2D) presented the highest average EBVs for Yield_{+N} (11.1 Mg ha^{-1}), NUE, and NUpE, but low EBV for Yield_{-N} (4.4 Mg ha^{-1}) and GU.

Estimated breeding value accuracy is an important method to compare the prediction reliability of desirable traits. Estimated breeding value accuracy ranged from 0.12 to 0.92 and, with the exception of $\text{NHI}_{-N,}$ EBV accuracies were similar among heterotic groups (Figure 1.6). While the majority of the inbreds exhibited high EBV accuracy, some genotypes did not. Skewness of EBV accuracy may be related to unbalanced data and genotypes with low stability across environments.

Although, precise estimates of H^2 and EBV accuracy are a function of genetic and residual variance, there was no relationship between EBV accuracy averaged across heterotic groups and H^2 (Figure 1.7). While the H^2 for NUtE and NHI_{-N} was both 0.11, their EBV accuracies were 0.61 and 0.28, respectively. Broad-sense heritability for Yield_{-N} was almost 50% less than H^2 for Yield_{+N}. However, these traits presented similar EBV accuracy (approximately 0.82). Discrepancies between H^2 and EBV accuracy can be associated with the genetic architecture of complex traits. Though large residual variance reduced H^2 of some phenotypic traits (e.g. $\text{Yield}_{-N,}$ NUpE, and NUtE), large additive variances increased their EBV accuracies.

CONCLUSIONS

Although 89 inbred lines were evaluated, there were certainly more ex-PVP lines available at the National Plant Germplasm System. Nonetheless, this subset was able to display large genetic variation among ex-PVP lines for most N-use traits. The large range of broad-sense heritabilities found for phenotypic traits highlights the importance of accurate phenotypic selection under field conditions. In addition, differences in the stability of N-use traits across environments will have important implications for phenotypic selection. Genetic utilization and NUE were stable across environments and were highly correlated with yield under low and high N conditions, respectively. Hybrids with high N-deficiency tolerance or high yield response to N fertilizer were associated with different phenotypic traits. Consequently, less than 2% of the hybrids evaluated exhibited high yield performance under both low and high N conditions. Nitrogen use efficiency is the end result of highly polygenic and complex traits. Future genetic improvement of NUE will require effective integration between accurate field phenotyping and marker-assisted breeding strategies, such as genome-wide prediction and metabolic profiling studies.

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TABLES AND FIGURES

Table 1.1. Maize line name, year of release, heterotic group, and proprietary company name of Ex-Plant Variety Protection (PVP) inbreds used as parents in this study.

Line [†]	**Year Released**	**Heterotic Group**[‡]	**Company**
PH207	2002	Iodent	Pioneer Hi-Bred International, Inc.
LH82	2003	Iodent	Holden's Foundation Seeds, Inc.
IB014	2004	Iodent	DeKalb-Pfizer Genetics
PHG29	2004	Iodent	Pioneer Hi-Bred International, Inc.
PHG72	2005	Iodent	Pioneer Hi-Bred International, Inc.
PHP02	2007	Iodent	Pioneer Hi-Bred International, Inc.
PHH93	2007	Iodent	Pioneer Hi-Bred International, Inc.
IBB15	2008	Iodent	DeKalb-Pfizer Genetics
IBC2	2008	Iodent	DeKalb-Pfizer Genetics
J8606	2008	Iodent	Novartis Seeds, Inc.
PHN82	2008	Iodent	Pioneer Hi-Bred International, Inc.
PHP76	2009	Iodent	Pioneer Hi-Bred International, Inc.
83IBI3	2010	Iodent	DeKalb-Pfizer Genetics
LH164	2010	Iodent	Holden's Foundation Seeds, Inc.
904	2010	Iodent	Novartis Seeds, Inc.
911	2010	Iodent	Novartis Seeds, Inc.
912	2010	Iodent	Novartis Seeds, Inc.
PHM81	2010	Iodent	Pioneer Hi-Bred International, Inc.
PHKE6	2011	Iodent	Pioneer Hi-Bred International, Inc.
Mo17	1973	Lancaster	Public
LH51	2001	Lancaster	Holden's Foundation Seeds, Inc.
MDF-13D	2002	Lancaster	DeKalb-Pfizer Genetics
LH123	2003	Lancaster	Holden's Foundation Seeds, Inc.
MBNA	2004	Lancaster	DeKalb-Pfizer Genetics
LH54	2005	Lancaster	Holden's Foundation Seeds, Inc.
LH52	2005	Lancaster	Holden's Foundation Seeds, Inc.
LH60	2005	Lancaster	Holden's Foundation Seeds, Inc.
PHG47	2005	Lancaster	Pioneer Hi-Bred International, Inc.
PHG84	2005	Lancaster	Pioneer Hi-Bred International, Inc.
PHJ40	2005	Lancaster	Pioneer Hi-Bred International, Inc.
PHZ51	2005	Lancaster	Pioneer Hi-Bred International, Inc.
78371A	2006	Lancaster	DeKalb-Pfizer Genetics
MBPM	2006	Lancaster	DeKalb-Pfizer Genetics
LH59	2006	Lancaster	Holden's Foundation Seeds, Inc.
LH65	2006	Lancaster	Holden's Foundation Seeds, Inc.
740	2006	Lancaster	Novartis Seeds, Inc.
S8324	2006	Lancaster	Novartis Seeds, Inc.
2MA22	2007	Lancaster	DeKalb-Pfizer Genetics
78551S	2008	Lancaster	DeKalb-Pfizer Genetics
E8501	2008	Lancaster	Novartis Seeds, Inc.
PHJ31	2008	Lancaster	Pioneer Hi-Bred International, Inc.
PHN73	2008	Lancaster	Pioneer Hi-Bred International, Inc.
LH128	2009	Lancaster	Holden's Foundation Seeds, Inc.
LH181	2009	Lancaster	Holden's Foundation Seeds, Inc.

Table 1.1 (Continued)

Line [†]	**Year Released**	**Heterotic Group**[‡]	**Company**
PHK56	2009	Lancaster	Pioneer Hi-Bred International, Inc.
MBSJ	2010	Lancaster	DeKalb-Pfizer Genetics
LH216	2010	Lancaster	Holden's Foundation Seeds, Inc.
LH213	2010	Lancaster	Holden's Foundation Seeds, Inc.
BCC03	2010	Lancaster	Novartis Seeds, Inc.
PHJ89	2010	Lancaster	Pioneer Hi-Bred International, Inc.
Lp215D	2010	Lancaster	Wilson Hybrids, Inc.
ICI581	2011	Lancaster	Advanta Technology Limited
LH214	2011	Lancaster	Holden's Foundation Seeds, Inc.
B73	1973	Stiff-stalk synthetic	Public
LH1	1994	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
FAPW	2002	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
LH145	2002	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
PHG39	2002	Stiff-stalk synthetic	Pioneer Hi-Bred International, Inc.
PHG35	2002	Stiff-stalk synthetic	Pioneer Hi-Bred International, Inc.
4676A	2004	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
78002A	2004	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
764	2005	Stiff-stalk synthetic	Novartis Seeds, Inc.
778	2005	Stiff-stalk synthetic	Novartis Seeds, Inc.
794	2005	Stiff-stalk synthetic	Novartis Seeds, Inc.
FBHJ	2006	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
PB80	2006	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
LH149	2006	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
807	2006	Stiff-stalk synthetic	Novartis Seeds, Inc.
790	2006	Stiff-stalk synthetic	Novartis Seeds, Inc.
793	2006	Stiff-stalk synthetic	Novartis Seeds, Inc.
H8431	2006	Stiff-stalk synthetic	Novartis Seeds, Inc.
S8326	2006	Stiff-stalk synthetic	Novartis Seeds, Inc.
PHT55	2006	Stiff-stalk synthetic	Pioneer Hi-Bred International, Inc.
W8304	2007	Stiff-stalk synthetic	Novartis Seeds, Inc.
2FACC	2008	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
W8555	2008	Stiff-stalk synthetic	Novartis Seeds, Inc.
LH220Ht	2009	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
LH208	2009	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
PHV07	2009	Stiff-stalk synthetic	Pioneer Hi-Bred International, Inc.
ICI441	2010	Stiff-stalk synthetic	Advanta Technology Limited
FBLA	2010	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
NL001	2010	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
F118	2010	Stiff-stalk synthetic	DeKalb-Pfizer Genetics
LH191	2010	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
LH197	2010	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.
PHGG7	2010	Stiff-stalk synthetic	Pioneer Hi-Bred International, Inc.
ICI193	2011	Stiff-stalk synthetic	Advanta Technology Limited
ICI740	2011	Stiff-stalk synthetic	Advanta Technology Limited
LH209	2011	Stiff-stalk synthetic	Holden's Foundation Seeds, Inc.

[†] Public lines were developed by Iowa State University (B73) and University of Missouri (Mo17).

[‡] Heterotic groups were defined using discriminant analysis of principal components (Jombart et al., 2010) and 26,768 single nucleotide polymorphism markers.

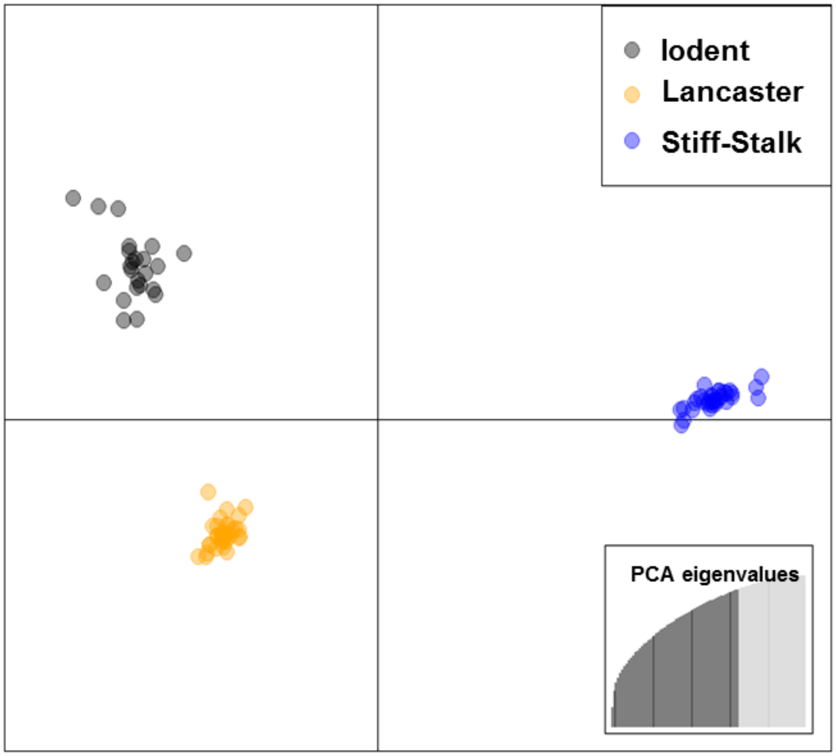


Figure 1.1. Scatterplots of the discriminant analysis of principal components of 89 ex-Plant Variety Protection (ex-PVP) maize inbred lines. Scatterplot displays the first two components using 26,768 single nucleotide polymorphism markers. Heterotic groups are represented by different colors: Iodent (black), Lancaster (yellow), and Stiff-stalk synthetic (blue), and each dot represents an individual inbred line.

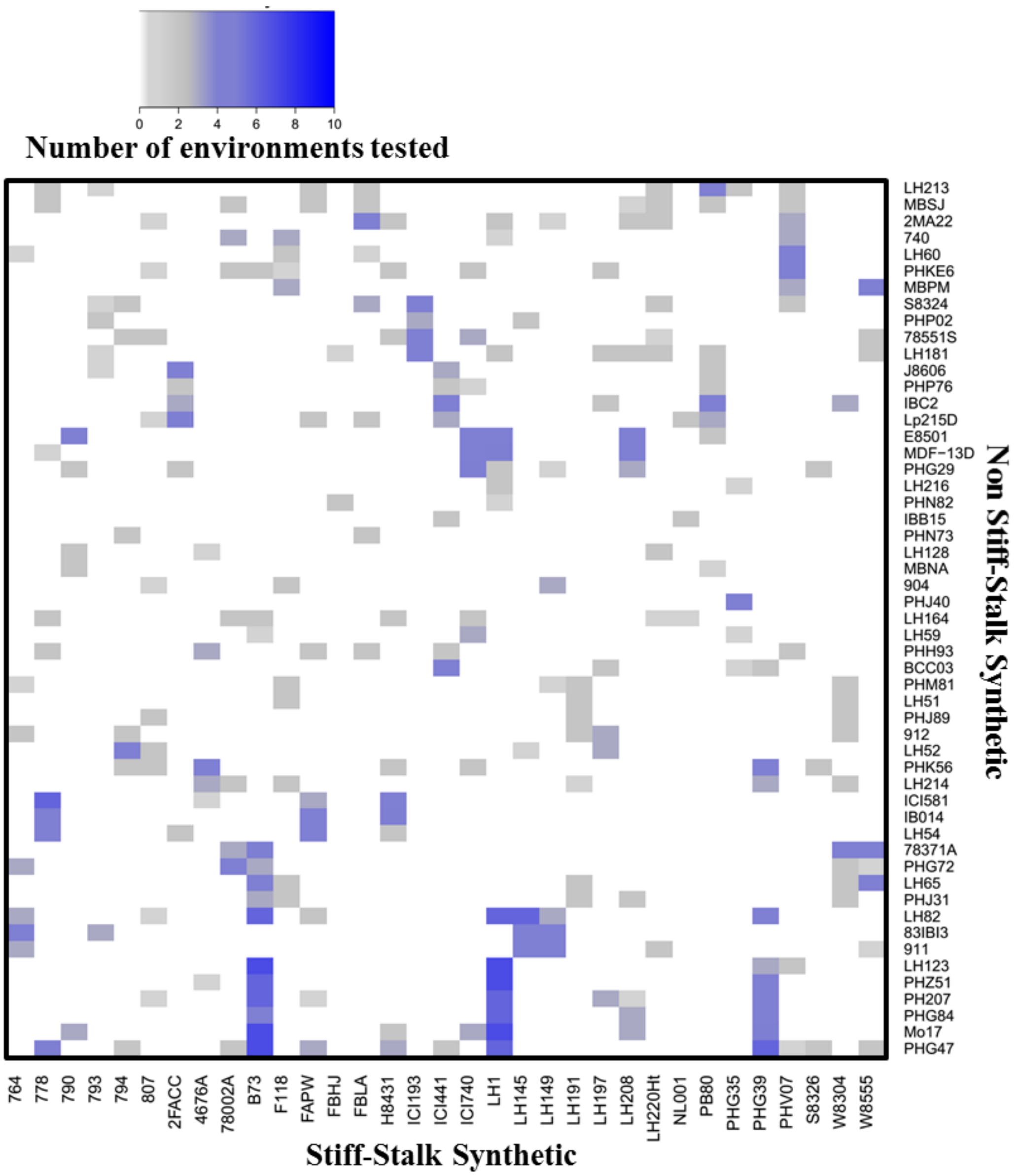


Figure 1.2. Heatmap showing maize hybrid combinations between 36 stiff-stalk synthetic and 53 non-stiff-stalk synthetic lines developed with the corresponding number of environments tested over three locations in Illinois from 2011 to 2015.

Table 1.2. Mean estimates and range for yield at low N (Yield$_{-N}$, Mg ha^{-1}), yield at high N (Yield$_{+N}$, Mg ha^{-1}), harvest index at low N (HI$_{-N}$, kg kg^{-1}), harvest index at high N (HI$_{+N}$, kg kg^{-1}), N harvest index at low N (NHI$_{-N}$, kg$_{grainN}$ kg$_{plantN}$$^{-1}$), N harvest index at high N (NHI$_{+N}$, kg$_{grainN}$ kg$_{plantN}$$^{-1}$), grain protein concentration at low N (Protein$_{-N}$, g kg^{-1}), grain protein concentration at high N (Protein$_{+N}$, g kg^{-1}), N-use efficiency (NUE, kg kg$_{Nfert.}$$^{-1}$), N-uptake efficiency (NUpE, kg$_{plantN}$ kg$_{Nfert}$$^{-1}$), N-utilization efficiency (NUtE, kg kg$_{plantN}$$^{-1}$), and genetic utilization (GU, kg kg$_{plantN}$$^{-1}$). Variance components for general and specific combining ability effects (GCA and SCA) were calculated using 36 stiff-stalk synthetic (SSS) and 53 non-SSS (NSSS) ex-PVP parental inbred lines across different N-use traits. Broad-sense heritability (H^2) for each trait was estimated in eight environments under low and high N conditions (0 and 252 kg N ha^{-1}, respectively).

		GCA$_{SSS}$†		**GCA$_{NSSS}$**		**SCA**					
Trait	**Mean ± SE**	**Range Min./Max.**	**σ^2_{SSS}**	**Range Min./Max.**	**σ^2_{NSSS}**	**Range Min./Max.**	**σ^2_{SCA}**	**σ^2_E**	**$\sigma^2_{SCA\times E}$**	**σ^2_R**	**H^2**
Yield$_{-N}$	4.9 ± 0.19	-0.7 / 0.7	0.13	-0.8 / 0.5	0.12	-	0.00	0.70	0.01	1.31	0.31
Yield$_{+N}$	9.1 ± 0.28	-0.9 / +0.9	0.25	-1.2 / 0.9	0.32	-0.2 / +0.3	0.06	2.12	0.36	1.07	0.61
HI$_{-N}$	0.36 ± 0.01	-0.05 / +0.09	6×10^{-4}	-0.10 / +0.06	1×10^{-3}	-0.01 / +0.01	1×10^{-4}	2×10^{-3}	3×10^{-4}	4×10^{-3}	0.63
HI$_{+N}$	0.47 ± 0.01	-0.02 / +0.03	2×10^{-4}	-0.06 / +0.03	4×10^{-4}	-0.01 / +0.01	5×10^{-5}	6×10^{-4}	1×10^{-5}	1×10^{-3}	0.73
NHI$_{-N}$	0.56 ± 0.01	-0.01 / +0.01	2×10^{-4}	-0.01 / +0.01	1×10^{-5}	-0.03 / +0.02	4×10^{-4}	9×10^{-3}	2×10^{-3}	8×10^{-3}	0.11
NHI$_{+N}$	0.68 ± 0.01	-0.04 / +0.02	3×10^{-4}	-0.05 / +0.03	5×10^{-4}	-0.02 / +0.01	1×10^{-4}	3×10^{-4}	1×10^{-4}	3×10^{-3}	0.44
Protein$_{-N}$	62 ± 1.3	-6.1 / +5.9	0.8	-5.2 / +5.4	0.8	-3.2 / +2.3	0.4	1.7	1.3	2.1	0.74
Protein$_{+N}$	85 ± 1.3	-3.8 / +4.9	0.7	-6.0 / +4.6	1.3	-2.5 / +2.5	0.2	5.5	0.5	3.0	0.77
NUE	16.7 ± 1.14	-3.6 / +3.9	3.81	-5.7 / +5.2	5.50	-0.95 / +1.20	0.80	10.13	4.97	18.56	0.60
NUpE	0.43 ± 0.03	-0.03 / +0.05	6×10^{-4}	-0.08 / +0.09	1×10^{-3}	-0.01 / +0.42	2×10^{-4}	2×10^{-3}	1×10^{-3}	0.01	0.27
NUtE	41.8 ± 1.79	-3.4 / +2.9	5.5	-2.7 / +4.2	5.2	-0.58 / +0.60	1.3	59.3	7.8	201.7	0.11
GU	59.0 ± 2.2	-7.8 / +8.9	17.8	-9.9 / +7.4	16.0	-3.2 / +2.9	5.5	29.1	7.8	88.7	0.58

† σ^2_{SSS}, σ^2_{NSSS}, σ^2_{SCA}, σ^2_E, $\sigma^2_{SCA\times E}$, σ^2_R, represent variance components for stiff-stalk lines, non-stiff-stalk lines, hybrid, environment, hybrid × environment interaction, and residual effects, respectively (Eq.[7]); SE, standard error of the mean; Min./Max., Minimum and maximum observed values compared to the respective means.

Table 1.3. Pearsons's pairwise correlations between the GCA effects of the N-use traits of yield at low N (Yield$_{\text{-N}}$), yield at high N (Yield$_{\text{+N}}$), harvest index at low N (HI$_{\text{-N}}$), harvest index at high N (HI$_{\text{+N}}$), N harvest index at low N (NHI$_{\text{-N}}$), N harvest index at high N (NHI$_{\text{+N}}$), grain protein concentration at low N (Protein$_{\text{-N}}$), grain protein concentration at high N (Protein$_{\text{+N}}$), N-use efficiency (NUE), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), and genetic utilization (GU) for 263 single-cross maize hybrids grown from 2011 to 2015 under low and high N conditions (0 and 252 kg N ha^{-1}, respectively).

	Yield$_{\text{-N}}$	**Yield$_{\text{+N}}$**	**HI$_{\text{-N}}$**	**HI$_{\text{+N}}$**	**NHI$_{\text{-N}}$**	**NHI$_{\text{+N}}$**	**Protein$_{\text{-N}}$**	**Protein$_{\text{+N}}$**	**NUE**	**NUpE**	**NUtE**
Yield$_{\text{+N}}$	0.31**	-	-	-	-	-	-	-	-	-	-
HI$_{\text{-N}}$	0.63***	-0.33**	-	-	-	-	-	-	-	-	-
HI$_{\text{+N}}$	0.49***	NS	0.77***	-	-	-	-	-	-	-	-
NHI$_{\text{-N}}$	NS	NS	NS	NS	-	-	-	-	-	-	-
NHI$_{\text{+N}}$	0.51***	NS	0.65***	0.78***	NS	-	-	-	-	-	-
Protein$_{\text{-N}}$	-0.38***	-0.22*	NS	NS	NS	NS	-	-	-	-	-
Protein$_{\text{+N}}$	NS	-0.39***	NS	NS	NS	0.37***	0.73***	-	-	-	-
NUE	-0.33**	0.74***	-0.73***	-0.42***	NS	-0.35***	NS	-0.26*	-	-	-
NUpE	NS	0.64***	-0.59***	-0.43***	NS	-0.27*	0.22*	NS	0.77***	-	-
NUtE	-0.29*	0.44***	-0.46***	NS	NS	-0.21*	NS	-0.47***	0.66***	NS	-
GU	0.67***	NS	0.82***	0.59***	NS	0.50***	-0.51***	NS	-0.59***	-0.48***	-0.32**

*Signifcant at $P \leq 0.05$.

**Signifcant at $P \leq 0.01$.

***Signifcant at $P \leq 0.001$.

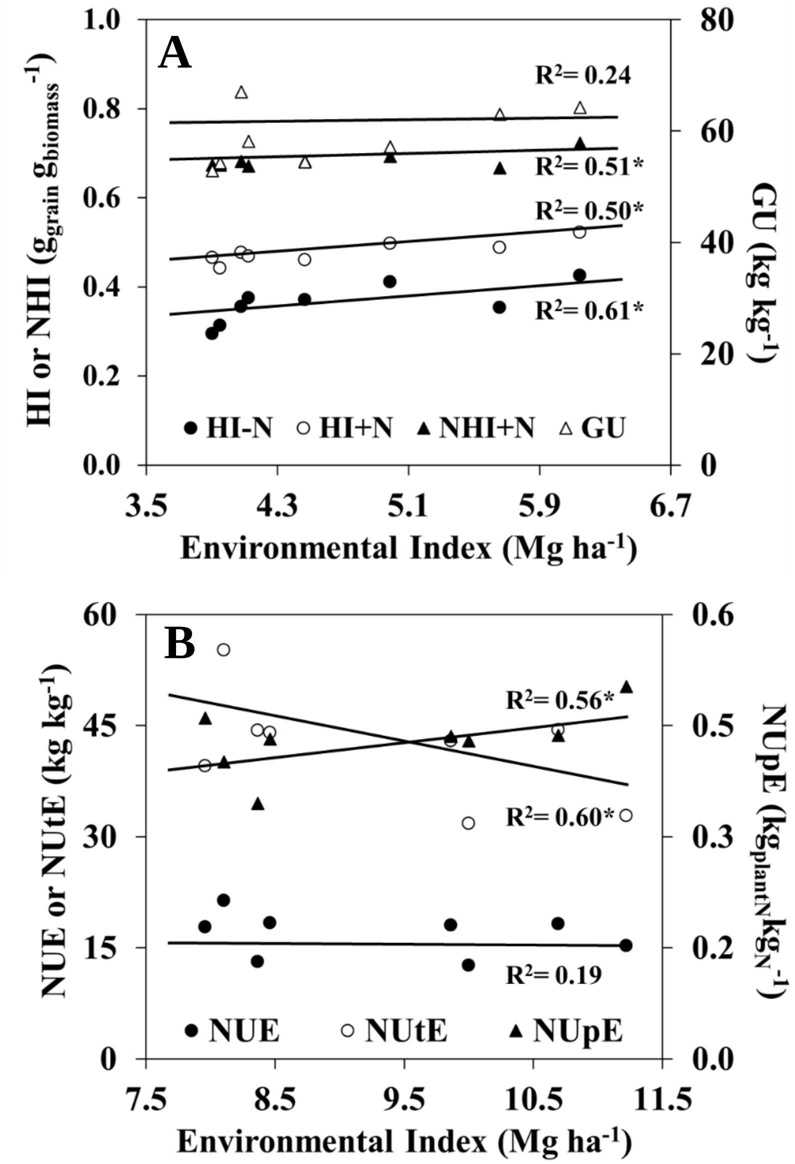
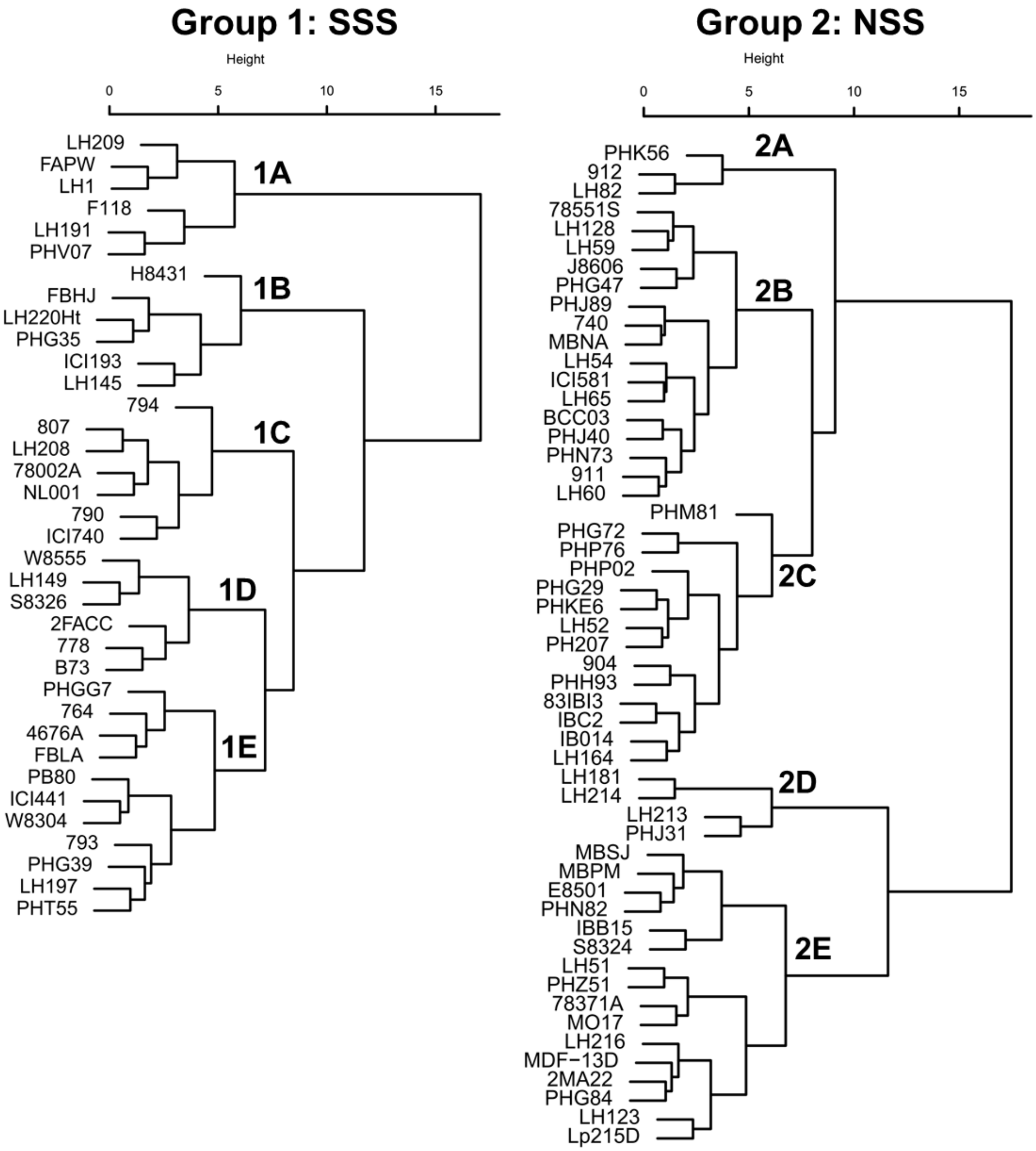


Figure 1.3. Influence of N supply and environment on selected N-use traits. A) Changes in harvest index at low and high N (HI_{-N} and HI_{+N}), N-harvest index at high N (NHI_{+N}), and genetic utilization (GU) due to the environmental index for maize hybrids grown at low N (0 kg N ha^{-1}); and B) Changes in N-use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE) due to the environmental index for maize hybrids grown with high N (252 kg N ha^{-1}). Values shown for each phenotypic trait are averaged over all hybrids grown in each of the eight environments from 2011 to 2015. * Indicates significant slopes at $P \leq 0.001$.


Group 1: SSS

Group 2: NSS

Figure 1.4. Hierarchical cluster analysis using different N-use traits of 36 stiff-stalk synthetic (SSS, Group 1) and 53 non-SSS (NSSS, Group 2) inbred lines. Clusters A, B, C, D, and E represent groups of inbreds with different N-use trait performances. Clusters were generated using the inbreds' GCA from 12 N-use traits. Inbred GCAs were calculated from 263 maize hybrids grown from 2011 to 2015 under low and high N conditions (0 and 252 kg N ha^{-1}, respectively).

Table 1.4. Variation in hybrid yield when grown at low and high N (Yield_{-N} and Yield_{+N}), harvest index at low and high N (HI_{-N} and HI_{+N}), N-harvest index at low and high N (NHI_{-N} and NHI_{+N}), grain protein concentration at low and high N (Protein_{-N} and Protein_{+N}), N-use efficiency (NUE), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), and genetic utilization (GU) based on parental inbred cluster groups for the stiff-stalk synthetic lines (SSS, Groups 1A-1E) and non-stiff-stalk synthetic lines (NSSS, Groups 2A-2E). A total of 263 maize hybrids were grown from 2011 to 2015 under low and high N conditions (0 and 252 kg N ha^{-1}, respectively).

Group	**N**	**Yield**		**HI**		**NHI**		**Protein**		**NUE**	**NUpE**	**NUtE**	**GU**
		Low N	**High N**	**Low N**	**High N**	**Low N**	**High N**	**Low N**	**High N**				
		Mg ha^{-1}		g g^{-1}		g_{grainN} g_{plantN}^{-1}		g kg^{-1}		kg $kg_{Nfert.}^{-1}$	kg_{plantN} kg_{Nfert}^{-1}	kg kg_{plantN}^{-1}	kg kg_{plantN}^{-1}
SSS													
1A	6	4.57	9.15	0.33	0.47	0.57	0.67	86.4	68.3	18.29	0.44	42.90	53.26
1B	6	5.05	8.82	0.39	0.48	0.57	0.69	85.0	64.1	15.37	0.42	41.10	64.41
1C	7	5.24	9.13	0.38	0.48	0.57	0.69	85.9	66.1	15.45	0.42	40.51	60.55
1D	6	4.93	9.29	0.37	0.49	0.57	0.69	82.2	64.8	17.90	0.42	43.42	60.44
1E	11	4.88	9.05	0.36	0.48	0.57	0.68	86.1	67.1	16.87	0.43	41.47	57.56
LSD $P \leq 0.05$		0.29	0.50	0.01	0.01	0.01	0.02	2.6	2.3	1.39	0.02	1.30	1.40
NSSS													
2A	3	5.31	9.32	0.42	0.50	0.57	0.70	83.5	64.0	16.21	0.40	42.70	64.92
2B	16	5.02	9.17	0.37	0.48	0.57	0.69	83.3	65.0	16.71	0.42	42.22	60.18
2C	14	5.08	8.74	0.40	0.49	0.57	0.70	88.1	67.2	14.85	0.42	40.22	61.26
2D	4	4.31	9.52	0.29	0.45	0.57	0.66	86.3	69.1	21.11	0.48	43.75	51.91
2E	16	4.78	9.18	0.34	0.47	0.57	0.67	84.5	66.1	17.47	0.44	42.06	56.65
LSD $P \leq 0.05$		0.21	0.52	0.02	0.02	0.00	0.02	3.2	2.3	1.38	0.03	1.05	1.47

[†] N, number of ex-PVP inbreds categorized within each cluster group.

[‡] LSD, Least significant difference was estimated from different cluster group within each phenotypic trait.

[§] NS, not significant (at $P \geq 0.05$).

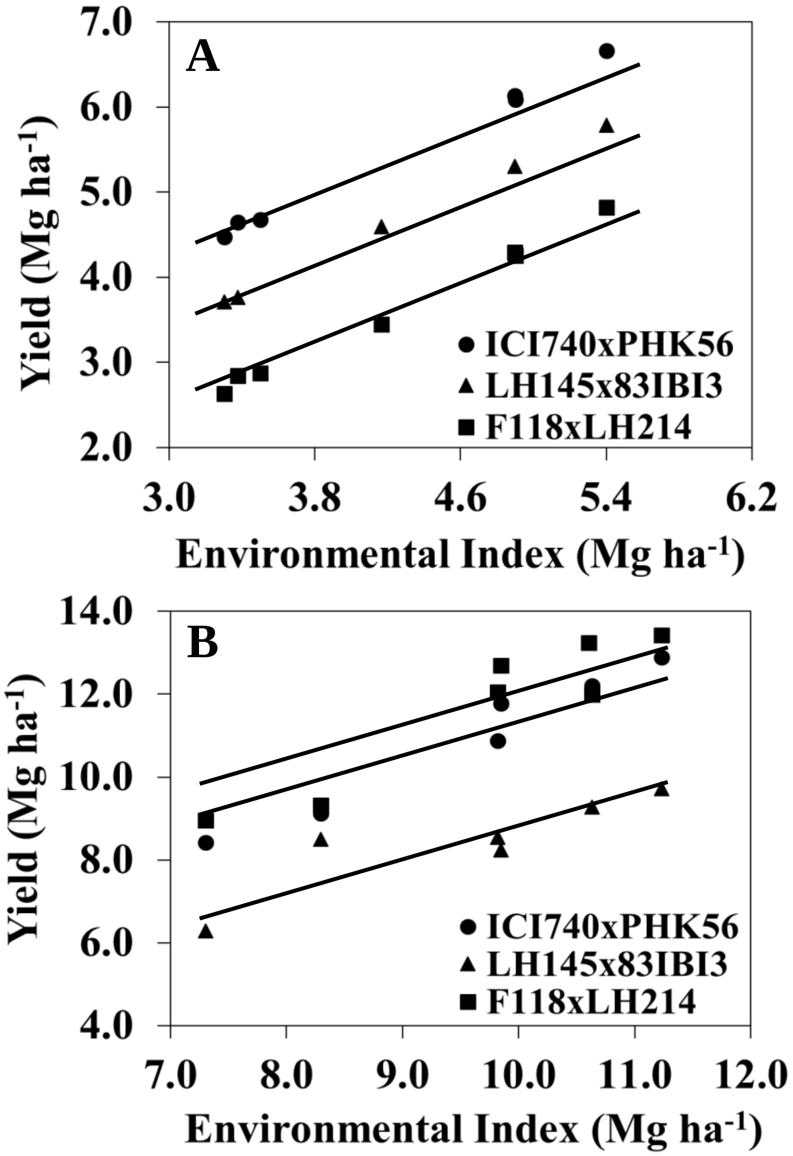


Figure 1.5. Changes in the ranking of yield performances of select hybrids across environmental indices when grown with A) low N (0 kg N ha^{-1}), and B) high N (252 kg N ha^{-1}). Data values are the average yields within an environment for ICI740×PHK56 (exhibiting high tolerance to N-deficiency and high positive response to N fertilizer), LH145×83IBI3 (exhibiting high tolerance to N-deficiency and low positive response to N fertilizer), and F118×LH214 (exhibiting low tolerance to N-deficiency and high positive response to N fertilizer).

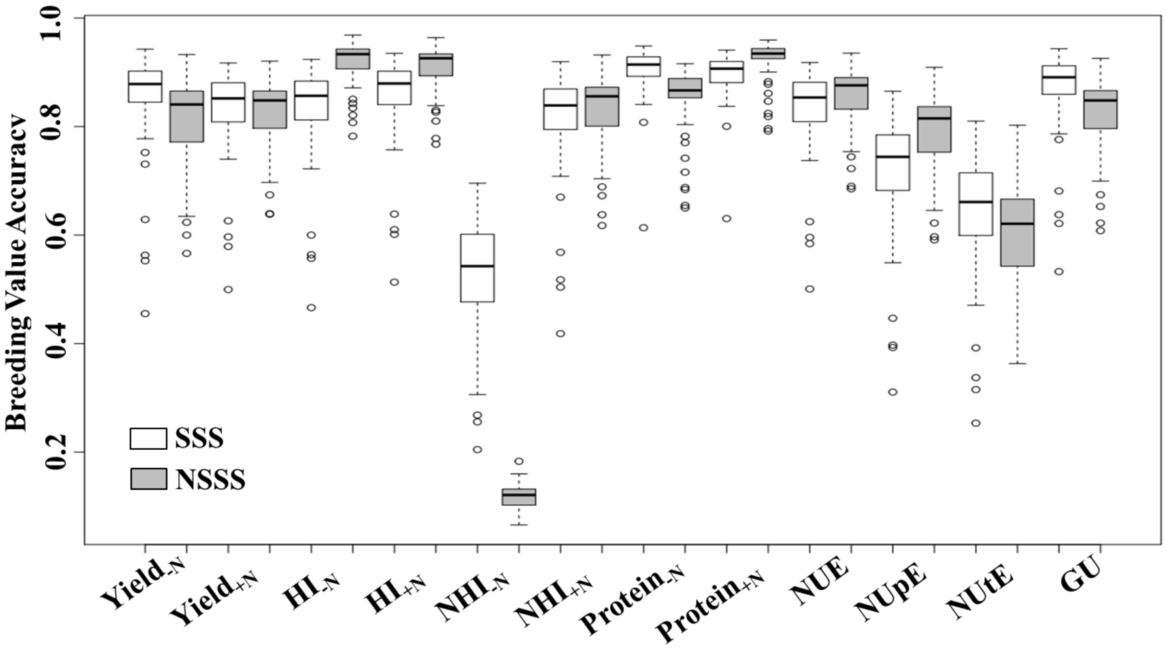


Figure 1.6. Box-plot of breeding value accuracies for yield at low and high N (Yield$_{-N}$ and Yield$_{+N}$), grain protein concentration at low and high N (Protein$_{-N}$ and Protein$_{+N}$), harvest index at low and high N (HI$_{-N}$ and HI$_{+N}$), N-harvest index at low and high N (NHI$_{-N}$ and NHI$_{+N}$), N-use efficiency (NUE), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), and genetic utilization (GU) in stiff-stalk synthetic (SSS) and non-stiff-stalk synthetic (NSSS) maize lines. Breeding value accuracy was estimated according to Eq. [9]. Values are based on the performance of 263 hybrids developed from these lines and grown in eight environments from 2011 to 2015 under low and high N conditions (0 and 252 kg N ha^{-1}, respectively).

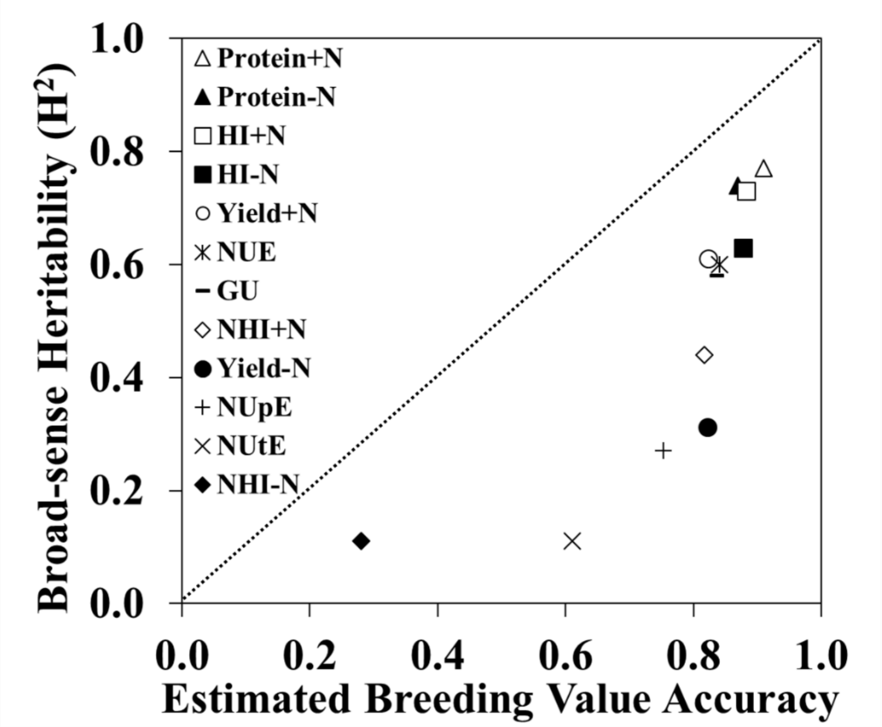


Figure 1.7. Relationship between broad-sense heritability and estimated breeding value accuracy in maize lines for different N-use traits. Breeding value accuracies were averaged across heterotic groups. Abbreviations include yield at low and high N ($\text{Yield}_{\text{-N}}$ and $\text{Yield}_{\text{+N}}$), grain protein concentration at low and high N ($\text{Protein}_{\text{-N}}$ and $\text{Protein}_{\text{+N}}$), harvest index at low and high N ($\text{HI}_{\text{-N}}$ and $\text{HI}_{\text{+N}}$), N-harvest index at low and high N ($\text{NHI}_{\text{-N}}$ and $\text{NHI}_{\text{+N}}$), N-use efficiency (NUE), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), and genetic utilization (GU) in stiff-stalk synthetic (SSS) and non-stiff-stalk synthetic (NSSS) maize lines. Breeding value accuracy was estimated according to Eq. [9]. Values are based on the performance of 263 hybrids developed from these lines and grown in eight environments from 2011 to 2015 under low and high N conditions (0 and 252 kg N ha^{-1}, respectively).

CHAPTER 2

GENOMIC SELECTION USING MAIZE EX-PLANT VARIETY PROTECTION GERMPLASM FOR THE PREDICTION OF N-USE TRAITS

ABSTRACT

Maize (*Zea mays* L) yield increases associated with better usage of nitrogen (N) fertilizer will require innovative breeding efforts. Genomic selection (GS) for N-use traits may speed up the breeding cycle of research programs targeting for improved N-use efficiency (NUE) in maize. The objective of this study was to evaluate GS accuracy of 12 N-use traits in response to different training composition (TC) scenarios and training population (TP) sizes. A total of 552 maize hybrids were planted under low (0 kg N ha^{-1}) and high N fertilizer (252 kg N ha^{-1}) conditions across 10 environments. Training composition scenarios included: T0 (hybrids in which none of the parents were included in the random subset of inbreds), T1 (hybrids in which one of their parents were included in the random subset of inbreds), and T2 (hybrids in which both of their parents were included in the random subset of inbreds). Training population sizes ranged from 10 to 40 or 30 to 90 hybrids, depending to the N-use trait. Across different TC, TP sizes, and N-use traits, GS accuracy ranged from -0.12 to 0.78 and was greatest with larger TP sizes when both parents of untested hybrids appeared in the training and validation sets (T2 hybrids). Moreover, GS accuracy in response to different TC and TP sizes was dependent on the N-use trait. Successful breeding for N-stress tolerance or improved yield response to N fertilizer level will require selection of specific N-use traits.

INTRODUCTION

The improvement of nitrogen use efficiency (NUE) in maize can provide environmental and economic advantages to agriculture, but requires advancements in multiple areas of the crop's production (e.g. fertilizer products, crop management, precision agriculture, and crop genetic improvement). Breeding for maize NUE improvement entails several challenges, such as the genetic complexity of the trait, the strong interaction with the environment, and the high cost for field phenotyping. However, conventional breeding approaches have reported significant genetic improvement in increasing N stress tolerance in maize hybrids (Bänziger and Lafitte, 1997; Bänziger et al., 1999).

Although maize breeding has indirectly improved NUE due to direct selection for greater yield (Moose and Below, 2009), the genetic improvement of NUE has also received substantial attention by maize breeders. Moreover, successful maize NUE breeding will require improved statistical designs, appropriate breeding schemes, and the integration of selection for secondary traits (Bänziger et al. 2000). Nitrogen use efficiency can be defined as the product of nitrogen uptake efficiency (NUpE, the ratio of the additional plant N content due to fertilizer N to the amount of fertilizer-applied N) and nitrogen utilization efficiency (NUtE, the ratio of yield increase to the difference in plant N content compared to those of an unfertilized crop) (Moll et al., 1982). Likewise, NUpE and NUtE are often considered as important secondary traits for the genetic characterization of maize NUE (Uribelarrea et al., 2007; Haegele et al. 2013). Other secondary traits relevant for NUE improvement include: harvest index, grain protein concentration, and genetic utilization (Haegele et al. 2013). Genetic utilization (GU) is defined as the plant physiological efficiency under N stress conditions to utilize N for grain production. In addition to the use of phenotypic traits, another method of indirect selection for improved NUE is by using molecular markers.

Marker-assisted breeding is becoming more commonly used in breeding programs as the cost of acquiring genotypic data becomes cheaper than phenotypic data (Bernardo, 2008). Linkage mapping studies were the first marker-assisted breeding efforts for improved NUE (Bertin and Gallais, 2001; Liu et al., 2002; Agrama, 2005). However, these linkage mapping studies provided inconsistent results. The identification of maize genes consistently associated with improved NUE is a complex task, since gene expression is dependent on the soil N level (Chen et al., 2015), the source of N fertilizer (Patterson et al., 2010), and the plant's growth stage (Amiour et al., 2012). In addition to linkage mapping, several proteome studies designed to better understand the genetics of NUE have shown no relationship between transcriptome or metabolomics results (Simons et al., 2014). Recent research efforts using constitutive gene promoters have been used in plants to improve NUE (Xu et al., 2012), but transgenic genotypes with improved NUE performance are yet to be available in the commercial seed market. Gene identification (linkage mapping) and biotechnological methods rely on phenotypic traits with large marker effects. However, marker-assisted breeding approaches with the ability to estimate small-effect genes (commonly associated in complex polygenic traits) may be another strategy for developing genotypes with superior NUE performance.

Genomic selection (GS) uses genome-wide markers to predict the genotype's breeding value (Meuwissen et al. 2001). Therefore, GS could potentially be used for NUE improvement where many loci with small effects may together contribute to the phenotypic expression of a genotype. Genomic estimated breeding values (GEBV) can then be predicted by using the genomic relationship or the marker effects from individuals that were both phenotyped and genotyped (i.e., a training population). The efficiency of the genomic prediction can be measured by the prediction accuracy, which is an important component in the response to selection and genetic gain of a breeding program (Falconer and Mackay, 1996). In addition, further improvement using appropriate statistical analysis is necessary to obtain accurate prediction of phenotypes (Lipka et al., 2015). The main questions that plant breeders have in developing GS models pertain to the optimal composition of the training sets and the training population size. These decisions are typically based on the trait of interest and the budget available to the breeding program.

Previous studies on genomic selection for different quantitative maize traits have demonstrated relatively high prediction accuracies for improving drought tolerance (Ziyomo and Bernardo, 2013), carotenoid grain content (Owens et al., 2014), and northern corn leaf blight resistance (Technow et al., 2013). These results suggest that GS may be a promising breeding technique for the genetic improvement of complex polygenic traits in maize. Nonetheless, there is no information available about the accuracy of GS for maize yield performance under different N fertilizer rates or for different N-use traits. This experiment used ex-Plant Variety Protection (ex-PVP) germplasm adapted to the U.S. Corn Belt to investigate the following objectives: i) evaluate GS accuracy of different N-use traits, ii) predict yield performance under different N fertilizer rates, and iii) investigate the application of GS in NUE maize breeding programs. In addition, the impact of training composition and population sizes on GS accuracy was evaluated. The N-use trait identified with the highest prediction accuracy could be integrated into marker-assisted breeding strategies to accelerate NUE improvement in maize.

MATERIALS AND METHODS

Germplasm collection and genomic data

A diversity panel of 89 ex-PVP and two public (B73 and Mo17) lines adapted to the U.S. Corn Belt were tested in this study (Table 1.1). The original seed source was acquired from the North Central Regional Plant Introduction Station (http://www.ars-grin.gov/npgs, verified 24 Aug.

2016). A collection of 12 important progenitor lines previously characterized by Hauck et al., (2014) were included in the diversity panel. Additionally, a random collection of inbreds originating from six different seed companies and released from 1972 and 2011 were also part of the diversity panel. While diversity panels can capture the historical genetic recombination performed by previous breeding programs (Lipka et al., 2015), the more recently released ex-PVP lines may contain the genetic diversity shifts observed during the past 20 yr (Smith et al., 2004).

For all inbreds, DNA was isolated from 14-day old seedlings. Inbreds were genotyped using the genotype by sequencing method (Elshire et al., 2011) and two enzyme combinations were used to reduce genomic complexity: PstI-HF,Bfal and PstI-HF, HinP1I. Sequenced data were obtained from Illumina HiSeq2000 (W.M. Keck Center for Comparative and Functional Genomics, Urbana, IL) and single-nucleotide-polymorphism (SNP) data were called using the GBS pipeline in TASSEL 3.0 (Bradbury et al., 2007). Minor allele frequency cutoff was set to 10%, and SNPs with more than 50% missing data were removed. A total of 26,769 SNPs were used for the analyses.

Principal component analysis using the full set of SNP markers was performed on all inbreds categorized to distinct different heterotic groups (Figure 2.1). Moreover, the genomic relationship matrix (K matrix) between all inbreds was calculated according to VanRaden (2008), (Figure 2.2). Different heterotic groups consisted of 36 stiff-stalk synthetic (SSS) lines originating from the B73 cluster, versus 53 non-SSS (NSSS) lines, in which 19 lines were from the Iodent sub-heterotic group (PH207 cluster), and 34 lines were from the Lancaster sub-heterotic group (Mo17 cluster).

Agronomic practices, ex-PVP hybrids, and experimental design

Field experiments were conducted in eleven environments from 2011 to 2016. Experimental data from 2012 was removed from the analysis due to drought stress. Plots were planted using a precision plot planter (SeedPro 360, ALMACO, Nevada, IA) for one environment at DeKalb, IL (41°47′ N, 88°50′ W; 19 May 2014), six environments at Champaign, IL (40°3′ N, 88°14′ W; 17 May 2011, 20 May 2013, 22 April 2014, 24 April 2015, 19 May 2015, and 18 April 2016), and three environments at Harrisburg, IL (37°43′ N, 88°27′ W; 29 May 2013, 23 May 2014, and 26 April 2016). Plots were 5.6 m in length with 0.76 m row spacing, and two rows in width. The previous crop planted in each environment was soybean [*Glycine max* (L.) Merr.]. Final population was adjusted to 79,000 plants ha^{-1}. Pre-emergence weed control consisted of the herbicide Lumax® EZ (mixture of S-Metolachlor, Atrazine, and Mesotrione; Syngenta Crop Protection, Greensburo, NC) applied at a rate of 7 L ha^{-1} to control early season weeds. Before planting, seeds were treated with Maxim® XL fungicide (Fludioxonil and Mefenoxam at rate of 0.07 mg active ingredient kernel^{-1}; Syngenta Crop Protection, Greensburo, NC) and Cruiser® 5FS insecticide (Thiamethoxam at 0.80 mg active ingredient kernel^{-1}; Syngenta Crop Protection, Greensburo, NC) for disease and insect damage protection, respectively. Additionally, Force 3G® insecticide [Tefluthrin 2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1α,3α)-(Z)-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; Syngenta Crop Protection, Greensburo, NC] was applied at planting in-furrow (at a rate of 0.15 kg active ingredient ha^{-1}) to prevent western corn rootworm (*Diabrotica virgifera virgifera*) larvae infestation.

Single-cross hybrid seeds were created between SSS and NSSS lines in nursery trials from 2011 to 2015 at the University of Illinois, Department of Crop Sciences Research and Education Center in Champaign, IL. Between 2011 and 2015, 259 hybrids were randomly tested (ex-PVP inbred combinations within and across seed companies) in a randomized complete block design with three replications. In 2016, 263 new hybrid combinations, in addition to 50 tested hybrids, were planted in an augmented design with four commercial hybrid checks randomly assigned in nine blocks. Although experimental error of genotypes cannot be estimated in augmented designs, this experimental design provides the opportunity to replicate more genotypes. New hybrids tested in 2016 were created based on the genomic prediction results using the phenotypic data obtained from the 2011 to 2014 experiments. A schematic representation of the incomplete hybrid combination factorial is shown in Figure 2.3. Across all 10 environments, a total of 522 single-cross maize hybrids were planted. On average, each SSS line was combined in 16 (range 6-57) and each NSSS line was combined in 9 (range 2-38) different hybrid combinations.

All research sites were planted at two N fertilizer rates (0 and 252 kg N ha^{-1}; designated low and high N, or -N and +N, respectively) in a split-plot arrangement. The main-plot was hybrid and the split-plot was N fertilizer rate. Nitrogen fertilizer was hand applied in a diffuse band as urea (46-0-0) during the V2 to V3 growth stages (Ritchie et al., 1997) on 17 June 2014 at DeKalb, IL, 02 June 2011, 04 June 2013, 04 June 2014, 18 May 2015, 10 June 2015, and 24 May 2016 at Champaign, IL, and 25 June 2013, 13 June 2014, and 01 June 2016 at Harrisburg, IL. At maturity, plots were harvested with a two-row plot combine (SPC40, ALMACO, Nevada, IA). Grain yield is reported as Mg ha^{-1} at 15.5% grain moisture. Grain protein concentration was estimated using a representative grain subsample from each plot collected during harvest using near infrared transmittance (NIT) spectroscopy (Infratec 1241, FOSS, Eden Prairie, MN).

Phenotypic data analysis and genomic prediction model

Nitrogen use traits were measured according to Haegele et al. (2013). Briefly, six whole plants from each experimental plot were harvested at the R6 growth stage (Ritchie et al., 1997) to measure biomass, grain weight, and plant N concentration. The twelve N-use traits measured in this study are described in Table 2.1. Adjusted means for hybrids tested in 2016 were calculated using best linear unbiased estimators (BLUEs) with hybrid as fixed effect and rows, columns, and environments used as random effects. General combining ability (GCA) and specific combining ability (SCA) were calculated using the phenotypic data obtained between 2011 and 2015 and the hybrid BLUEs from 2016. Best linear unbiased predictors (BLUPs) were calculated using the restricted maximum likelihood method according to the model described by Reif et al. (2013). The estimated breeding value of each hybrid was calculated according to Eq. [1]:

$$EBV_{kl} = \mu + GCA_k + GCA_l + SCA_{kl} \qquad [1]$$

where EBV_{kl} is the estimated breeding value of *kl*th hybrid; μ is the grand mean; GCA_k is the general combining ability effect of *k*th SSS inbred (*k*=1 to 57); GCA_l is the general combining ability effect of *l*th NSSS inbred (*l*=1 to 38), and SCA_{kl} is the specific combining ability effect of *kl*th hybrid (*kl*=1 to 522). All variance components were determined using the lmer4 package in R Studio (R Development Core Team, 2015). Phenotypic variance was calculated as the sum of all variance components, except the variance component for block effect and environment (Holland et al., 2003). Therefore, broad-sense heritability was calculated according to Eq. [2]:

$$H^2 = 2 \times (\sigma^2_{GCAk} \times \sigma^2_{GCAl} \times \sigma^2_{SCA}) / (\sigma^2_{GCAk} + \sigma^2_{GCAl} + \sigma^2_{SCA} + \sigma^2_{GCA \times E} + \sigma^2_{SCA \times E} + \sigma^2_{R}) \qquad [2]$$

where H^2 is the broad-sense heritability, and σ^2_{GCAk}, σ^2_{GCAl}, σ^2_{SCA} are the variance components for the SSS GCA, NSSS GCA, and SCA, respectively. Similarly, $\sigma^2_{GCA \times E}$, $\sigma^2_{SCA \times E}$, σ^2_{R} are variance components for GCA × E interaction, SCA × E interaction, and residual, respectively.

Genomic best linear unbiased prediction (G-BLUP) of untested hybrids (y_u) was calculated according to Eq.[3]:

$$y_u = C_{UT} C_{TT}^{-1} y_T \qquad [3]$$

where C_{UT} is the covariance matrix among untested and tested hybrids, C_{TT}^{-1} is the inverse of the variance-covariance matrix of the tested hybrids, and y_T is the EBV of a set of tested hybrids. The hybrid's EBV will vary according to the phenotypic information and the additive effect between the individuals (Lynch and Walsh, 1998). Therefore, the genomic relationship coefficients between SSS and NSSS inbreds (Figure 2.2) were assigned to C_{UT} and C_{TT}^{-1} according to Bernardo (1996).

Cross validation

The estimation of prediction accuracy was performed in R Studio (R Development Core Team, 2015) using the cross validation approach described by Technow et al. (2014). For investigating the effect of training composition (TC) in the prediction accuracy, a random subset of 16 SSS and 30 NSSS lines were selected in each iteration. The 522 hybrids were categorized into T0 (hybrids in which none of the parents were included in the random subset of inbreds), T1 (hybrids in which one of their parents were included in the random subset of inbreds), and T2 (hybrids in which both of their parents were included in the random subset of inbreds). For investigating the effect of training population (TP) size in the prediction accuracy, a random subset of either 10, 20, 30, or 40 T2 hybrids (for the traits of HI, NHI, NUE, NUpE, NUtE, and GU) or 30, 50, 70, or 90 T2 hybrids (for the traits of yield and grain protein concentration) were used to predict the T0, T1, or the remaining T2 hybrids. Prediction accuracy was calculated as the Pearson correlation between a hybrid's EBV and predicted values (GEBV). Moreover, the cross-validation process was repeated 1,000 times. On average, predictions of T0, T1, and T2 consisted 65, 129, and 63 hybrids, respectively across iterations.

RESULTS

Genetic relationship and population structure between ex-PVP lines

Principal component analysis using SNP markers of all inbreds revealed distinct clusters among heterotic groups (Figure 2.1). Mean relationship coefficients between SSS, NSSS, and SSS by NSSS lines were 0.59, 0.31, and 0.31 with standard deviations of 0.31, 0.23, and 0.15, respectively (Figure 2.4). Inbreds from the SSS group were more genetically related than inbreds from NSSS group. This variation in genetic relatedness between heterotic groups is due to the fact that most SSS inbreds are B73 descendants and the inbreds of the NSSS group originated from two separate sub-heterotic groups (Iodent and Lancaster).

Phenotypic variation and correlation

Variance components and broad-sense heritability (H^2) varied across N-use phenotypic traits for the 522 hybrids when grown with differing N supplies in 10 environments (Figure 2.5). Broad-sense heritability ranged from 0.11 to 0.77. Genetic variance and H^2 were greater for traits when hybrids were grown under high N compared to low N conditions. Across all phenotypic traits, residual and GCA variances accounted for the majority of the total phenotypic variance, regardless of the N treatment. In contrast, SCA and genetic by environment interaction variances had a small contribution to the total phenotypic variance.

A biplot depiction of the principal component analysis revealed different variations and correlations among phenotypic traits due to fertilization level for the field-grown hybrids (Figure 2.6). Overall, N-use traits associated with plant N partitioning and redistribution to the grain (i.e., GU, HI, and NHI_{+N}) were highly correlated to yield under low N, while traits associated with the yield response to N fertilizer (i.e., NUE, NUtE, and NUpE) were correlated to yield under high soil N conditions. Within each N fertilizer treatment, grain protein concentration was negatively correlated to yield. In addition, NHI_{-N} accounted for a small phenotypic variation, likely due to a large residual variance (Figure 2.5).

Genomic prediction accuracy

Across all N-use traits and TP schemes, G-BLUP accuracy ranged from -0.12 to 0.78 (Tables 2.2 and 2.3). Prediction accuracy increased 13% when the TC changed from T0 to T1 and increased 10% when the TC changed from T1 to T2 hybrids when averaged across N-use traits and TP sizes (Figure 2.7). Increasing TP size was more effective when more genetic information (TC) was available in the TP. Consequently, increased TP size (from 30 to 90 or 10 to 40 hybrids) improved GS accuracy by 5, 19, and 31% using T0, T1, and T2 hybrids in the TC, respectively.

Changes in prediction accuracy as a result of variation in TC and TP sizes were dependent on the N use trait. For example, HI_{-N} and GU, compared to other traits, exhibited a greater increase in prediction accuracy due to increased TP size compared to increased inclusion of parents in the TC. In contrast, $Protein_{-N}$ exhibited a greater increase in prediction accuracy by changing the TC rather than by increasing the training size. The differences found in the prediction accuracy response to the increased training population size and training composition among N-use traits will have direct implications for NUE breeding programs.

DISCUSSION

Prediction accuracy response to different training composition

Hybrid performance prediction is mostly driven by the coancestry coefficient between individuals (Bernardo, 1996). In addition, genomic prediction for maize performance has also noted the importance of training composition on prediction accuracy (Riedelsheimer et al., 2013; Technow et al., 2013; Technow et al., 2014). Similar to these studies, more genotypes that are shared between the training and validation sets provided higher prediction accuracy (Figure 2.7). The related genetic constitution between training and validation sets allows for similar linkage phases between markers and QTL among these groups (Technow et al. 2013). Nonetheless, differences in prediction accuracy associated with TC were trait specific.

High H^2 and genetic relatedness between individuals are important factors for increased prediction accuracy in additive genetic models (Daetwyler at al. 2010). Using ex-PVP germplasm resulted in low genetic relatedness between the NSSS lines (Figure 2.4) and a large range of H^2 for N-use traits (Figure 2.5). Technow et al. (2014) compared different cross-validation methods using G-BLUP by changing the training composition and found higher prediction accuracy values than reported here, due to a greater number of both hybrids and environments. High prediction accuracies obtained by Technow et al. (2014) were associated with higher H^2 and realized relationship between parental lines. Similarly, a low amount of phenotypic observations decrease genomic prediction accuracy (Meuwissen et al., 2001).

Prediction accuracy response to increased training size

Increased TP size has an important effect on GS accuracy with both animals (VanRaden et al., 2008) and crops (Lorenzana and Bernardo, 2009). However, increased TP size was minimally or not effective when no parental information was available in the training population (T0 hybrids), (Figure 2.7). Increased TP size may gain more importance than prediction accuracy when training and validation populations become more genetically related.

One of the possible reasons for the success of increasing prediction accuracy by increasing TP size is due to the fact that the SCA effect had only a small contribution to the total phenotypic variance (Figure 2.5). Therefore, increasing TP size will increase the precision to estimate the GCA effects (Technow et al., 2014). The greater importance of GCA than SCA for genomic prediction is mainly associated in plant species with genetically distinct heterotic patterns (Reif et al., 2007). This heterotic-pattern condition may be one explanation why prediction accuracy benefits more from increasing TP size using the T2 hybrids in a TP than the T0 hybrids.

Another interesting finding from this study was the negative prediction accuracy found for NUtE and NHI$_{-N}$ (Table 2.3). While negative prediction accuracy for NUtE was observed only when using unrelated parents (T0 hybrids) in the TP, NHI$_{-N}$ provided negative accuracies regardless of TC or TP size. Previous reports have also reported negative prediction accuracy in maize (Riedelsheimer et al., 2013; Daetwyler et al., 2015). The large residual variance and small genetic variance of NUtE and NHI$_{-N}$ traits may have provided a poor genetic signal for the training model and reduced the GS accuracy.

Use of secondary traits for NUE breeding

One strategy for a maize NUE breeding program is to integrate into the genotype the desirable phenotypic traits that are correlated to increased yield under N-stress (Yield$_{-N}$) or high N conditions (Yield$_{+N}$). Moreover, the use of secondary traits may improve the precision to identify a genotype, identify the degree of the N stress, and aid plant breeders in making selection. Under low-N stress conditions, GU and HI$_{-N}$ both displayed higher GS accuracy and correlation to Yield$_{-N}$. These N-use traits may be integrated into maize breeding programs targeting improved performance under N-stress conditions. Alternatively, NUE and NUpE were the secondary traits that provided the highest GS accuracy under high N and correlation to Yield$_{+N}$. These traits will be desirable in breeding programs developing hybrids for agricultural systems using high N fertilizer inputs.

CONCLUSIONS

Nitrogen use traits are highly polygenic and complex. Phenotyping for maize NUE under field conditions is time consuming and requires great research effort. The use of GS for NUE improvement holds great promise, since it can reduce the number of breeding cycles and the cost for field phenotyping. Thus, the identification of highly heritable and predictable N-use traits is important and must be targeted according to the breeding objectives (tolerance to N-stress or yield response to N fertilizer). Training composition and size are important factors for GS accuracy, but their usefulness will vary according to the N-use trait desired. Future research integrating the G × E effect and crop growth models into GS may improve the prediction accuracy of N-use traits.

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TABLES AND FIGURES

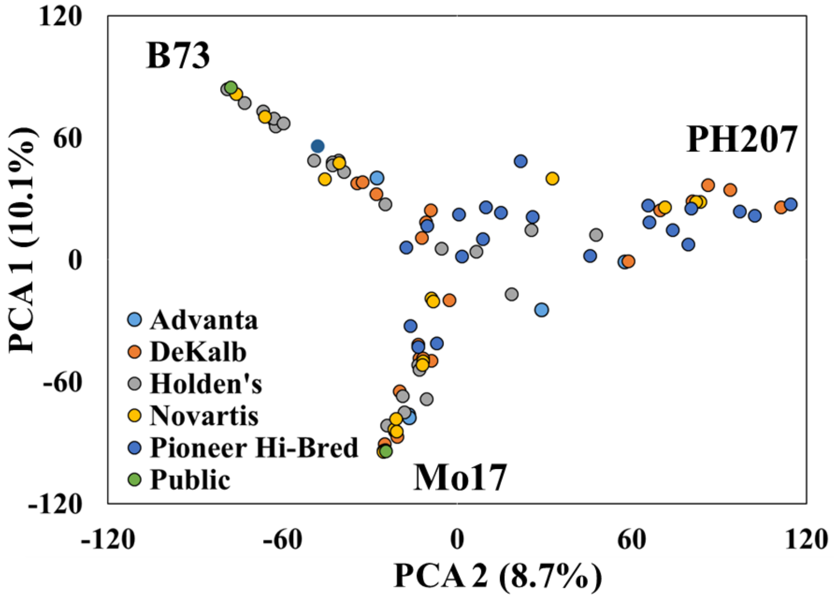


Figure 2.1. Principal component analysis (PCA) of 89 ex-PVP and 2 public (B73 and Mo17) inbred lines using 26,769 single-nucleotide polymorphisms. The main progenitor lines for each cluster are identified: B73 for the stiff-stalk synthetic (SSS), PH207 for the Iodent, and Mo17 for the Lancaster heterotic groups. Colors represent the origin of the different inbred lines.

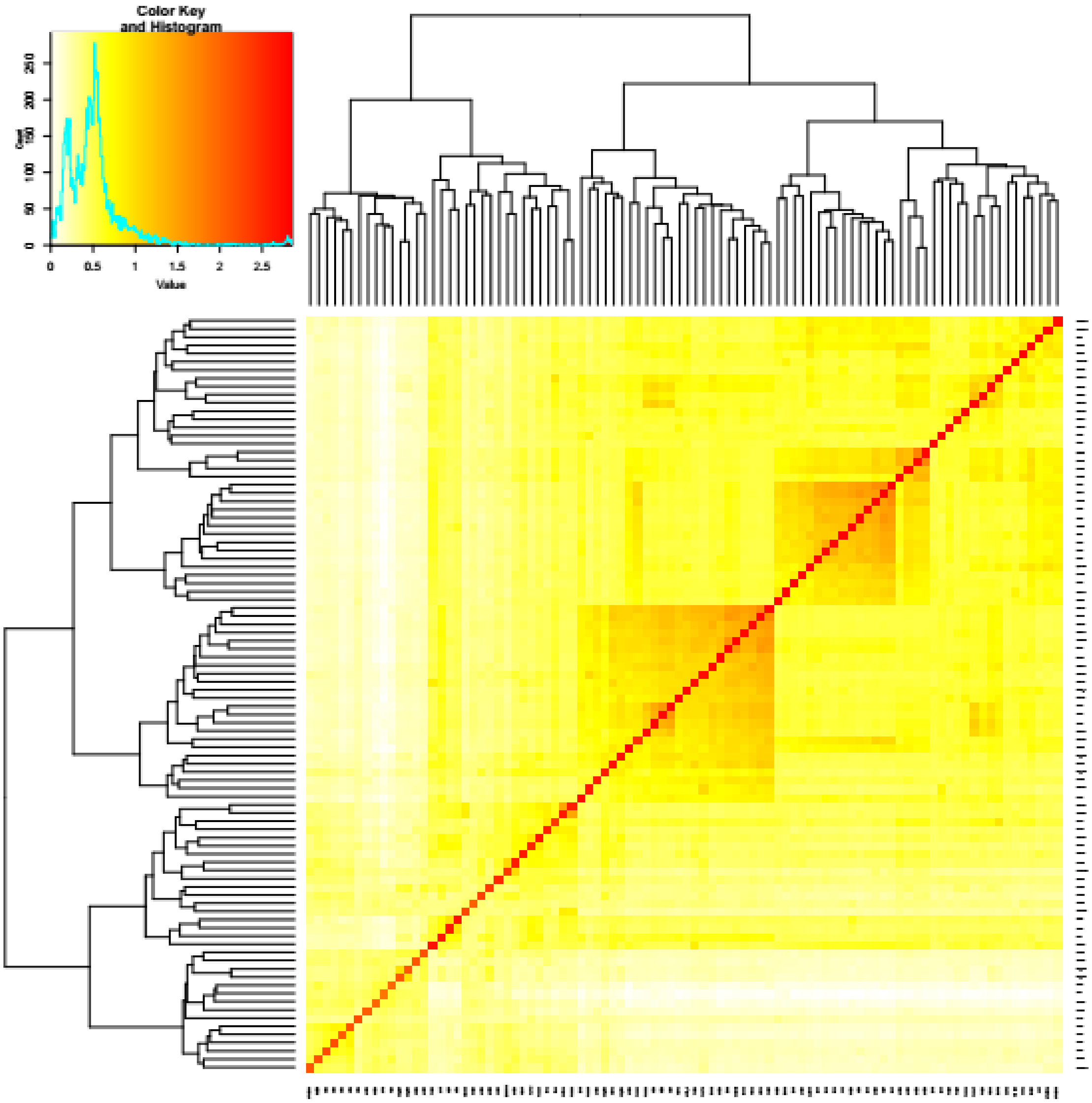


Figure 2.2. Heat map of the genomic relationship coefficients (K-matrix) for 91 maize inbred lines using 26,769 single-nucleotide polymorphisms. Rows and columns represent different inbred lines and genomic coefficients were calculated between each inbred combination. Dark red represents high genomic coefficients (high genetic relatedness between inbred lines) and white and light-yellow represent low genomic coefficients (low genetic relatedness between inbred lines). Hierarchical clusters using the K-matrix are represented by different heterotic groups (Stiff-stalk synthetic, Iodent, and Lancaster).

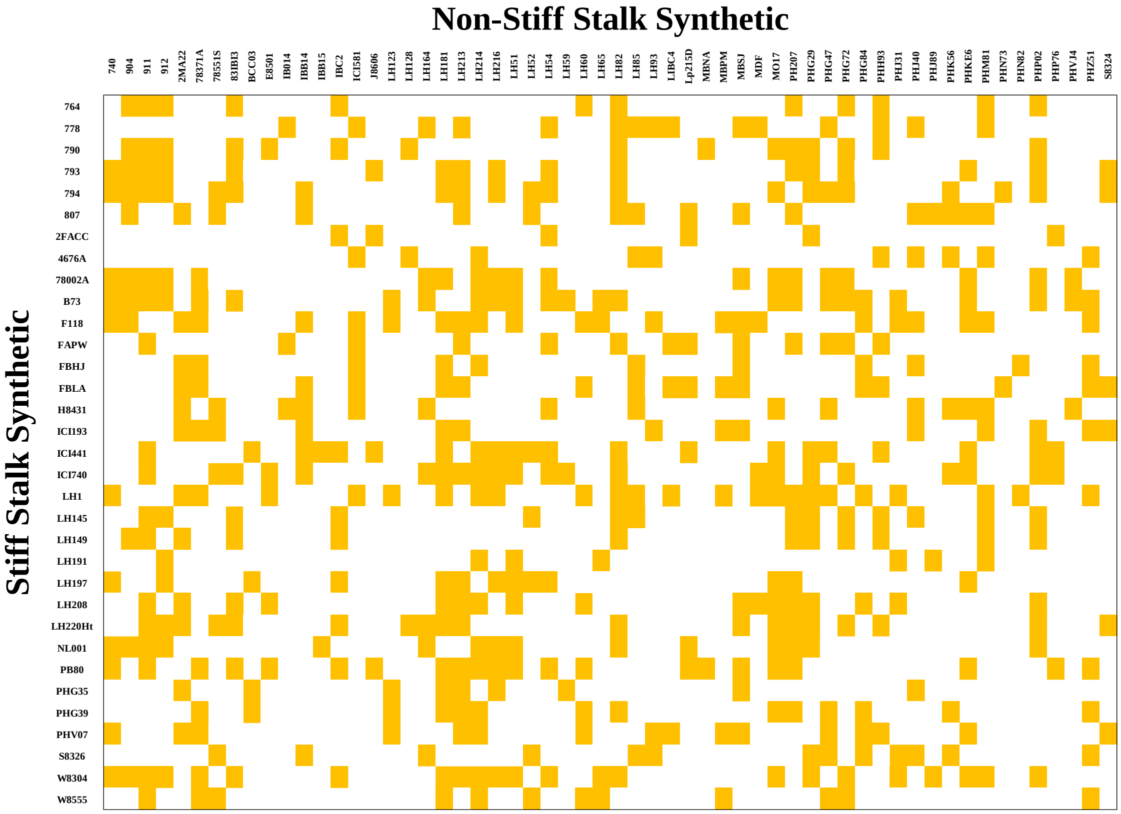


Figure 2.3. Schematic illustration of the mating factorial of 36 stiff-stalk synthetic by 53 non-stiff-stalk synthetic lines. A total of 522 single-cross hybrids indicated by the orange squares were planted at two N fertilizer rates (0 and 252 kg N ha^{-1}) across 10 environments in Illinois from 2011 to 2016. Rows and columns represent different inbred lines.

Table 2.1. List of 12 phenotypic traits, units, acronyms, and formulas. Differerent trait measurements included: grain weight at low and high N (Grain$_{-N}$ and Grain$_{+N}$, respectively), total above-ground dry biomass at low and high N (BM$_{-N}$ and BM$_{+N}$, respectively), and total plant biomass N content at low and high N (PN$_{-N}$ and PN$_{+N}$, respectively).

Trait name, unit	**Trait acronym**	**Formula**
Yield at low N, Mg ha^{-1}	Yield$_{-N}$	-
Yield at high N, Mg ha^{-1}	Yield$_{+N}$	-
Harvest index at low N, kg kg^{-1}	HI$_{-N}$	$\frac{Grain_{-N}}{BM_{-N}}$
Harvest index at high N, kg kg^{-1}	HI$_{+N}$	$\frac{Grain_{+N}}{BM_{+N}}$
Nitrogen harvest index at low N, kg$_{grainN}$ kg$_{plantN}^{-1}$	NHI$_{-N}$	$\frac{GrainN_{-N}}{PN_{-N}}$
Nitrogen harvest index at high N, kg$_{grainN}$ kg$_{plantN}^{-1}$	NHI$_{+N}$	$\frac{GrainN_{+N}}{PN_{+N}}$
Grain protein concentration at low N, g kg^{-1}	Protein$_{-N}$	-
Grain protein concentration at high N, g kg^{-1}	Protein$_{+N}$	-
Nitrogen use efficiency, kg kg$_{Nfert}^{-1}$	NUE	$\frac{Yield_{+N} - Yield_{-N}}{N\ rate}$
Nitrogen uptake efficiency, kg$_{plantN}$ kg$_{Nfert}^{-1}$	NUpE	$\frac{PN_{+N} - PN_{-N}}{N\ rate}$
Nitrogen utilization efficiency, kg kg$_{plantN}^{-1}$	NUtE	$\frac{Yield_{+N} - Yield_{-N}}{PN_{+N} - PN_{-N}}$
Genetic utilization, kg kg$_{plantN}^{-1}$	GU	$\frac{Yield_{-N}}{PN_{-N}}$

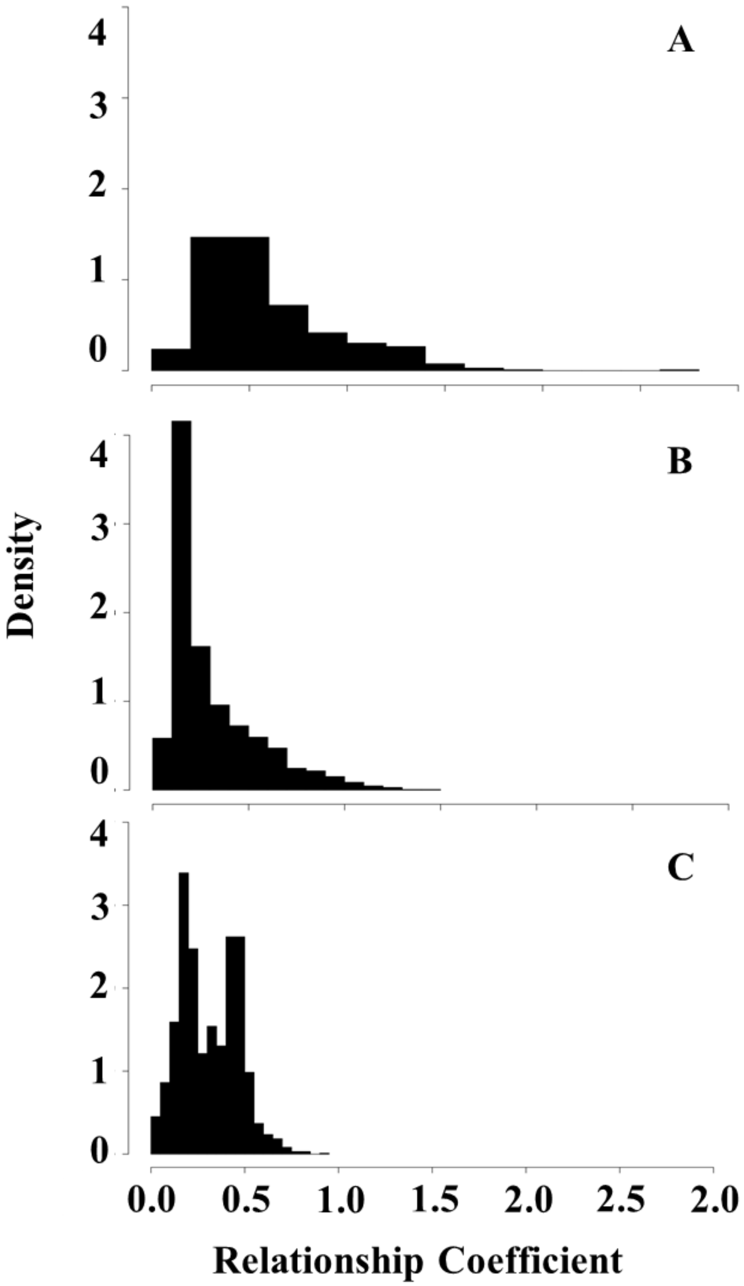
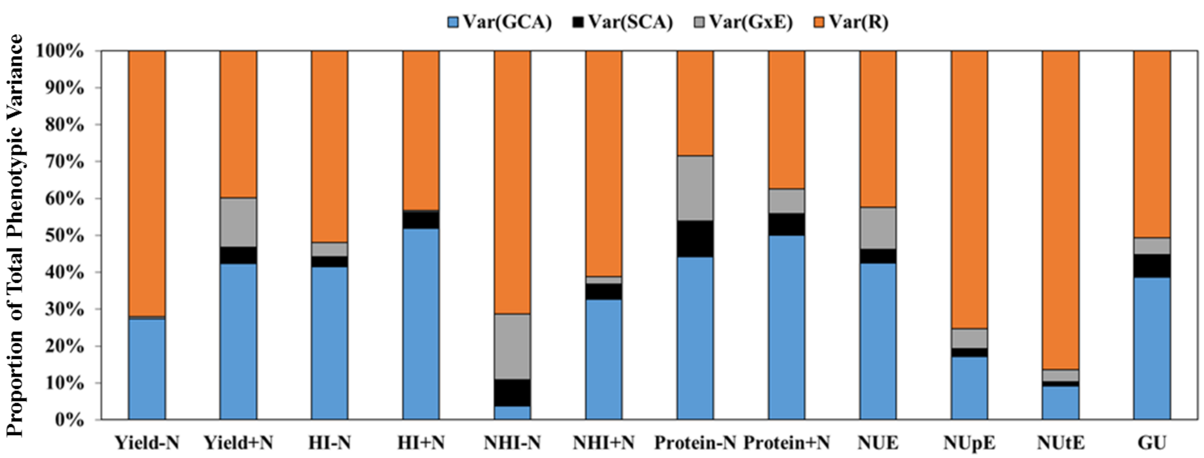


Figure 2.4. Density histogram of pairwise genomic relationship coefficients between SSS lines (A), NSSS lines (B), and SSS by NSSS lines (C). Coefficients are elements of the K-matrix as computed in the G-BLUP model.



H^2	0.31	0.61	0.63	0.73	0.11	0.44	0.74	0.77	0.60	0.27	0.11	0.58

Figure 2.5. Relative contribution to total phenotypic variance of general combining ability [Var(GCA)], specific combining ability [Var(SCA)], genetic by environment interaction [Var(G × E)], and residual variances [Var(R)] for 12 different N-use traits averaged over 522 maize hybrids grown at low (0 kg N ha^{-1}) or high N (252 kg N ha^{-1}) from 2011 to 2016. The measured traits included: yield at low N (Yield$_{-N}$), yield at high N (Yield$_{+N}$), harvest index at low N (HI$_{-N}$), harvest index at high N (HI$_{+N}$), nitrogen harvest index at low N (NHI$_{-N}$), nitrogen harvest index at high N (NHI$_{+N}$), grain protein concentration at low N (Protein$_{-N}$), grain protein concentration at high N (Protein$_{+N}$), nitrigen use efficiency (NUE), N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), and genetic utilization (GU). Broad-sense heritabilities (H^2) were estimated on a entry mean basis for the same hybrids described above.

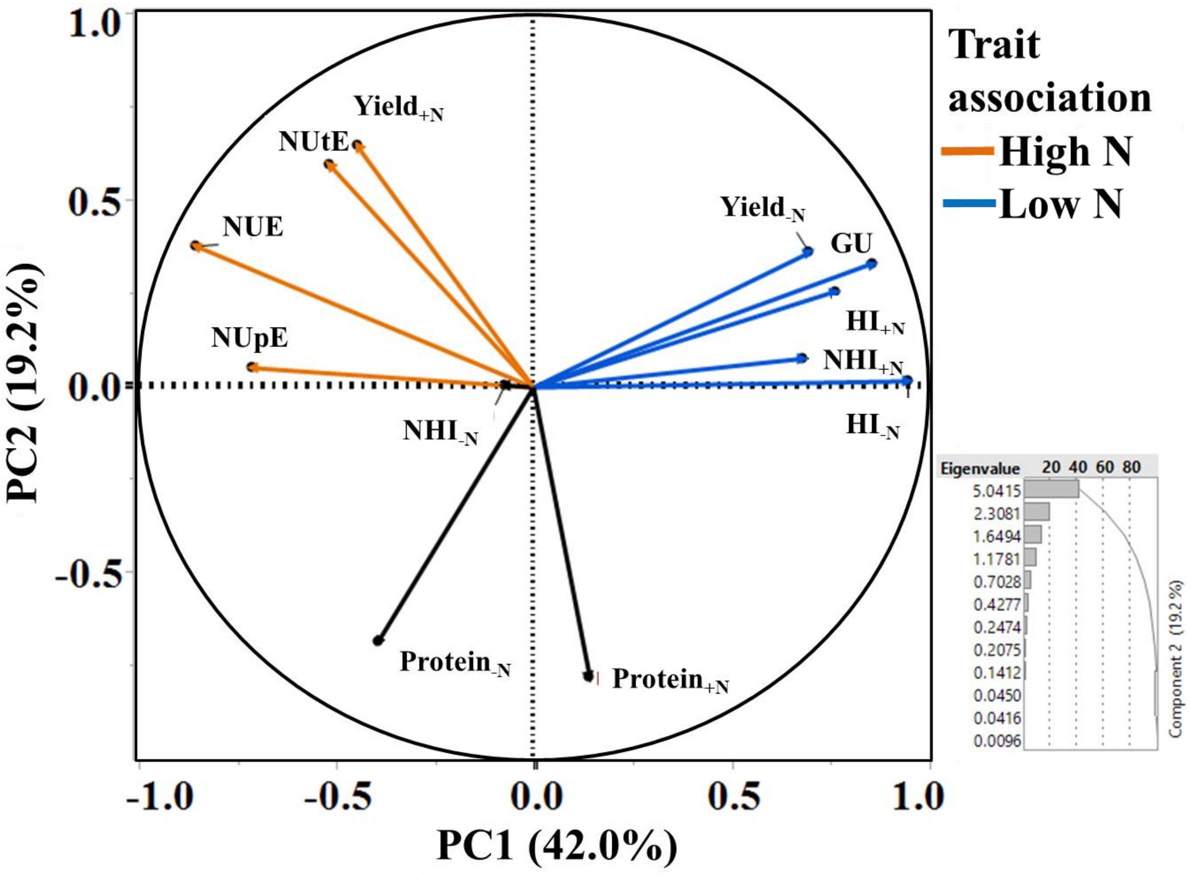


Figure 2.6. Biplot derived from principal component analysis of 12 phenotypic traits. Principal component analyses were performed comparing N-use phenotypic traits to 522 single-cross hybrids receiving either low N (0 kg N ha^{-}1) or high N (252 kg N ha^{-1}) fertilizer and averaged across 10 environments from 2011 to 2016. Phenotypic traits positively correlated to yield at low N and yield at high N are respresented by blue and orange arrows, respectively. Phenotypic traits not positively correlated to yield (Low or High N) are represented by black arrows.

Table 2.2. Genomic prediction accuracies of maize yield when grown under low (Yield$_{-N}$; 0 kg N ha^{-1}) or high N (Yield$_{+N}$; 252 kg N ha^{-1}) and grain protein concentration under low (Protein$_{-N}$) and high N (Protein$_{+N}$) conditions in response to different training composition (TC) schemes and training population (TP) sizes.

TC[†]	**TP**[‡]	**Yield$_{-N}$**	**Yield$_{+N}$**	**Protein$_{-N}$**	**Protein$_{+N}$**
T0	30	0.17[§]	0.17	0.13	0.16
	50	0.20	0.21	0.13	0.17
	70	0.21	0.24	0.15	0.19
	90	0.22	0.26	0.13	0.20
T1	30	0.23	0.29	0.24	0.23
	50	0.29	0.39	0.30	0.30
	70	0.34	0.45	0.35	0.35
	90	0.37	0.50	0.37	0.39
T2	30	0.27	0.37	0.36	0.28
	50	0.38	0.53	0.53	0.41
	70	0.47	0.64	0.67	0.53
	90	0.53	0.72	0.76	0.62

[†] Training composition (TC) for T0, T1, and T2 represent groups of hybrids with zero, one, and both parents that were tested in the training set, respectively.

[‡] Training population (TP) size constituted from a random selection of 30, 50, 70, and 90 single-cross hybrids set from a constant number of 16 SSS and 30 NSSS inbreds. The total data set consisted of a group of 522 hybrids.

[§] Accuracy values represent the Pearson correlation mean between observed and predicted values averaged over 1,000 cross-validation runs.

Table 2.3. Genomic prediction accuracies of maize harvest index when grown with low N (HI_{-N}; 0 kg N ha^{-1}) or high N (HI_{+N}; 252 kg N ha^{-1}), nitrogen harvest index under low N (NHI_{-N}) and high N (NHI_{+N}), N-use efficiency (NUE), N-uptake efficiency (NUtE), N-utilization efficiency (NUtE), and genetic utilization (GU) in response to different training composition (TC) schemes and training population (TP) sizes.

TC[†]	**TP**[‡]	**HI_{-N}**	**HI_{+N}**	**NHI_{-N}**	**NHI_{+N}**	**NUE**	**NUpE**	**NUtE**	**GU**
T0	10	0.26[§]	0.16	-0.08	0.06	0.05	0.06	-0.06	0.06
	20	0.34	0.20	-0.12	0.08	0.07	0.08	-0.05	0.13
	30	0.38	0.22	-0.12	0.11	0.09	0.09	-0.06	0.17
	40	0.41	0.23	-0.10	0.12	0.11	0.09	-0.05	0.23
T1	10	0.28	0.18	-0.05	0.09	0.11	0.08	0.03	0.15
	20	0.45	0.28	-0.07	0.18	0.21	0.16	0.05	0.30
	30	0.57	0.35	-0.09	0.28	0.28	0.21	0.07	0.41
	40	0.63	0.42	-0.06	0.35	0.36	0.25	0.07	0.48
T2	10	0.31	0.19	-0.04	0.11	0.14	0.10	0.08	0.19
	20	0.53	0.33	-0.07	0.23	0.28	0.20	0.11	0.42
	30	0.69	0.46	-0.08	0.38	0.40	0.28	0.15	0.56
	40	0.78	0.55	-0.05	0.48	0.51	0.35	0.15	0.68

[†] Training composition (TC) for T0, T1, and T2 represent groups of hybrids with zero, one, and both parents that were tested in the training set, respectively.

[‡] Training population (TP) size constituted from a random selection of 10, 20, 30, and 40 single-cross hybrids set from a constant number of 16 SSS and 30 NSSS inbreds. Total data set consisted of a group of 259 hybrids.

[§] Accuracy values represent the Pearson correlation mean between observed and predicted values averaged over 1,000 cross-validation runs.

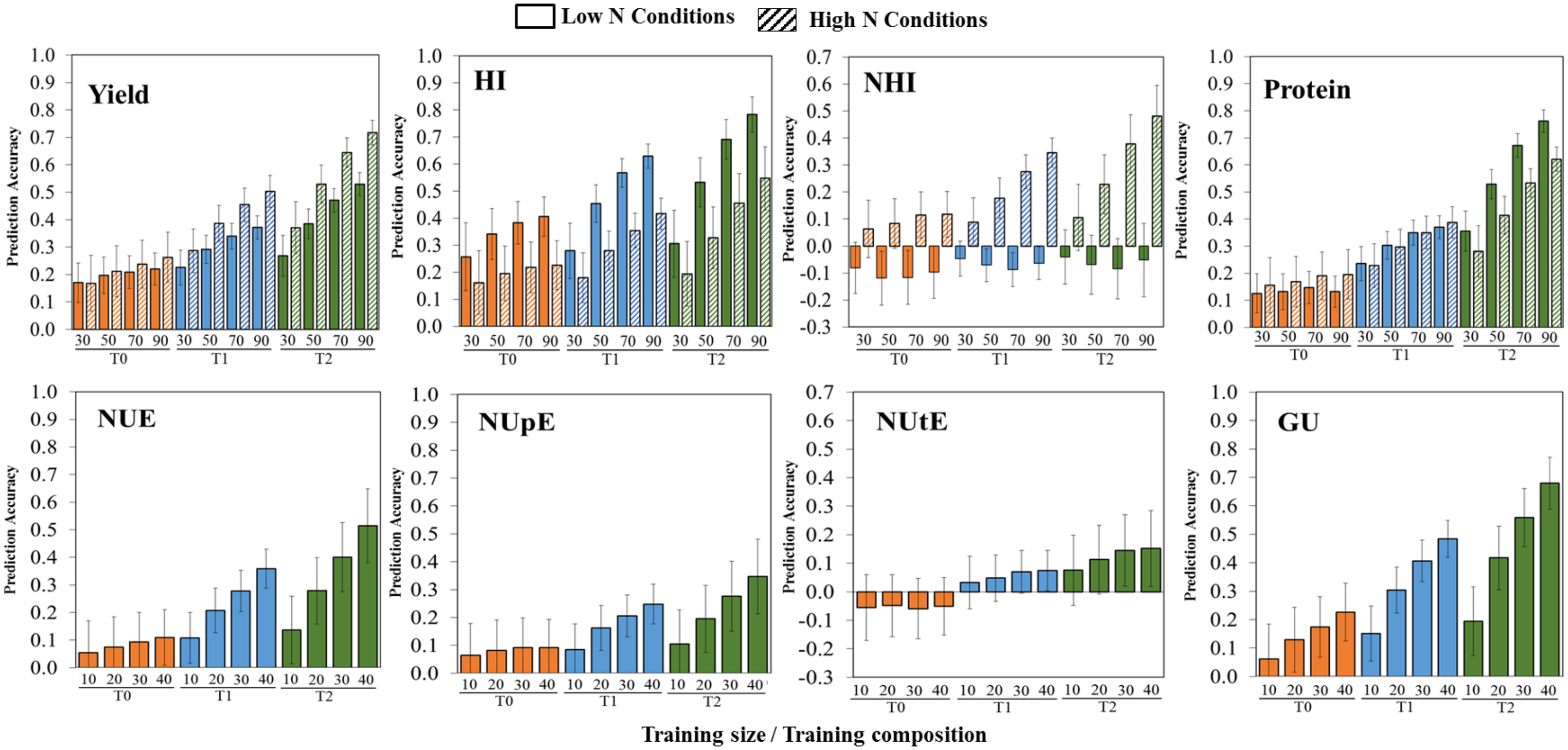


Figure 2.7. Prediction accuracy response to different training population sizes and compositions for 12 phenotypic traits of maize when grown with either low N (0 kg N ha^{-1}) or high N (252 kg N ha^{-1}) fertilization. Training composition schemes were categorized into T0 (hybrids in which none of the parents were included in the random subset of inbreds), T1 (hybrids in which one of their parents were included in the random subset of inbreds), and T2 (hybrids in which both of their parents were included in the random subset of inbreds). A total of 522 hybrids were used for the prediction of yield and grain protein, and 259 hybrids were used for HI, NHI, NUE, NUpE, NUtE, and GU. Vertical bars represent the standard deviation of the accuracy mean.

CHAPTER 3

SELECTING MAIZE GENOTYPES WITH IMPROVED YIELD RESPONSE TO NITROGEN FERTILIZER AND PLANT DENSITY

ABSTRACT

Continued yield increases in modern commercial maize (*Zea mays* L.) hybrids will require increased plant density, improved N use efficiency, and breeding for a hybrid's potential yield response to this management. The objective of this study was to determine the genetic variation of commercial hybrids in response to plant density and N fertilizer levels to assist breeding programs selecting for hybrids with high yield stability or adaptability to crop management. From 2011 to 2014, 101 hybrids were grown in eight different environments at two planting densities (79,000 and 110,000 plants ha^{-1}), three N rates (0, 67, and 252 kg N ha^{-1}), and four blocks. Broad-sense heritability increased with increased N rate and plant density. Increased plant density altered yield from -0.60 Mg ha^{-1} to +0.58 Mg ha^{-1} under high N conditions, while the yield response to increased N ranged from +4.47 to +5.64 Mg ha^{-1}. Hybrids that combined above average yield under low N conditions and to the initial N fertilizer increment exhibited greater yield stability under high N conditions. Hybrid yield stability variance was larger under high N than low N conditions due to greater genetic by environment interaction. Selecting hybrids with high yield stability may be challenging since yield under lower N levels and yield increases with high N fertilization were negatively correlated. Hybrids that are adaptable to high plant density and N conditions exhibited greater yield potential, but also greater stability variance.

INTRODUCTION

Maize yield increases since the 1930s have been due to a combination of genetic improvement and better crop management practices (Duvick, 2005). Because maize genotypes interact with crop management in producing yield, understanding the dynamics between plant genetics and agronomic management will provide the opportunity to obtain the maximum yield potential of a hybrid using a corresponding recommended agricultural management system. In addition, continued increases in corn yield will depend on a hybrid's ability to utilize resources more efficiently when grown under greater planting densities (Tollenaar and Lee, 2002) and under favorable agronomic conditions (Boomsma et al., 2009). Nonetheless, increased plant density needs to be in synergy with other intensified management factors, such as better soil fertility, in order to minimize the current maize yield gap that exists in the U.S. Corn Belt (Ruffo et al., 2015).

For maximum yield, nitrogen (N) is the nutrient required in the greatest amount for maize production (286 kg N to produce 14.4 Mg ha^{-1} of grain) accompanied by a high N harvest index (58%) in the grain (Bender et al., 2013). Increased N fertilizer rate was one of the major crop management practices that contributed to increased maize yield over the past 20 years (Egli, 2008). In comparisons of the genetic gain of maize hybrids under different levels of N fertility, newer hybrids (1990's) exhibited greater yield than older hybrids (1970's) under low and high N conditions (Tollenaar et al., 1997; O'Neil et al., 2004). Although this genetic improvement of maize did not increase plant total N uptake, current maize hybrids have greater N utilization, and approximately 70% of the genetic gain of maize yield under high N conditions is due to yield improvement under low N conditions (Haegele et al., 2013).

Current commercial breeding programs select and develop elite hybrids under optimal agronomic inputs (high N fertilizer level and standard plant density), only evaluating a hybrid's yield responses to different crop management practices at the pre-commercial stage. Therefore, there are limited reports of genetic variability of elite hybrids for different N-use traits (Bertin and Gallais, 2000). The genetic improvement in the yield response to N fertilizer in maize hybrids is well documented (Ding et al., 2005; Coque and Gallais, 2007; Haegele et al., 2013). However, these previous studies used a small representation of elite maize hybrids across a limited number of environments, which may have underestimated the genetic variation of current maize hybrid yields in response to N fertilizer supplementation.

In addition to the genetic improvements in nitrogen use efficiency, tolerance to increased plant density is one of the most valuable agronomic advances since the development of the maize hybrid (Duvick, 1977). The most recent maize yield record in the U.S. (31.5 Mg ha^{-1}) was achieved with a plant density (128,000 plants ha^{-1}) much greater than common agronomic practices (National Corn Growers Association, 2015), indicating that the average yield of maize is still far from a plateau and that current maize hybrids exhibit greater crowding stress tolerance when compared to their predecessors (Tollenaar and Wu, 1999).

One of the possible reasons for the success of increasing plant density to improve maize yield is due to the fact that the yield potential of individual plants has not increased in the past 80 years, rather maize hybrids have better stress tolerance, including the ability to tolerate higher plant densities (Tollenaar and Lee, 2002; Duvick, 2005). Increased plant density typically reduces the yield of individual plants, but increases light interception, and as a result, kernels produced per ground area, therefore increasing the area-wide source-sink ratio (Borras et al., 2003). In turn, the greater number of potential kernels produced per area in density- tolerant hybrids may minimize the yield decreases from environmental stresses.

Genotypes that are tolerant to abiotic and biotic stresses are expected to have more yield stability, i.e., greater ability to maintain consistent yield across different environmental conditions (Tollenaar and Lee, 2002). On the other hand, genotypes that are responsive to high yield environments are defined as adaptable genotypes and are expected to have greater yield under favorable agronomic conditions. Stability and adaptability classifications were first proposed by Finlay and Wilkinson (1963) and are based on a hybrid's performances compared to the corresponding environmental indices (average performance of multiple hybrids at a certain environment). High stability genotypes have been further characterized as "work-horse" hybrids, while hybrids with high adaptability are "race-horse" hybrids (Tollenaar and Lee, 2002).

One of the future challenges of maize breeding will be selecting genotypes with greater yields in response to increased plant density with concurrent yield stability across environments (Tokatlidis and Koutroubas, 2004). Genetic gain for yield has been found to be reduced when maize hybrids were grown under high plant density conditions (De Leon and Coors, 2002; Fasoula and Tollenaar, 2005). These authors attributed the higher genetic gain observed at low plant densities to improved prolificacy and the yield potential of individual plants. However, most current elite hybrids are single-eared plants and are better adapted to increased plant densities than older hybrids. Breeding programs targeting for maize hybrids with improved tolerance to high plant density and utilization of N fertilizer need a comprehensive evaluation including a representative number of commercial maize hybrids, N rates, plant densities, and environments. Therefore, the objective of this research was to evaluate the genetic × environment × management interaction on current maize hybrid yields, yield stability, and adaptability.

MATERIALS AND METHODS

Cultural practices

Eight environments were used for the experiment, covering the years 2011 to 2014. Data from 2012 of the trial was excluded from the analysis due to severe drought stress. Research sites were planted for two years at DeKalb, IL (DK; 41°47′ N, 88°50′ W; 15 May 2013 and 20 May 2014), three years at Champaign, IL (CH; 40°3′ N, 88°14′ W; 18 May 2011, 19 May 2013, and 08 May 2014), and three years at Harrisburg, IL (HB; 37°43′ N, 88°27′ W; 01 June 2011, 29 May 2013, and 23 May 2014). Field trials from 2011 and 2013 were conducted by Dr. Jason Haegele. Soil types at the research sites generally were Flanagan silt loam at DeKalb, IL, Drummer silty clay loam at Champaign, IL, and Patton silty clay loam at Harrisburg, IL. The previous crop planted in each environment was soybean [*Glycine max* (L.) Merr.].

The experiment was planted using a precision plot planter with variable seeding rate capability (SeedPro 360, ALMACO, Nevada, IA). Plots were 5.6 m in length with 0.76 m row spacing and two rows in width. At planting, Force 3G insecticide [(tefluthrin 2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1α,3α)-(Z)-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; Syngenta Crop Protection, Greensboro, NC] was applied in-furrow at a rate of 0.15 kg a.i. ha^{-1} to control soil pests. Pre-emergence herbicide Lumax EZ (mixture of S-metolachlor, atrazine, and mesotrione; Syngenta Crop Protection, Greensboro, NC) was applied at a rate of 7 L ha^{-1} to control early season weeds. Post-emergence herbicide Roundup (N-phosphonomethyl, glycine; Monsanto, St. Louis, MO) was applied at a rate of 1.75 L ha^{-1} when necessary.

A set of representative elite single-cross maize hybrids commercially available at the time adapted to the state of Illinois were evaluated. These 101 commercial maize hybrids had a variety of biotechnology traits and seed treatment technologies, were from eleven different seed brands, and had relative maturities ranging from 101 to 117 days (Table 3.1). On average, 42 hybrids were planted at each environment.

Treatments

To assess the ability of the hybrids to tolerate high plant density conditions (Ruffo et al., 2015), two plant densities (79,000 and 110,000 plants ha^{-1}, denoted as standard and high plant density, respectively) were used. Final plant stands were determined prior to harvest. Nitrogen stress tolerance was measured by check plot yield (0 kg N ha^{-1}), while 67 and 252 kg N ha^{-1} were used to estimate the yield response to initial and maximum N fertilizer, respectively. Nitrogen treatments were broadcast applied as urea (46-0-0) between the V2 to V4 developmental stages in each environment (Ritchie et al., 1997). Nitrogen application dates were 18 June 2013 and 20 May 2014 at DeKalb, IL, 03 June 2011, 13 June 2013, and 08 May 2014 at Champaign, IL, and 01 July 2011, 25 June 2013, and 23 May 2014 at Harrisburg, IL.

Yield and yield component measurements

At maturity, plots were harvested with a two-row plot combine (SPC40, ALMACO, Nevada, IA). Grain yield is reported as Mg ha^{-1} at 15.5% grain moisture. A representative grain subsample from each plot was collected during harvest from which 300 random kernels were selected and weighed to estimate average kernel weight (KW). Kernel number (KN) per area was estimated from the total plot grain weight, individual kernel weight, and final plant density. Seed protein and oil concentrations were estimated using Near Infrared Transmittance (NIT) spectroscopy (Infratec 1241, FOSS, Eden Prairie, MN) from the same subsample used for yield component measurements.

Statistical design, derived measurements, and analysis

The experimental design was a strip-plot with a split plot arrangement in four randomized complete blocks within each environment. The main plot was hybrid, the split plot was N fertilizer rate, and the split-split plot was plant density level. Statistical analysis was performed using a linear mixed model approach in PROC MIXED in SAS version 9.4 (SAS Institute, 2013). Nitrogen fertilizer rates and plant density levels were included in the model as fixed effects, while environment, block, and hybrid were considered random effects. The interactions between fixed effects and random effects were included in the model as random effects. The normality of residuals, outlier observations, and assumptions of homoscedasticity were assessed using PROC UNIVARIATE in SAS.

Since not all hybrids were planted in every environment, and the objective of this study was to make an inference about the distribution of current maize hybrid performances, best unbiased linear predictors (BLUP’s) were calculated within each N fertilizer and plant density treatments using restricted estimation of maximum likelihood. Therefore, the phenotypic observations (y_{ijk}) were modeled according to Eq. [1]:

$$Y_{ijk} = \mu + E_i + B_{(i)j} + G_k + (G \times E)_{ik} + \varepsilon_{ijk} \quad [1]$$

in which Y_{ijk} is the phenotypic observation of *i*th environment within *j*th block, for *k*th hybrid, μ is the overall mean, E_i is the random effect of *i*th environment (*i*=1, 2,…, and 8), $B_{(i)j}$ is the random effect of *j*th block nested within *i*th environment (*j*= 1, 2, 3, and 4), G_k is the genetic random effect of *k*th hybrid (*k*=1, 2, … , and 101), $(G \times E)_{ik}$ is random effect of the interaction between *k*th hybrid and *i*th environment, and ε_{ijk} is the random error term. Variance component estimates from this model were used to calculate the broad-sense heritability (H^2) per hybrid mean basis (Holland et al., 2003).

Nitrogen use efficiency was calculated as the ratio of grain yield increase from the amount of N fertilizer supplied relative to the unfertilized control treatment (Moll et al., 1982). In addition, yield stability and adaptability were calculated in PROC REG regressing the BLUP estimates from each hybrid (sum of the E_i, G_k, and $G \times E_{ik}$ effects) against the environmental indices (E_i). Pearson's pairwise correlation coefficients (r) between the hybrid *b*-values (slopes derived from the yield across environments arranged in increasing average yield order) at different N rates and population densities were calculated using PROC CORR. The mean of all hybrid *b*-values evaluated was set to 1.0 and hybrids with a *b*-value equal to 1 were considered average hybrids. High stability hybrids were defined as those with regression slopes of $b < 1$, i.e., "work-horse"; while hybrids with $b > 1$ were classified as high adaptability, or "race-horse" hybrids (Tollenaar and Lee, 2002).

Hybrids were separated into four groups based on comparing their yield performance to the mean distribution across environments for four phenotypic traits. The phenotypic traits considered in this study were: check plot yield (yield at 0 kg N ha^{-1} at 79,000 plants ha^{-1}), initial N yield response (yield change between 0 and 67 kg N ha^{-1} at 79,000 plants ha^{-1}), yield response to maximum N (yield change between 0 and 252 kg N ha^{-1} at 79,000 plants ha^{-1}), and yield response to plant density (yield change between 79,000 and 110,000 plants ha^{-1} at 252 kg N ha^{-1}). Mean separation for different hybrid groups were analyzed in PROC MIXED using hybrid group as fixed effect at the 95% significance level. The test for equal stability variances across hybrid groups was performed using the Brown-Forsythe method in PROC GLM (Brown and Forsythe, 1974).

RESULTS AND DISCUSSION

Weather conditions

Air temperature and rainfall amounts from all environments were obtained from the Water and Atmospheric Resources Monitoring Program (Illinois Climate Network, 2016) and presented in Table 3.2. During July 2011, precipitation at Champaign was 10.6 mm below the 10-yr average. While in 2011, both minimum and maximum average daily temperatures during July were greater than the 10-yr average at Champaign (min. +2.95 °C, max.+3.18 °C) and Harrisburg (min. +2.34°C, max.+2.51°C) (Table 3.2). Late vegetative and early reproductive development of maize usually occur during July and August in the U.S. Corn Belt, and are important growth stages in determining maize yield. In contrast, the weather of 2013 provided ideal environmental conditions for maize development and yield at all three sites, with daily temperatures similar to the 10-year average and above- average precipitation during July (+15.0, +4.8, and +9.9 mm at DeKalb, Champaign, and Harrisburg, respectively). In 2014, above average precipitation occurred during June (+17.9, +17.1, and +5.9 mm at DeKalb, Champaign, and Harrisburg, respectively), and below average temperature occurred in July with minimum daily average temperatures deviating from the mean by -2.25, -2.47, and -3.37 °C and maximum average daily temperature deviating from the mean by -2.46, -2.62, and -2.17 °C for DeKalb, Champaign, and Harrisburg, respectively. The environmental conditions in 2014 led to a statewide record corn yield 12,500 kg ha^{-1}.

Hybrid yields and variance components

Averaged across environments, N fertilizer increased maize yield, but hybrids exhibited similar yields within N rates regardless of the plant density (Table 3.3). Environments provided different crop growing conditions (Table 3.1). Consequently, over the eight location-year environments, environmental indices for the average yield with high N conditions (252 kg N ha^{-1}) deviated from the overall mean from -1.6 to +2.4 Mg ha^{-1} at the standard plant density (79,000 plants ha^{-1}) and from -1.9 to +2.3 Mg ha^{-1} at the higher plant density (110,000 plants ha^{-1}) (Figure 3.1). Although hybrids exhibited similar yields at both plant densities, the highest-yielding hybrids within each environment were usually grown at the higher plant density and with the highest N supply (data not shown).

Maize genetic improvement for NUE has been more attributed to improved yield under low N than to yield increases with N fertilizer (Haegele et al., 2013). In this study, maize yield under low N conditions (0 kg N ha^{-1}) accounted for, on average, 55 and 52% of the yield under high N at the standard and high plant densities, respectively (Table 3.3). The initial yield response to N (i.e., the yield increase between 0 and 67 kg N ha^{-1}) accounted for +2.7 and +2.9 Mg ha^{-1}, and the yield response to maximum N (i.e., the yield increase between 0 and 252 kg N ha^{-1}) accounted for +5.1 and +5.6 Mg ha^{-1} at the standard and high plant densities, respectively. As a result, the NUE for the initial yield response to N was greater than the NUE for the yield response to maximum N. Increased plant density may increase NUE under greater N fertilizer rates (165 and 330 kg N ha^{-1}) (Boomsma et al., 2009). Accordingly, commercial hybrids increased in NUE from 34.0 to 37.7 kg kg N^{-1} for the initial N response and from 16.5 to 18.4 kg kg N^{-1} for the maximum N response under standard and high plant densities, respectively (Table 3.3), supporting the idea that maize hybrids have improved their tolerance to crowding stress (Tollenaar and Lee, 2002) in synergy with NUE improvement (Tollenaar and Wu, 1999).

Environmental (σ^2_{E}), genetic (σ^2_{G}) and the genetic × environmental interaction ($\sigma^2_{G \times E}$) variances for yield differed across N rates and plant densities (Table 3.3). While the environmental variance for yield decreased from low to high N conditions, the genetic variance tended to increase. Other investigators have also reported a reduction in environmental variance for yield due to better agronomic conditions (Banziger and Cooper, 2001). Also, high N conditions may have reduced the soil heterogeneity and allowed for an increased genetic effect (Bertin and Gallais, 2000). At the standard plant density (79,000 plant ha^{-1}), N fertilizer increased yield $\sigma^2_{G \times E}$ over the unfertilized control, but at the high plant density the $\sigma^2_{G \times E}$ remained similar regardless of the N fertilizer rate.

High N conditions can increase heritability (Brun and Dudley, 1989; Bänziger et al., 1997; Bertin and Gallais, 2000) or decrease heritability (Agrama et al., 1999) depending on the germplasm and the agronomic condition evaluated. Higher heritability under high N can increase the effectiveness of selection and be used as indirect selection for maize genetic improvement under low N (Gallais et al., 2008). Conversely, a reduced response to selection has been found for maize hybrids grown under high plant densities due to reduced stand uniformity and individual plant yield (Fasoula and Tollenaar, 2005). In this study, genetic variance for yield increased 41% and broad-sense heritability increased 15% with increased plant density (Table 3.3). Therefore, current maize hybrids are more tolerant of crowding stress as a group, and under these conditions, the greater variability of yield potential allows for improved NUE and yield.

Yield components and grain quality

Although plant density treatments did not affect overall yields, they influenced yield components (Figure 3.2). Increased plant density has been found to reduce total leaf area per plant, individual kernel weight (KW), and kernel number (KN) per plant (Borrás et al. 2003). Under low N, KW and KN were similar across plant densities. However, the maximum N rate increased both KW and KN by 16 and 34% at the standard plant density, and by 13 and 40% at high plant density, respectively, over the unfertilized control. Kernel number often exhibits greater plasticity than KW when maize hybrids are exposed to different agronomic conditions (Sadras et al., 2009; Boomsma et al., 2009). In a similar manner, KN was more correlated to yield than KW across all N and plant density treatments (Table 3.4). Maize seed set and the resulting KN is sensitive to N-stress (Below et al., 1981). Accordingly, the highest correlation coefficients were found between yield at low N and KN at both plant densities. Correlations between KW and KN to yield tended to decrease with increased N rate regardless of the plant density, but KW was more correlated to yield at the standard plant density than at the high plant density. Reduced correlations between yield components and yield with increased N fertilizer may be associated with increased genetic variance (Table 3.3) and to specific hybrid grain characteristics. Individual KW was under greater genetic control than KN at 0 and 67 kg N ha^{-1}, but under high N both yield components were highly heritable.

In addition to yield components, N fertilizer and plant density had an effect on grain quality (Table 3.5). Grain oil concentration was stable across plant density treatments. Therefore, due to increased yield, maximum N fertilizer increased oil content by approximately 35% over the control regardless of the plant density. In addition, N fertilizer increased grain protein content by 60% at both plant densities, due to increases in both yield and protein concentration. Although grain protein and oil proportions have a negative relationship (Simmonds, 1995), increases in N availability increased the content per area of both traits, while no changes to grain quality were observed with increased plant density. Nitrogen fertility affected protein more than oil content, since protein contains N. These results using 101 current commercial hybrids reveal less genetic variation in grain protein and oil concentration than previous studies (Below et al., 2004; Uribelarrea et al., 2004).

Hybrid characterization

The large genetic variance for yield response to N fertilization and plant density highlights the importance of hybrid characterization to identify proper agronomic management. There was no difference among maize hybrids' responses to N fertilizer and plant density when comparing different seed brands and relative maturities (data not shown). At high N, the majority of the 10 highest-yielding and the 10 lowest-yielding maize hybrids were observed at the higher plant density condition (Table 3.6), indicating that hybrids with tolerance to crowding stress under high N can express high yield performance, while hybrids that are susceptible to crowding stress may have a more limited yield, regardless of N fertility. While full-season compared to short-season hybrids can exhibit greater biomass plasticity and partitioning to the grain in response to plant density (Sarlangue et al., 2007), there was no significant relationship for yield found in this study between hybrid maturity (CRM) and tolerance to crowding stress (data not shown). Contrasting results obtained by Sarlangue et al. (2007) may be attributed to the genetic improvement of current early maturity hybrids with increased reproductive sink capacity or to different agronomic conditions.

Although the 10 highest-yielding hybrids achieved similar yields under high N conditions, these hybrids exhibited different NUE and yield component proportions across N rates and plant densities (Table 3.6). The 10 highest-yielding hybrids exhibited higher NUE ($P \leq 0.01$) under initial N (NUE between 0 and 67 kg N ha^{-1}) and high N (NUE between 0 and 252 kg N ha^{-1}) than the 10 lowest-yielding hybrids. However, not all of the top-yielding hybrids expressed the same NUE at initial or high N conditions. Similarly, the 10 highest-yielding hybrids had greater KN at initial and high N conditions (KN change from 0 to 67 and 0 to 252 kg N ha^{-1}, respectively) compared to the 10 lowest-yielding hybrids, but there was no significant difference in KW within each group. Differences in NUE between hybrids when grown under high N are attributed to both the difference in a hybrid's yield performance at low N and its yield response to N fertility.

In order to identify hybrids with good agronomic performance under conditions associated with lower soil N availability (i.e. lower rates of N fertilizer application and/or weather conditions leading to N loss), yields were compared at low N versus the response to initial N (Figure 3.3A). Yields at low N ranged from 5.87 to 6.78 Mg ha^{-1} and the initial N response ranged from +2.40 to +3.14 Mg ha^{-1} among all hybrids when averaged across all environments. Subsequently, hybrids were divided into four groups: group 1A (42% of the hybrids) included those that yielded below average at low N but had an above average yield response to the initial N increment, group 2A (9% of the hybrids) included those with above average responses for both yield at low N and initial N increment, group 3A (4% of the hybrids) included those with below average responses for both yield at low N and the initial N increment, and group 4A (45% of the hybrids) included those with above average yield at low N but below average response to initial N. Since yield at low N and the yield response to the initial N increment were negatively correlated ($r = –0.35$), selecting hybrids with above average performance for both traits (group 2A) may be challenging in a maize breeding program.

Hybrids adaptable to intensive crop management were identified as those with high yield response to both maximum N and increased plant density (Figure 3.3B). Among all hybrids, and averaged across environments, the yield response to maximum N fertilizer ranged from +4.47 to +5.64 Mg ha^{-1} and the yield response to increased plant density ranged from -0.60 to +0.58 Mg ha^{-1}. Compared to results from previous studies under similar agronomic conditions, the findings reported here identified substantially greater yield ranges for both response to maximum N fertilizer (Haegele et al., 2013) and increased plant density (Ruffo et al., 2015), demonstrating greater genetic variability for these traits in current elite maize hybrids. Moreover, four groups of hybrids were identified: group 1B (25% of the hybrids) included those with below average yield response to maximum N but above average response to increased plant density, group 2B (24% of the hybrids) included those with above average yield responses to both maximum N and increased plant density, group 3B (25% of the hybrids) included those with below average yield response to both maximum N and plant density, and group 4B (26% of the hybrids) included those with above average yield response to maximum N but below average response to increased plant density. Under standard plant density and high N, groups 2B and 3B obtained the same average yield (11.64 Mg ha^{-1}), which was significantly greater ($P < 0.001$) than groups 1B and 4B (11.32 and 11.19 Mg ha^{-1}, respectively). However, under high N and high plant density, group 2B (11.99 Mg ha^{-1}) obtained a significantly greater yield ($P < 0.001$) than groups 3B, 4B, and 1B (11.15, 11.59, and 11.67 Mg ha^{-1}, respectively). Maize hybrids that were adaptable to increased N fertilizer and plant density exhibited greater yield and would be more suitable for intensive crop management practices.

Hybrid stability analysis

Yield stability among different N rates and plant densities was evaluated for 61 maize hybrids that were grown in at least three environments. Hybrids exhibited similar yield stability correlations within N levels across different plant densities (Table 3.7). Overall, phenotypic correlations for hybrid stability (*b*-values) across N treatments and plant densities for yield ranged from non-significant to 0.85. Correlation coefficients tended to be greater at standard compared to high plant density. In addition, hybrid yield stability appeared to be more associated with a hybrid's tolerance to N stress at low N, than a hybrid's response to N fertilizer or tolerance to crowding stress. Increased $\sigma^2_{G \times E}$ at high N and high plant density may have resulted in decreased correlation coefficients for yield stability.

Hybrids characterized with different crop management responses also exhibited different characteristics of yield stability across N and plant density treatments (Table 3.8). Group 2A (hybrids with above average yield at low N and initial N increment response) had greater yield stability (smaller *b*-value) than groups 1A, 3A and 4A at 67 kg N ha^{-1} (both plant densities) and greater yield stability than group 4A at 252 kg N ha^{-1} and standard plant density. Yield stability from hybrid group 2A tended to increase with additional N fertilizer regardless of the plant density, indicating that hybrids with above average tolerance to N loss will provide consistent performance across high yield environments and this stability is plant density independent. On the other hand, hybrid group 2B (hybrids with above average yield response to both maximum N and increased plant density) exhibited average yield stability across N and plant density treatments. Hybrids with above average response to maximum N and below average response to increased plant density (group 4B) had greater yield stability than hybrids with below average yield response to maximum N and above average response to increased plant density (group 1B).

Previous authors have attributed less response to selection for yield at high plant density due to reduced stand uniformity, increased plant-to-plant variability, and reduced plant prolificacy (Hallauer and Sears, 1969; De Leon and Coors, 2002; Fasoula and Tollenaar, 2005). However, the improvement of maize agronomic management over time has increased stand uniformity and reduced plant-to-plant variability. In addition, current maize genotypes appear to be more density dependent, exhibit reduced barrenness, and are mostly single-ear hybrids. Hybrids with a high yield response to maximum N fertilizer and that are also plant density independent (their yield is less hindered by increased plant density) may have greater yield stability under standard plant density. Yield stability variance significantly increased ($P \leq 0.05$) with additional N fertilizer at both plant densities (Table 3.8). Under high N conditions, yield stability variance was notably greater at standard compared to the high plant density condition.

CONCLUSIONS

Current elite maize hybrids expressed large genetic variation for yield in tolerance to N loss, N fertilizer response, and tolerance to crowding stress when grown across different environments in Illinois. Yield stability was more associated with a hybrid's ability to tolerate N stress and respond to N fertilizer than a hybrid's ability to tolerate high plant density conditions. Selecting hybrids with above average yield performance under both low N and high N conditions may be challenging in a maize breeding program, since less than 10% of the hybrids evaluated in this study were characterized as such. These hybrids achieved greater yield stability under high yield environments. Although hybrids with an above average yield response to high N fertilizer supply and increased plant density exhibited greater yield performance, these hybrids also revealed larger stability variance suggesting that hybrid selection will be more advantageous under specific growing environments. Future research evaluating hybrids' responses to agronomic management (e.g. N fertilizer, plant density, and row spacing) may be integrated with different hybrid selection methods for the development of maize hybrids with improved agronomic performance.

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TABLES AND FIGURES

Table 3.1. Hybrid names, seed company names, and crop relative maturity (CRM), for hybrids planted at DeKalb (DK), Champaign (CH), and Harrisburg (HB) in 2011, 2013 and 2014.

Hybrid	Company†	CRM	DK		CH			HB			Stability
			2013	2014	2011	2013	2014	2011	2013	2014	Analysis
N45P-4011	Syngenta	101	x			x					
D42SS42	CPS	102				x					
N49W-3000GT	Syngenta	102	x			x					
DKC55-09SSTX	Monsanto	105	x			x					
206-78STXRIB	Monsanto	106				x					
5415SS	Winfield	106	x	x		x	x			x	x
5516SS	Winfield	106		x			x			x	x
5828RIB	Monsanto	107	x			x			x		x
85V88-3000GT	Syngenta	107			x			x			
D47SS23	CPS	107	x	x		x	x			x	x
G07F23-3111	Syngenta	107	x			x			x		x
G07V88-5122	Syngenta	107					x				
N60F-3111	Syngenta	107		x						x	
N61P-3000GT	Syngenta	107	x			x			x		x
5875SS/RIB	Winfield	108	x			x					
W5787RIB	Wyffels	108	x			x					
209-46STXRIB	Monsanto	109		x			x			x	x
209-53STXRIB	Monsanto	109	x	x		x	x		x	x	x
5975VT3P	Winfield	109		x			x			x	x
84S08-4011	Syngenta	109			x			x			
N63H-3111	Syngenta	109	x			x			x		x
N63R-3000GT	Syngenta	109	x	x		x	x		x	x	x
N64M-5122	Syngenta	109					x				
210-95STXRIB	Monsanto	110	x			x			x		x
6175VT3P	Winfield	110	x			x					
DKC60-67RIB	Monsanto	110	x	x		x	x		x	x	x
N65D-3122	Syngenta	110	x			x			x		x
W6487RIB	Wyffels	110	x	x		x	x			x	x
W6627RIB	Wyffels	110	x			x					
211-24STXRIB	Monsanto	111	x	x		x	x		x	x	x
211-35STXRIB	Monsanto	111		x			x			x	x
6065SS	Winfield	111		x			x			x	x
6148RIB	Monsanto	111		x			x			x	x
61BV3	Growmark	111			x			x			

Table 3.1. (Continued)

Hybrid	Company	CRM	DK		CH			HB			Stability Analysis
			2013	2014	2011	2013	2014	2011	2013	2014	
D51VP32	CPS	111	x	x		x	x		x	x	x
DKC61-16SSTX	Monsanto	111	x			x			x		x
DKC61-21SSTX	Monsanto	111			x			x			
DKC61-54GENSSRIB	Monsanto	111		x			x			x	x
N68A-3000GT	Syngenta	111			x			x			
N68B-3111	Syngenta	111	x	x	x	x	x	x		x	x
P1184XR	Pioneer	111			x			x			
W6917RIB	Wyffels	111	x			x			x		x
212-86STXRIB	Monsanto	112	x			x			x		x
6160VT3P	Winfield	112			x			x			
6258SS	Monsanto	112	x	x			x			x	x
6265SS	Winfield	112	x	x		x	x		x	x	x
6274RIB	Winfield	112	x	x		x	x		x	x	x
D52SS91	CPS	112	x	x		x	x		x	x	x
DKC62-08RIB	Monsanto	112	x	x		x	x		x	x	x
DKC62-63VT3P	Monsanto	112			x	x		x	x		x
DKC62-77GENSSRIB	Monsanto	112		x			x			x	x
DKC62-97RIB	Monsanto	112	x	x	x	x	x	x	x	x	x
H-90023110	Syngenta	112	x			x			x		x
H-90114011	Syngenta	112	x			x			x		x
N69Z-5222	Syngenta	112					x				
N70J-4011	Syngenta	112	x			x			x		x
N72A-3111	Syngenta	112			x			x			
P1221AMXT	Pioneer	112		x			x			x	x
P1236XR	Pioneer	112			x			x			
W7477RIB	Wyffels	112	x	x		x	x		x	x	x
213-28STXRIB	Monsanto	113		x			x			x	x
213-59STXRIB	Monsanto	113	x			x			x		x
33Z74XR	Pioneer	113			x			x			
6358RIB	Monsanto	113	x			x			x		x
6364RIB	Monsanto	113	x			x			x		x
6378RIB	Monsanto	113		x			x			x	x
63MV4	Growmark	113			x			x			
6594SS	Winfield	113		x			x			x	x
6640VT3P	Winfield	113	x	x		x	x		x	x	x
83S06-3000GT	Syngenta	113				x			x		
DKC63-33RIB	Monsanto	113	x	x		x	x		x	x	x
DKC63-55GENDGVT2P	Monsanto	113		x			x			x	x

Table 3.1. (Continued)

Hybrid	Company	CRM	DK		CH			HB			Stability Analysis
			2013	2014	2011	2013	2014	2011	2013	2014	
DKC63-84VT3	Monsanto	113			x			x			
H-91383000GT	Syngenta	113			x	x		x	x		x
N71U-3122	Syngenta	113	x			x			x		x
N72Q-3111	Syngenta	113		x						x	
N74G-3000GT	Syngenta	113	x			x			x		x
P1395XR	Pioneer	113			x			x			
6448RIB	Monsanto	114		x			x			x	x
6914AS3000GT	Winfield	114			x	x		x	x		x
6926VT3P	Winfield	114	x	x		x	x		x	x	x
6960VT3P	Winfield	114			x			x			
7087VT3P	Winfield	114		x		x	x		x	x	x
83R38-3000GT	Syngenta	114				x			x		
DKC64-69VT3P	Monsanto	114			x			x			
DKC64-87GENSSRIB	Monsanto	114		x			x			x	x
G14H66-GTA	Syngenta	114	x			x			x		x
H-93413000GT	Syngenta	114	x			x			x		x
N74R-3000GT	Syngenta	114		x	x		x	x		x	x
N75H-5122A	Syngenta	114					x				
N77P-3000GT	Syngenta	114				x			x		
215-52VT3P	Monsanto	115	x			x			x		x
215-82VT3PRIB	Monsanto	115	x			x			x		x
7505VT3	Winfield	115			x			x			
DKC65-63VT3	Monsanto	115			x			x			
N79Z-3000GT	Syngenta	115		x		x	x		x	x	x
H-95743111	Syngenta	116				x			x		
N78S-3111	Syngenta	116		x						x	
8505VT3P	Winfield	117				x			x		
D57VP51	CPS	117				x			x		

† CPS, Crop Productions Services; Monsanto, Monsanto Company; Pioneer, DuPont Pioneer; Winfield, Winfield United.

Table 3.2. Average monthly minimum (Tmin.) and maximum (Tmax.) temperatures, and precipitations for different locations and years used in this study. Values in parenthesis indicate deviation from 10 yr- average within each location and year.

		DeKalb			**Champaign**			**Harrisburg**		
Year	**Month**	**Tmin.**	**Tmax.**	**Preciptation**	**Tmin.**	**Tmax.**	**Preciptation**	**Tmin.**	**Tmax.**	**Preciptation**
		°C		cm	°C		cm	°C		cm
2011	**May**	-	-	-	11.44 (+0.50)	22.44 (-0.95)	15.54 (+4.38)	12.88 (+0.16)	23.50 (-0.97)	24.09 (+7.42)
	June	-	-	-	17.54 (+1.09)	28.40 (+0.01)	14.00 (+1.09)	18.55 (+1.27)	30.35 (+1.41)	25.66 (+16.89)
	July	-	-	-	21.09 (+2.95)	32.89 (+3.18)	5.03 (-10.66)	21.41 (+2.34)	32.83 (+2.51)	14.67 (+0.08)
	Aug.	-	-	-	17.59 (-0.13)	31.02 (+1.77)	5.70 (-5.38)	18.92 (+0.01)	30.85 (+0.34)	13.00 (+3.12)
	Sept.	-	-	-	11.97 (-1.38)	23.84 (-2.52)	9.30 (-0.83)	12.97 (-1.42)	24.60 (-2.36)	22.10 (9.22)
2013	**May**	10.41 (+1.43)	22.92 (+1.47)	11.67 (-1.10)	12.08 (+1.14)	23.98 (+0.58)	15.09 (+3.93)	12.85 (+0.13)	24.57 (+0.09)	13.74 (-2.93)
	June	14.72 (-0.05)	25.98 (-0.69)	26.03 (+15.00)	16.16 (-0.27)	27.94 (-0.44)	17.80 (+4.89)	17.39 (+0.11)	29.03 (+0.08)	18.70 (+9.92)
	July	15.50 (-0.47)	27.03 (-0.90)	5.41 (-5.08)	17.56 (-0.57)	28.26 (-1.44)	11.22 (-4.47)	17.67 (-1.39)	28.68 (-1.62)	13.38 (-1.20)
	Aug.	14.32 (-0.64)	27.23 (+0.27)	14.03 (+0.44)	16.85 (-0.87)	29.51 (+0.25)	1.54 (-9.54)	18.24 (-0.67)	29.33 (-1.17)	21.06 (+11.19)
	Sept.	10.48 (+0.31)	24.64 (+0.23)	4.60 (-5.17)	14.01 (+0.66)	28.35 (+1.98)	1.63 (-8.50)	15.28 (+0.88)	27.50 (+0.53)	6.30 (-6.58)
2014	**May**	9.48 (+0.51)	22.86 (+1.41)	7.87 (-4.91)	11.99 (+1.05)	23.76 (+0.37)	13.80 (+2.63)	13.79 (+1.07)	25.42 (+0.93)	8.58 (-8.09)
	June	15.47 (+0.68)	27.05 (+0.37)	29.00 (+17.96)	17.76 (+1.31)	28.61 (+0.22)	30.10 (+17.19)	18.84 (+1.56)	28.72 (-0.22)	14.76 (+5.99)
	July	13.72 (-2.25)	25.47 (-2.46)	8.03 (-2.47)	15.72 (-2.41)	27.08 (-2.62)	25.87 (+10.17)	15.70 (-3.37)	28.13 (-2.17)	6.87 (-7.72)
	Aug.	15.66 (+0.69)	26.94 (-0.01)	13.16 (-0.42)	18.13 (+0.40)	28.70 (-0.54)	4.54 (-6.54)	18.97 (+0.06)	30.50 (-0.01)	12.77 (+2.9)
	Sept.	9.15 (-1.01)	22.96 (-1.43)	9.70 (-0.07)	11.98 (-1.36)	25.16 (-1.20)	11.73 (+1.60)	13.24 (-1.15)	26.51 (-0.45)	9.40 (-3.48)

Table 3.3. Plant density and N fertilizer effects on yield, variance components, broad-sense heritability (H^2, estimated on hybrid-mean basis), and nitrogen use efficiency (NUE). Values are averaged across 101 maize hybrids grown at three locations (DeKalb, Champaign, and Harrisburg, IL) and three years (2011, 2013, and 2014).

Plant density	**N Rate**	**Yield**[†]	σ^2_E	σ^2_G	$\sigma^2_{G \times E}$	σ^2_ε	H^2	**NUE**
plant ha^{-1}	kg N ha^{-1}	------------------	Mg ha^{-1}	------------------				kg kg N^{-1}
79,000	**0**	6.3 ± 0.6	2.50	0.09	0.15	1.05	0.38 ± 0.10	-
	67	9.1 ± 0.6	2.39	0.05	0.26	1.21	0.20 ± 0.11	34.0 ± 4.1
	252	11.4 ± 0.4	1.62	0.16	0.23	0.75	0.52 ± 0.09	16.5 ± 2.2
110,000	**0**	6.0 ± 0.6	2.68	0.17	0.29	1.10	0.45 ± 0.09	-
	67	8.9 ± 0.7	3.34	0.19	0.35	1.42	0.43 ± 0.09	37.7 ± 3.9
	252	11.6 ± 0.5	1.94	0.27	0.26	0.83	0.61 ± 0.06	18.4 ± 2.2

[†] Yield, H^2, and NUE average values are shown with ± 1 standard error for 95% significance level.

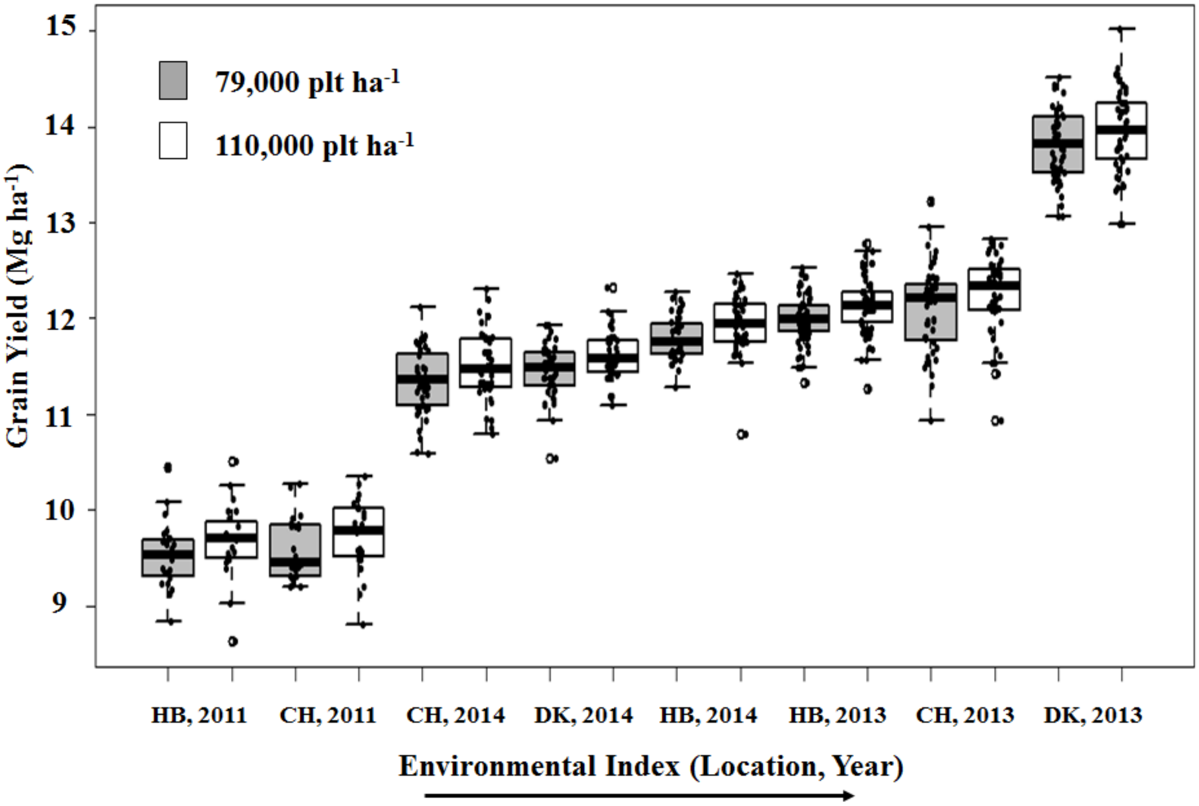

Figure 3.1. Influence of location, year, and plant population on grain yield, arranged by increasing average yield (Environmental index) for 101 maize hybrids grown at DeKalb (DK), Champaign, (CH), and Harrisburg (HB) under high N conditions (252 kg N ha^{-1}) in 2011, 2013, and 2014. Dots represent yield estimates for individual hybrids within each environment and population. Horizontal lines in the box plot indicate the median, top and bottom edges of the box refer to 75th and 25th percentiles, and whiskers refer to 10th and 90th percentiles.

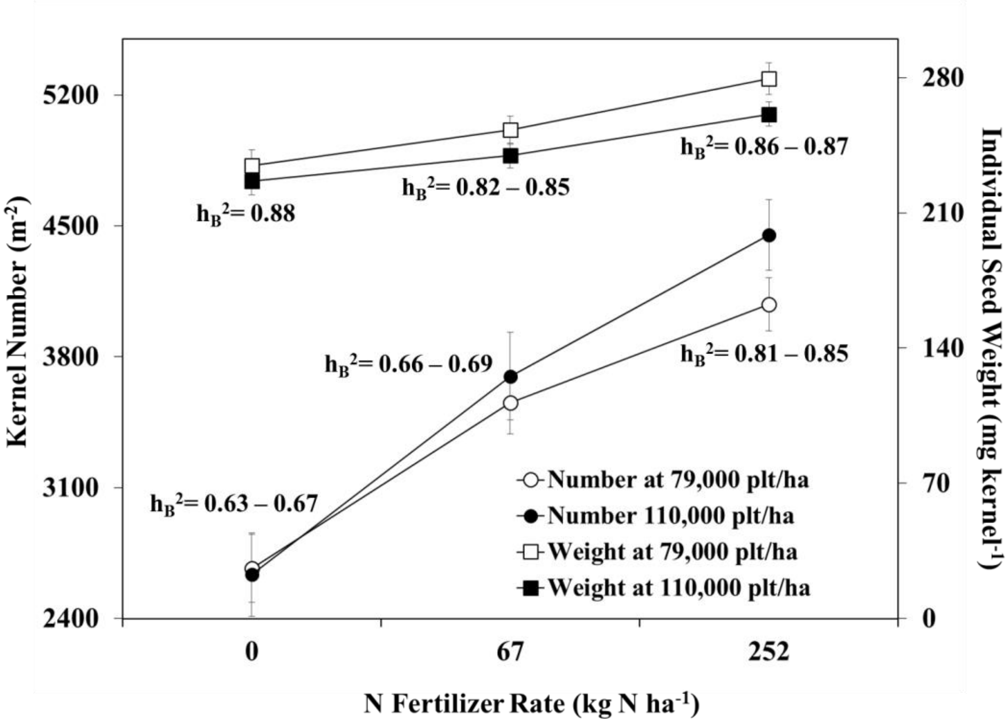

Figure 3.2. Plant density and N fertilizer rate effects on kernel number, kernel weight, and broad-sense heritability (estimated on hybrid-mean basis) for 101 maize hybrids grown at three locations (DeKalb, Champaign, and Harrisburg, IL) and three years (2011, 2013, and 2014). Bars extending from data points indicate ±1 standard error for 95% significance level.

Table 3.4. Pearson's pairwise correlation coefficients (r) between maize yield and yield components (kernel number and individual kernel weight) at different N fertilizer rates and plant densities. Values are averaged across 101 hybrids grown at three locations (DeKalb, Champaign, and Harrisburg, IL) over three years (2011, 2013, and 2014).

	N Fertilizer Rate (kg N ha^{-1})		
	0	**67**	**252**
Kernel parameter		**79,000 plant ha^{-1}**	
Number, kernel m^{-2}	0.90***	0.85***	0.74***
Weight, mg kernel^{-1}	0.55***	0.54***	0.38***
		110,000 plant ha^{-1}	
Number, kernel m^{-2}	0.93***	0.89***	0.75***
Weight, mg kernel^{-1}	0.38***	0.46***	0.30***

***Significant at $P \leq 0.001$.

Table 3.5. Maize grain oil and protein concentration and content responses to N fertilizer supply and plant density. Values are averaged across 101 hybrids grown at three locations (DeKalb, Champaign, and Harrisburg, IL) over three years (2011, 2013, and 2014).

	N Fertilizer Rate (kg N ha^{-1})		
Kernel parameter	**0**[†]	**67**	**252**
	79,000 plant ha^{-1}		
Oil concentration, g kg^{-1}	38.5 ± 0.1	38.0 ± 0.1	38.1 ± 0.1
Oil content, kg ha^{-1}	246 ± 25	347 ± 25	437 ± 18
Protein concentration, g kg^{-1}	58.9 ± 0.2	63.6 ± 0.1	77.5 ± 0.1
Protein content, kg ha^{-1}	376 ± 38	583 ± 44	890 ± 42
	110,000 plant ha^{-1}		
Oil concentration, g kg^{-1}	38.0 ± 0.1	37.3 ± 0.1	36.7 ± 0.1
Oil content, kg ha^{-1}	230 ± 26	335 ± 28	426 ± 17
Protein concentration, g kg^{-1}	58.0 ± 0.2	62.0 ± 0.1	75.5 ± 0.1
Protein content, kg ha^{-1}	348 ± 35	557 ± 45	876 ± 40

[†] Oil and protein (concentration and content) average values are shown with ± 1 standard error for 95% significance level.

Table 3.6. Plant density effects on yield, N-use efficiency (NUE), kernel number (KN), and kernel weight (KW) from the 10 highest and lowest-yielding maize hybrids grown under high N conditions (252 kg N ha^{-1}). Values are averaged over three locations (DeKalb, Champaign, and Harrisburg, IL) and three years (2011, 2013, and 2014). Initial and high N for NUE indicate the ratio between yield and N fertilizer supplied at 67 – 0 and 252 – 67 kg N ha^{-1}, respectively. Initial and high N values for both ΔKN and ΔKW indicate the change in kernel number and kernel weight between 67 – 0 and 252 – 67 kg N ha^{-1}, respectively.

Rank	**CRM**	**Plant density**	**Yield**	**NUE_{LowN}**	**$\text{NUE}_{\text{HighN}}$**	**$\Delta\text{KN}_{\text{LowN}}$**	**$\Delta\text{KN}_{\text{HighN}}$**	**$\Delta\text{KW}_{\text{LowN}}$**	**$\Delta\text{KW}_{\text{HighN}}$**
		plant ha^{-1}	Mg ha^{-1}	kg kg N^{-1}		kernel m^{-2}		mg kernel^{-1}	
1	113	111,000	12.5	45.4	24.6	1235.1	2027.0	6.6	35.5
2	115	111,000	12.3	46.8	25.4	1252.7	2021.5	15.9	51.5
3	110	111,000	12.3	38.9	24.2	1040.3	1909.4	-0.3	41.6
4	112	111,000	12.2	40.9	23.0	945.5	1765.1	8.8	29.3
5	113	111,000	12.2	47.7	24.1	1169.9	1968.7	13.7	33.0
6	111	111,000	12.2	44.4	23.8	1199.5	2075.5	8.8	33.5
7	111	111,000	12.2	45.9	24.3	1353.1	2599.5	9.3	17.9
8	113	111,000	12.2	44.4	23.5	1041.2	1838.2	23.9	44.9
9	114	111,000	12.2	42.2	21.7	1039.8	1714.8	17.5	39.5
10	109	111,000	12.1	45.5	24.6	979.7	1540.6	19.1	59.0
193	107	111,000	11.0	38.8	19.0	1001.1	636.1	2.8	15.5
194	102	79,000	10.9	41.6	18.6	906.4	493.4	15.5	29.4
195	115	111,000	10.9	42.0	22.6	1194.3	973.6	-3.7	17.0
196	109	111,000	10.9	39.4	19.9	888.9	804.0	14.3	21.7
197	111	111,000	10.9	40.0	20.7	1003.3	824.0	14.6	32.4
198	113	79,000	10.8	40.1	18.6	665.3	562.7	22.9	45.3
199	109	111,000	10.8	43.6	21.1	1185.5	960.4	12.7	23.9
200	113	111,000	10.7	41.2	20.8	968.8	797.1	7.7	20.4
201	114	111,000	10.5	45.3	19.7	1141.9	440.2	16.2	34.9
202	112	111,000	10.4	41.7	19.7	923.7	545.3	11.5	25.6
Average from 10 highest-yielding			12.2	44.2	23.9	1125.7	1946.0	12.3	38.6
Average from 10 lowest-yielding			10.8	41.4	20.1	987.9	703.7	11.4	26.6
Least square difference ($P \leq 0.01$)			0.1	3.0	1.2	161.4	299.7	7.7	12.5

†Least significant difference was estimated from groups of 10 hybrids in ascending yield order under high N conditions (252 kg N ha^{-1}).

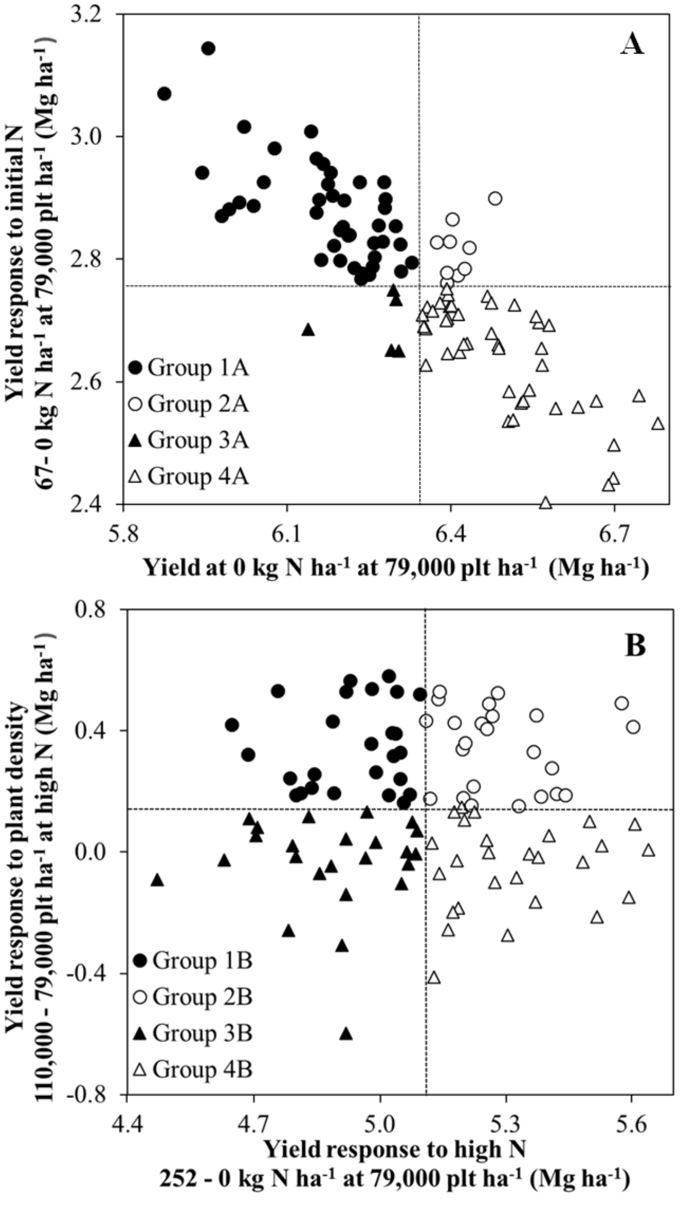

Figure 3.3. Characterization of 101 elite maize hybrids under different agronomic conditions: A) relationship between yield at low N (0 kg N ha^{-1}) and initial N response (67 – 0 kg N ha^{-1}) and B) relationship between yield response to plant density (110,000 – 79,000 plt ha^{-1}) at high N conditions and yield response to maximum N (252 – 0 kg N ha^{-1}). Hybrids were grouped based on below or above average performance for each phenotypic trait. Values are averaged across three locations (DeKalb, Champaign, and Harrisburg, IL) and three years (2011, 2013, and 2014). Dashed lines represent the average performance from all hybrids within each phenotypic trait.

Table 3.7. Pearson's pairwise correlation coefficients (r) for yield stability (*b*-values) from sixty-one maize hybrids grown at different N rates and plant densities and averaged across three locations (DeKalb, Champaign, and Harrisburg, IL) and three years (2011, 2013, and 2014).

		79,000 plt ha^{-1}			**110,000 plt ha^{-1}**	
		Nitrogen fertilizer rate (kg N ha^{-1})				
Plant density	**N rate**	**0**	**67**	**252**	**0**	**67**
plant ha^{-1}	kg N ha^{-1}					
79,000	**67**	0.54***	-	-	-	-
	252	0.34**	0.46***	-	-	-
110,000	**0**	0.85***	0.47***	0.36**	-	-
	67	0.44***	0.82***	0.48***	0.43***	-
	252	NS	NS	0.56***	NS	0.32*

NS, non- significant

* Significant at $P \leq 0.10$.

**Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

Table 3.8. Plant density and N fertilizer effects on yield stability (*b*-value) from 61 maize hybrids averaged across eight environments and three years. Hybrids were grouped based on their average yield response to N fertilizer and plant density. Yield stability variance was calculated from all hybrids tested within each N fertilizer and plant density treatment.

		Plant density (plant ha^{-1})					
		79,000			**110,000**		
		N fertilizer rate (kg N ha^{-1})					
Group	**N**	**0**	**67**	**252**	**0**	**67**	**252**
		Yield response to initial N vs. at 0 N at 79,000 plt ha^{-1}					
1A	26	0.98	1.03	0.97	0.96	1.06	1.03
2A	4	1.01	0.8	0.38	1.06	0.73	0.55
3A	3	1.08	1.17	0.94	1.07	1.11	0.85
4A	29	1.01	1.03	1.22	1.01	1.02	1.09
LSD ($P \le 0.05$)[†]		NS	0.18	0.68	NS	0.25	0.56
		Yield response to increased density vs. to maximum N					
1B	17	1.05	1.08	1.36	1.1	1.1	1.14
2B	17	0.99	1.02	1.05	0.96	1.04	1.1
3B	12	0.94	0.99	0.97	0.91	1	0.84
4B	16	1	0.98	0.77	0.96	0.95	0.93
LSD ($P \le 0.05$)		0.06	NS	0.45	0.1	NS	NS
Variance[‡]		0.01b	0.03b	0.41a	0.02b	0.06b	0.27a

[†] Least square difference for yield stability between hybrid groups within N and plant density treatments ($P \le 0.05$); NS, Non- significant.

[‡] Within plant density level, variances followed by the same letter are not significant different according to the Brown-Forsythe method ($P \le 0.05$).

CHAPTER 4

USING THE SMITH-HAZEL INDEX FOR THE AGRONOMIC CHARACTERIZATION OF MAIZE HYBRIDS

ABSTRACT

Intensive crop management systems and accurate agronomic characterization of hybrids are important factors for continued maize yield increases. The yield response to nitrogen (N) fertilizer and to increased plant density are among the most important traits for maize improvement. The objectives of this research were to evaluate the effect of N fertilizer, increased plant density, and narrower row spacing on maize yield, and develop selection indices for better agronomic characterization of maize hybrids. In 2015 and 2016, 67 commercial maize hybrids were planted in three locations at three N fertilizer rates (0, 67, 312 kg N ha^{-1}), three plant densities (79,000, 94,000, and 108,000 plants ha^{-1}), and two row spacing arrangements (56 and 70 cm). Hybrids exhibited large genotypic variation and broad-sense heritability ranged from 0.66 to 0.87 across different agronomic conditions. Smith-Hazel indices using the yield response to different agronomic conditions were generated to characterize hybrids for N stress tolerance and high yield potential. The hybrid response to N fertilizer was the most important agronomic factor influencing yield increases. The yield response to maximum N fertilizer and narrower row spacing averaged +5.9 and +0.53 Mg ha^{-1} yield increases over the unfertilized control, respectively across hybrids and environments. The large genetic variation and high heritability observed for the yield response to high N fertilizer supply combined with narrower row spacing suggests that further selection under this agronomic condition may provide high genetic gain and improve hybrid performance under intensive crop management systems.

INTRODUCTION

Maize yield and plant density in the U.S. Corn Belt have steadily increased since 1930 (Troyer, 2004). However, the success of increased plant density affecting maize yield during the last seven decades was not associated with an equivalent increase in the individual plant yield potential (Duvick, 1997; Hernández et al., 2014). Rather, this yield increase over time was correlated with a greater number of kernels per unit area (Carlone and Russell, 1987; Li et al., 2011), and to a lesser extent, increased kernel size (Barker et al., 2005). Because the sink capacity of an individual maize plant (ear size and number of ears per plant) has not increased, greater plant densities will be important for continued maize yield increases, and will involve future crop management challenges (Egli, 2015).

The yield response to increased plant density is usually related to the individual plant yield potential and the genotype’s tolerance to crowding stress (Hernández et al., 2014). Therefore, while little genotypic differences are observed for yield potential on an individual plant basis, previous research has reported large genotypic differences to crowding stress tolerance (Hernández et al., 2014). In addition, increased plant density can be associated with several plant physiological changes, such as increased plant-to-plant variability (Boomsma et al., 2009), changes in yield components (Haegele et al., 2014), increased anthesis-to-silking interval, and increased barrenness (Mansfield and Mumm, 2014). These physiological changes may reduce the crop growth rate during reproductive development (Rossini et al., 2011) by decreasing individual plant leaf area and light interception (Westgate et al. 1997), and increasing the crop senescence rate (Borrás et al., 2003). Alternatively, narrower row spacing (<0.76 m) may lead to less plant-to-plant variability by altering the light quality (red:far-red ratio) within the crop canopy (Borrás et al., 2003) and reducing plant-to-plant competition (Haegele et al., 2014). As a result, the combination between increased plant density and narrower row spacing may be one strategy to increase the number of plants per area, while achieving the individual plant yield potential.

Maize genetic improvement and better crop management practices have both contributed to maize yield increases (Tollenaar and Lee, 2002). In addition to improved crowding stress tolerance in maize hybrids (Tollenaar and Lee, 2002), genetic improvement and crop management have contributed to cold tolerance and earlier planting dates (Kucharik, 2008), prolonged seed fill duration (Echarte et al., 2008), and greater tolerance to abiotic stresses such as nitrogen (N) deficiency (Haegele et al., 2013).

While plant density in maize has continually increased, N fertilizer consumption in the U.S. has remained constant for the last three decades, indicating successful genetic improvement for maize nitrogen use efficiency (Haegele et al., 2013). In addition to improved nitrogen use efficiency, newer maize hybrids have exhibited greater tolerance to N stress than older hybrids (Ciampitti and Vyn, 2011). More recently, Mueller and Vyn (2016) have shown that newer hybrids have a greater ability to uptake N during late reproductive development and exhibited greater yield under N stress and higher plant density conditions than older hybrids.

The integration between plant genetics and crop management in maize breeding programs is becoming an important research topic in the seed industry. Hybrid selection and positioning into an agronomic management arrangement will require a better understanding of the interactions between important agronomic factors and maize yield, such as increased plant density, narrower row spacing, and different N fertility conditions. As such, maize hybrids that combine a high increased yield response to increased plant density, narrower row spacing, and high N fertility conditions may express an adaptability to intensified crop management practices and high yield potential. These hybrid types are often called 'Racehorses'. On the other hand, hybrids that exhibit a high tolerance to N stress conditions may have stable yield performance across a wider range of environments. These hybrid types are often called 'Workhorses'.

The objectives of this study were to investigate the relative merits of increased plant density, narrower row spacing, and different N fertilizer conditions on commercial hybrids' yield performance, understand the phenotypic and genotypic variation of these traits, and develop a selection framework for better agronomic characterization of hybrids. Selection indices (Smith, 1936; Hazel, 1943) for each hybrid were generated, which considered the phenotypic variation and relationship of each agronomic factor across environments and their genetic contributions to the observed yield increases. The Smith-Hazel index selection has been commonly used in plant breeding for simultaneous selection of traits in a variety of crops (Smith et al., 1981). However, to the best of our knowledge, this method has not been used yet for characterization of hybrid responses to different agronomic factors. Smith-Hazel indices may be used to identify hybrids that are responsive to crop management ('Racehorses') or tolerant to N deficiency ('Workhorses'). These hybrid indices may assist agronomists for better hybrid management recommendations.

MATERIALS AND METHODS

Cultural practices

Six environments were used for the experiment, covering the years 2015 and 2016. Research sites were planted for one year at DeKalb, IL (DK; 41°47′ N, 88°50′ W; 22 May 2015), one year at Yorkville, IL (YV; 41°44′ N, 88°40′ W; 20 May 2016), and two years each at Champaign, IL (CH; 40°3′ N, 88°14′ W; 06 May 2015 and 24 April 2016), and Harrisburg, IL (HB; 37°43′ N, 88°27′ W; 02 June 2015 and 26 April 2016). Soil types at the research sites were Flanagan silt loam at DeKalb and Yorkville, IL, Drummer silty clay loam at Champaign, IL, and

Patton silty clay loam at Harrisburg, IL. The previous crop planted in each environment was soybean [*Glycine max* (L.) Merr.], and conventional tillage was used.

The experiment was planted using a precision plot planter with variable seeding rate capability (SeedPro 360, ALMACO, Nevada, IA). Plots were 5.6 m in length and two rows in width. At planting, Force 3G insecticide [(tefluthrin 2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1α,3α)-(Z)-(±)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate; Syngenta Crop Protection, Greensboro, NC] was applied in-furrow at a rate of 0.15 kg a.i. ha^{-1} to control soil pests. Pre-emergence herbicide Lumax EZ (mixture of S-metolachlor, atrazine, and mesotrione; Syngenta Crop Protection, Greensboro, NC) was applied at a rate of 7 L ha^{-1} to control early season weeds. Post-emergence herbicide Roundup (N-phosphonomethyl, glycine; Monsanto, St. Louis, MO) was applied at a rate of 1.75 L ha^{-1} when necessary.

A set of 67 representative elite single-cross maize hybrids commercially available at the time in the state of Illinois were evaluated (Table 4.1). These hybrids originated from four different seed companies, had a variety of biotechnology traits and seed treatment technologies, and ranged in relative maturities from 104 to 117 days. On average, 41 hybrids were planted at each environment and 15 hybrids were planted in all environments.

Treatments

To assess the ability of the hybrids to respond to increased plant density conditions, three plant densities (79,000, 94,000, and 108,000 plants ha^{-1}, denoted as standard, intermediate, and maximum plant density, respectively) were used. Final plant stands were determined prior to harvest. To assess the ability of the hybrids to respond to narrower row spacing, two row spacing configurations (0.76 and 0.50 m) were used. Nitrogen stress tolerance was measured by check plot yield (0 kg N ha^{-1}), while 67 and 312 kg N ha^{-1} were used to estimate the yield response to initial and maximum N fertilizer, respectively.

Treatments included: (i) 79,000 plants ha^{-1} at 76-cm row spacing with 0 kg N ha^{-1}, (ii) 79,000 plants ha^{-1} at 76-cm row spacing with 67 kg N ha^{-1}, (iii) 79,000 plants ha^{-1} at 76-cm row spacing with 312 kg N ha^{-1}, (iv) 94,000 plants ha^{-1} at 76-cm row spacing with 312 kg N ha^{-1}, (v) 108,000 plants ha^{-1} at 76-cm row spacing with 312 kg N ha^{-1}, and (vi) 108,000 plants ha^{-1} at 50-cm row spacing with 312 kg N ha^{-1}. In 2016, treatment (vi) at Champaign was planted at 170,000 plants ha^{-1} instead of the desired 108,000 plants ha^{-1} due to a problem in the configuration of the planter's software and was removed from the data analysis. Nitrogen treatments were broadcast applied as urea (46-0-0) between the V2 to V4 developmental stages in each environment (Ritchie et al., 1997). Nitrogen application dates were 11 June 2015 at DeKalb, IL, 16 June 2016 at Yorkville, IL, 28 May 2015, and 24 May 2016 at Champaign, IL, and 02 June 2015 and 01 June 2016 at Harrisburg, IL.

Yield and yield component measurements

At maturity, plots were harvested with a two-row plot combine (SPC40, ALMACO, Nevada, IA). Grain yield is reported as Mg ha^{-1} at 15.5% grain moisture. A representative grain subsample from each plot was collected during harvest from which 300 random kernels were selected and weighed to estimate kernel weight (KW). Kernel number (KN) per area was estimated from the total plot grain weight, individual kernel weight, and final plant density.

Statistical design, derived measurements, and analysis

The experimental design was a strip-plot with a split plot arrangement in four randomized complete replications within each environment. The main plot was hybrid, the split plot was N fertilizer rate, and the split-split plot was plant density level. Statistical analysis was performed using a linear mixed model approach in PROC MIXED in SAS version 9.4 (SAS Institute, 2013). Plant density, row spacing, and N fertilizer levels were included in the model as fixed effects, while environment, replication, and hybrid were considered random effects. The interactions between fixed effects and random effects were included in the model as random effects. The normality of residuals, outlier observations, and assumptions of homoscedasticity were assessed using PROC UNIVARIATE in SAS.

Since not all hybrids were planted in every environment, and the objective of this study was to make an inference about all possible current maize hybrids performance, best unbiased linear predictors (BLUP’s) were calculated within each N fertilizer, plant density, and row spacing treatment using restricted estimation of maximum likelihood. Therefore, the phenotypic yield observations (Y_{ijk}) within each treatment were modeled according to Eq. [4.1]:

$$Y_{ijk} = \mu + E_i + R_{(i)j} + G_k + (G \times E)_{ik} + \varepsilon_{ijk} \qquad [4.1]$$

in which Y_{ijk} is the phenotypic observation of *i*th environment within *j*th replication, for *k*th hybrid, μ is the overall mean, E_i is the random effect of *i*th environment (i=1, 2,…, and 6), $R_{(i)j}$ is the random effect of *j*th replication nested within *i*th environment (j= 1, 2, 3, and 4), G_k is the genetic random effect of *k*th hybrid (k=1, 2, … , and 67), $(G \times E)_{ik}$ is random effect of the interaction between *k*th hybrid and *i*th environment, and ε_{ijk} is the random error term. Variance component estimates from this model were used to calculate the broad-sense heritability (H^2) per hybrid mean basis. Phenotypic variance (σ^2_P) was calculated according to Eq. [4.2]:

$$\sigma^2_P = \sigma^2_G + (\sigma^2_{G\times E} / e) + (\sigma^2_\varepsilon / er) \qquad [4.2]$$

where σ^2_G is the genotypic variance, $\sigma^2_{G\times E}$ is the genotypic by environment interaction variance, σ^2_ε is the residual variance, e is the harmonic mean for the number of environments, and r is the harmonic mean for the number of replications. Broad-sense heritability (H^2) was calculated as the ratio between σ^2_G and σ^2_P. Pearson's pairwise correlation coefficients (r) between the hybrid yield at different treatments and yield components were calculated using PROC CORR. Principal component analysis was performed to identify patterns among hybrid yield performance across different agronomic conditions using R Studio (R Development Core Team, 2015).

The identification of 'Racehorse' hybrids, or hybrids with adaptability to high yield environments (i.e. responsive to crop management), and 'Workhorse' hybrids, or hybrids with acceptable yields in a low fertility environment (i.e. tolerant to N loss) was achieved based on a hybrid's yield response to different agronomic management conditions. Accordingly, 'Racehorse' hybrids are the genotypes that have greater yield increases in the following categories: (i) yield response to maximum N fertilizer (RTN, yield change between 0 and 312 kg N ha^{-1} when grown at 79,000 plants ha^{-1}), (ii) an intermediate plant density (IntRTD, yield response between 79,000 and 94,000 plants ha^{-1} when grown with 312 kg N ha^{-1}), (iii) maximum plant density (MaxRTD, yield change between 94,000 and 108,000 plants ha^{-1} when grown with 312 kg N ha^{-1}), and (iv) narrower row spacing (RTR, yield change between 76 and 50-cm row spacing when grown at 108,000 plants ha^{-1} and 312 kg N ha^{-1}). Conversely, hybrids with high Check Plot (Check, yield at 0 kg N ha^{-1}) and a high initial yield increase when grown with moderate N (InitN, yield change between 0 and 67 kg N ha^{-1} at 79,000 plants ha^{-1}) supply were considered 'Workhorse' hybrids.

Hybrids were categorized into decile ranks from 1 to 10 using the PROC RANK procedure of SAS to generate scores for Check Plot, initial N response (InitN), response to maximum N (RTN), yield response to intermediate population (IntRTD), yield response to maximum plant population (MaxRTD), and yield response to row spacing (RTR). This assessment provided scores for each parameter and hybrid within each year, and combined across all years using the overall yields for the hybrid. Scores for each parameter (and the indices) ranged from 1 to 10, with 1 being the least yield increase compared to the overall average, and 10 being the greatest yield increase compared to the overall average, with average ranking equaling 5.

‘Racehorse’ and ‘Workhorse’ indices were estimated using a multiple regression approach with the Smith-Hazel index (Smith, 1936; Hazel, 1943). The data for the yield response to narrower row spacing at Champaign in 2016 was not included in the ‘Racehorse’ index analysis. Moreover, the genotypic and phenotypic variance-covariance matrices among all the parameters were estimated using restricted maximum likelihood in PROC MIXED of SAS (Holland, 2006). Index weights (vector) for each parameter were estimated by multiplying the phenotypic covariance matrix, the genotypic covariance matrix, and a vector of weight 1 Eq. [4.3]. ‘Racehorse’ and ‘Workhorse’ indices were calculated using the relative weights of each parameter multiplied by their corresponding scores (Eq. [4.4] and [4.5]).

$$b_n = P^{-1} G a \qquad [4.3]$$

$$\textit{`Workhorse index'} = b_1\text{Check} + b_2\text{InitN} \qquad [4.4]$$

$$\textit{`Racehorse index'} = b_3\text{IntRTP} + b_4\text{MaxRTP} + b_5\text{RTN} + b_6\text{RTR} \qquad [4.5]$$

Where b_n is the vector of the weights of the indices, P^{-1} is the inverse matrix of the phenotypic variance-covariance, G is the matrix of the genetic variance-covariance, and a is the initial weight (a = 1).

RESULTS AND DISCUSSION

Yield performance and variance components

When hybrids were grown at 79,000 plants ha^{-1}, N fertilizer increased yield by 60 and 74% with the initial (67 kg N ha^{-1}) and maximum (312 kg N ha^{-1}) N fertilizer conditions, respectively (Table 4.2). These yield increases indicates that the initial N fertilizer amount had a greater contribution to yield than the additional (maximum) N fertilizer. When hybrids were grown with maximum N, average yield remained unchanged, regardless of increasing the plant density or providing narrower row spacing. Yet notably, the top 8 highest yields of hybrids (ranging from 16.3 to 16.7 Mg ha^{-1}), when averaged across all environments and treatments, were obtained under the narrower row spacing and the highest plant density (108,000 plants ha^{-1}), (data not shown). Continued maize yield increases will require a synergistic integration between important agronomic practices (increased plant density, better soil fertility, and plant protection), (Ruffo et al. 2015). Although this study did not evaluate different fertilizer sources and foliar protection, it is possible to achieve even greater yields under higher plant density and narrower row spacing conditions than the current national average (National Corn Growers Association, 2015).

Similar to previous research (Boomsma et al., 2009), this study highlights the importance of breeding efforts to simultaneously target maize genetic improvement in tolerance to crowding stress, N-stress, and the yield response to N fertilizer. Large genotypic variation was found for yield performance across the diverse N fertilizer rates and plant density conditions provided in this study. Previous studies have also reported a large genotypic variation in maize hybrids in their response to N fertilizer supply levels (Uribelarrea et al., 2007) and increased plant density (Sarlangue et al., 2007). However, genotypic variance (σ^2_G) increased with increasing N fertilizer rate and plant density (Table 4.2). Also, the σ^2_G for yield, when hybrids were grown at the narrower row spacing, high plant density, and maximum N conditions was at least two fold greater than when grown with any other agronomic treatment. The large σ^2_G found under these conditions may be due to higher yield potential conditions and decreased plant-to-plant competition with abundant soil N availability. Therefore, maize genotypes when grown under narrower row spacing were able to express more of their individual plant yield potential and via different physiological mechanisms (e.g. root growth, photosynthetic activity, and biomass accumulation), (Borras et al., 2003).

In addition to σ^2_G, residual variance (σ^2_ε) for yield increased under the narrower row spacing, high plant density, and high N conditions compared to other agronomic treatments (Table 4.2). The large σ^2_ε for yield found when plants were grown under narrower row spacing may be associated with a greater border effect between plots (influenced by the neighboring plots' plant height and plant size). This study used two-row plots; although it was blocked by hybrid, it is possible that four-row plots may decrease the border effect and σ^2_ε, and increase broad-sense heritability (H^2). When averaged across all the agronomic conditions studied, yield H^2 ranged from 0.66 to 0.87 and was less under low and initial N conditions (0 and 67 kg N ha^{-1}, respectively). Similar to previous reports (Brun and Dudley, 1989; Bänziger et al., 1997), high N fertilizer conditions decreased soil heterogeneity and increased H^2. Conversely, increased plant density when plants were grown under maximum N and 76-cm row spacing conditions had no effect on H^2.

Yield components

When averaged across all hybrids and environments, the relationship between yield and yield components changed in response to different row spacing, plant density, and N fertilizer conditions (Table 4.3). Under N stress conditions, a reduction in dry matter redistribution to the reproductive organs may result in decreased kernel number (KN) and kernel weight (KW), (Below et al., 2000). As such, the Pearson's correlation between yield, KN, and kernel number per plant (KNPP) was higher when hybrids were grown under low N than high N conditions; suggesting that N-deficient tolerant hybrids have greater seed-set under N-stress conditions than hybrids that are susceptible to N deficiency.

Yield components are more affected by N fertilizer supply level than increased plant density (Ciampitti and Vyn, 2011). When hybrids were grown under conventional row spacing (76 cm) and high N conditions, the correlation between yield and KN, KNPP, and KW were similar at 79,000 and 94,000 plants ha^{-1} (Table 4.3). But, when hybrids were grown at the highest plant density (108,000 plants ha^{-1}) and conventional row spacing only KW was correlated to yield. Under the narrower row spacing condition, the average yield correlation coefficients for KN was greater than for KNPP or KW. The lower correlation found between yield and KN or KNPP at high plant densities (94,000 and 108,000 plants ha^{-1}) may be associated with greater plant-to-plant variability than when the plants were grown at the lowest plant density (79,000 plants ha^{-1}).

Yield response to different agronomic conditions

Yield increases in response to high plant densities and supplemental fertility are only possible with hybrids that are tolerant to crowding stress and have high yield potential (Haegele et al., 2014). Therefore, hybrid characterizations as to how they are affected by different agronomic conditions are essential for better agronomic recommendations. The hybrid yield response to maximum N fertilizer (312 kg N ha^{-1}) was the agronomic parameter with the greatest impact on yield (Table 4.4). Across all hybrids and environments, the initial (InitN) and maximum (RTN) yield responses to N fertilizer averaged +5.1 and +5.9 Mg ha^{-1}, respectively. In contrast, the intermediate (IntRTD) and maximum (MaxRPD) response to increased plant density changed yield by +0.30 and -0.12 Mg ha^{-1}, respectively. In addition, the average yield response of the hybrids to being grown at the narrower row spacing (RTR) was +0.53 Mg ha^{-1}.

Broad-sense heritability (H^2) for yield ranged from 0.40 to 0.91 across the different agronomic parameters provided, suggesting that additive and dominant effects will differ depending on the agronomic trait (Table 4.4). The low H^2 found for hybrids' MaxRTD may have increased the shrinkage effect on the BLUP's estimation (Robinson, 1991) reducing the range of this phenotypic value. Alternatively, there was high H^2 exhibited for yield averaged over the hybrids and environments in their response to maximum N supply (RTN) and/or the narrower row spacing (RTR).

Agronomic hybrid characterization using selection indices

The evaluation of the crop responses to different agronomic factors gives growers and agronomists the knowledge to better position their hybrids and to obtain the maximum yield potential of the hybrid using the recommended agricultural management. Typical variety testing methods using ‘standard’ agronomic conditions (e.g., 312 kg N ha^{-1} at 79,000 plants $acre^{-1}$ and 76-cm row spacing) are used to determine a hybrid’s yield potential, but do not provide information regarding a hybrid’s responses to N loss, increased plant density, or narrower row spacing. Intensive crop management practices are necessary in order to decrease the current corn yield gap existing in the U.S Corn Belt (Ruffo et al., 2015). Moreover, hybrid selection based on agronomic management performance is a key component to the success of intensive farming practices.

Using the Smith-Hazel Index Selection method (Smith et al., 1981), this study identified the impact on yield due to changes in the most important agronomic factors (e.g. N rate, hybrid selection, plant population, and row spacing) using the factors’ phenotypic and genotypic correlations and variance components. The average hybrid phenotypic and genotypic correlation coefficients for yield between check plot (Check) and InitN (used to calculate ‘Workhorse’ indices), and RTN, IntRTD, MaxRTD, and RTR (used to calculate ‘Racehorse’ indices) are presented in Table 4.5. Although producing high yields when grown in unfertilized (Check) and low N (InitN) conditions are desirable traits for ‘Workhorse’ hybrids, their negative phenotypic and genotypic correlations highlight the challenge for breeding for N stress tolerance. Similarly, the negative correlations found between RTR and IntRTD and MaxRTD, suggests that hybrids that are population-dependent (need increased plant density for greater yields) may not produce greater yields in response to narrower row spacing or to situations where plants are more equally distant. However, the positive genetic correlation between RTN and MaxRTD indicates that hybrids that produce greater yields in response to maximum N fertilizer provided may also make use of high plant densities in order to efficiently use the N available.

Using the phenotypic and genotypic correlations between each desirable trait, relative weights for each agronomic factor were calculated (Eq.[4.3]). Relative weights for each agronomic factor were calculated within each year and across years (2015 and 2016) in order to understand the effect of varying environments (Table 4.6). Averaged across years, relative weights for the

Check, InitN, RTN, IntRTD, MaxRTD, and RTR were 0.735, 0.260, 0.615, 0.104, 0.039, and 0.242, respectively. As a result, Check had a higher relative weight than InitN for the estimation of the 'Workhorse' index; while RTN and RTR were more important than IntRTD and MaxRTD to the 'Racehorse' index estimation. Changes in the relative weights among agronomic parameters are associated with differences in H^2 and their effects on yield.

The primary objective for using the Smith-Hazel index was to estimate the value of a genotype using a linear function of multiple traits with their genetic effect (Baker, 1974). Using 'Workhorse' and 'Racehorse' indices, maize hybrids were categorized based on their yield stability and response to different agronomic conditions (Figure 4.1). Biplot analysis using average yield across the different agronomic conditions and environments revealed that the yield variation was a function of N fertilizer rate, rather than different plant densities or row spacing arrangements. As such, hybrids with high 'Workhorse' indices (WHI > 7) were grouped between yield vectors for 0 and 67 kg N ha^{-1}. Hybrids with high 'Racehorse' indices (RHI > 7) were grouped closer to all vectors at 312 kg N ha^{-1} with the planting densities of 79,000, 94,000, and 108,000 plant ha^{-1}. Interestingly, only one hybrid across all genotypes evaluated combined high 'Workhorse' and 'Racehorse' indices (WHI > 7 and RHI > 7). This hybrid combined tolerance to N-stress conditions with a high yield response to intensive crop management conditions (high N fertilizer, increased plant density, and narrower row spacing conditions).

Selection indices correlation across environments

The understanding of the correlation between 'Workhorse' and 'Racehorse' indices across environments is important for the accurate application of hybrid characterization. 'Workhorse' indices were positively correlated across environments within years (Table 4.7). However, 'Racehorse' index exhibited higher correlations coefficients than the 'Workhorse' index within and across years. Low correlation coefficients with the DeKalb, 2015 location (DK, 2015) may be due to the unusual environmental conditions there (excessive rainfall and water logging).

CONCLUSIONS

Commercial maize hybrids exhibited a large genotypic variation in response to different crop management factors. Having a large genotypic variation pool provides the foundation for continued genetic selection and hybrid improvement for different agronomic conditions. Nitrogen fertilizer supply level was the agronomic factor with the greatest effect on yield and H^2. In addition, the yield response to narrower row spacing under high plant density conditions exhibited large genotypic variance and was highly heritable. Future breeding efforts for intensive agronomic management should focus on increased plant density at narrower row spacing. The Smith-Hazel index has categorized maize hybrids for their tolerance to N stress (‘Workhorse index’) and their response to intensive crop management practices (‘Racehorse index’). These selection indices may be used by agronomists for better hybrid positioning according to the desired agronomic management style.

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TABLES AND FIGURES

Table 4.1. Hybrid names, seed company names, and crop relative maturity (CRM), for hybrids planted at, DeKalb (DK), Yorkville (YV), Champaign (CH), and Harrisburg (HB) in 2015 and 2016.

			2015			**2016**		
Hybrid	**Company†**	**CRM**	**DK**	**CH**	**HB**	**YV**	**CH**	**HB**
P0419AMX	Pioneer	104	x	x	x			
5369SS	Winfield	105	x	x	x			
4644DGVT2P	Winfield	106				x	x	
5516SS	Winfield	106	x	x	x			
G06N80-3111	Syngenta	106	x	x	x			
207-27STXRIB	Monsanto	107	x	x	x			
G07B39-3111A	Syngenta	107	x	x	x	x	x	x
G07F23-3111	Syngenta	107	x	x	x	x	x	x
208-23STXRIB	Monsanto	108				x		x
4895SS/RIB	Winfield	108				x	x	x
5887VT2P	Winfield	108	x	x	x	x	x	x
DKC58-06RIB	Monsanto	108	x	x	x			
5938RIB	Monsanto	109	x	x	x			
5978VT3P	Winfield	109	x	x	x			
DKC59-50SS	Monsanto	109				x	x	x
G09E98-3122-EZ0	Syngenta	109	x	x	x	x	x	x
P0987AMX	Pioneer	109	x	x	x			
6068RIB	Monsanto	110				x	x	x
6110SS/RIB	Winfield	110	x	x	x	x	x	x
DKC60-87SS	Monsanto	110					x	x
G10S30-3220-EZ0	Syngenta	110	x	x	x	x	x	x
G10T63-3000GT	Syngenta	110	x	x	x	x	x	x
6065SS	Winfield	111	x	x	x	x	x	x
6148RIB	Monsanto	111	x	x	x			
DKC61-54RIB	Monsanto	111	x	x	x			
G11F16-3111A	Syngenta	111				x	x	x
G11K47-3110	Syngenta	111	x	x	x			
P1197AMXT	Pioneer	111				x	x	x
5290DGVT2P	Winfield	112				x	x	x
6265SS/RIB	Winfield	112	x	x	x	x	x	x
6288RIB	Monsanto	112	x	x	x			
D52VC91RIB	CPS	112				x	x	x
DKC62-77RIB	Monsanto	112	x	x	x			
DKC62-97RIB	Monsanto	112	x	x	x			
G12J11-3111A	Syngenta	112	x	x	x	x	x	x

Table 4.1. (Continued)

			2015			2016		
Hybrid	**Company**	**CRM**	**DK**	**CH**	**HB**	**YV**	**CH**	**HB**
G12W66-3000GT	Syngenta	112				x	x	x
P122AMXT	Pioneer	112	x	x	x			
P1257AMXT	Pioneer	112				x	x	x
P1339AMI	Pioneer	112	x	x	x			
213-19STXRIB	Monsanto	113				x		x
6594SS/RIB	Winfield	113	x	x	x	x	x	x
6640VT3P	Winfield	113	x	x	x	x	x	x
DKC63-33RIB	Monsanto	113	x	x	x			
DKC63-71SS	Monsanto	113	x	x	x	x	x	x
G13G41-3000GT	Syngenta	113	x	x	x			
P1311AMXT	Pioneer	113				x	x	x
214-45STXRIB	Monsanto	114	x	x	x			
6448RIB	Monsanto	114	x	x	x			
6458RIB	Monsanto	114				x	x	x
7087VT2P/RIB	Winfield	114	x	x	x	x		x
D54DC94RIB	CPS	114				x	x	x
DKC64-34SS	Monsanto	114				x	x	x
DKC64-87RIB	Monsanto	114	x	x	x			
G14H66-3010A	Syngenta	114	x	x	x			
G14R38-3122GT	Syngenta	114	x	x	x	x	x	x
G14Y81-3000GT	Syngenta	114	x	x	x			
P1479AM	Pioneer	114				x	x	x
215-05STXRIB	Monsanto	115	x	x	x			
D55VC77RIB	CPS	115				x	x	x
DKC63-60SS	Monsanto	115				x	x	x
216-36STXRIB	Monsanto	116					x	x
D56VC46RIB	CPS	116				x	x	x
DKC66-74SS	Monsanto	116				x	x	x
G16C59-3010	Syngenta	116	x	x	x			
6718RIB	Monsanto	117				x	x	x
7927VT3P/RIB	Winfield	117	x	x	x	x	x	x
8621VT2P/RIB	Winfield	117	x	x	x	x	x	x

† CPS, Crop Productions Services; Monsanto, Monsanto Company; Pioneer, DuPont Pioneer; Winfield, Winfield United.

Table 4.2. Row spacing, plant density, and N fertilizer effects on yield, variance components and broad-sense heritability (H^2). Values are averaged across 67 maize hybrids grown at four locations (DeKalb, Yorkville, Champaign, and Harrisburg, IL) and two years (2015 and 2016).

Row spacing	**Plant density**	**N rate**	**Yield**	σ^2_E	σ^2_G	$\sigma^2_{G \times E}$	σ^2_ε	H^2
cm	plant ha^{-1}	kg N ha^{-1}	------------------ Mg ha^{-1} ------------------					
76	**79,000**	**0**	8.5 ± 1.2[†]	8.5	0.15	0.13	1.53	0.69[‡]
76	**79,000**	**67**	13.6 ± 1.3	4.5	0.20	0.25	1.39	0.66
76	**79,000**	**312**	14.5 ± 0.9	4.3	0.68	0.34	0.91	0.85
76	**94,000**	**312**	14.8 ± 0.9	4.8	0.69	0.43	1.14	0.82
76	**108,000**	**312**	14.7 ± 0.9	4.3	0.71	0.43	1.31	0.82
50	**108,000**	**312**	14.7 ± 1.1	5.9	1.61	0.67	2.57	0.87

[†] Yield average values are shown with ± 1 standard error for 95% significance level.

[‡] H^2 was calculated using the harmonic mean values for environments ($e = 3.43$) and replications ($r = 13.31$) across six environments in Illinois.

Table 4.3. Pearson's pairwise correlation coefficients between maize yield and kernel number per area (m^{-2}), kernel number per plant, and kernel weight ($g\ m^{-2}$) at different row spacing, plant densities, and N fertilizer rates. Values are averaged across 67 commercial hybrids grown at four locations (DeKalb, Yorkville, Champaign, and Harrisburg, IL) for two years (2015 and 2016).

Row spacing	**Plant density**	**N rate**	**Kernel Number**		**Kernel Weight**
cm	plant ha^{-1}	kg N ha^{-1}	m^2	$plant^{-1}$	$g\ m^{-2}$
76	**79,000**	**0**	0.80*	0.79	0.48
76	**79,000**	**67**	NS	NS	0.53
76	**79,000**	**312**	0.47	0.41	0.40
76	**94,000**	**312**	0.48	0.30	0.42
76	**108,000**	**312**	NS	NS	0.55
50	**108,000**	**312**	0.69	0.49	0.56

*Significant at $P \leq 0.05$.

Table 4.4. Yield increase from initial N fertilizer (InitN), yield response to maximum N fertilizer (RTN), yield response to intermediate increased plant population (IntRTD), yield response to maximum increased plant population (MaxRTD), and yield response to narrower row spacing (RTR) effects on yield, variance components and broad-sense heritability (H^2). Values are averaged across 67 maize hybrids grown at four locations (DeKalb, Yorkville, Champaign, and Harrisburg, IL) and two years (2015 and 2016).

Parameter	**Yield change**	σ^2_E	σ^2_G	$\sigma^2_{G \times E}$	σ^2_ε	H^2
	------------------ Mg ha^{-1} ------------------					
InitN	+5.1 ± 1.2[†]	3.9	0.09	0.01	1.45	0.71[‡]
RTN	+5.9 ± 1.0	6.5	0.53	0.06	2.30	0.91
IntRTD	+0.3 ± 0.2	4.3	0.04	0.00	0.93	0.66
MaxRTD	-0.12 ± 0.1	0.1	0.02	0.00	1.18	0.40
RTR	+0.53 ± 0.3	0.3	0.47	0.00	3.84	0.91

[†] Yield average values are shown with ± 1 standard error for 95% significance level.

[‡] H^2 was calculated using the harmonic mean values for environments ($e = 3.43$) and replications ($r = 13.31$) across six environments in Illinois.

Table 4.5. Phenotypic (lower triangle) and genotypic (upper triangle) correlation coefficients between yield under low N (Check), initial yield increase with N fertilizer (InitN), maximum yield response to N fertilizer (RTN), yield response to intermediate increased plant population (IntRTD), yield response to maximum increased plant population (MaxRTD), and yield response to narrower row spacing (RTR). Coefficients were calculated using 67 hybrids at DeKalb, Yorkville, Champaign, and Harrisburg in 2015 and 2016.

Traits†	**Check**	**InitN**	**RTN**	**IntRTD**	**MaxRTD**	**RTR**
Check		-0.20	-	-	-	-
InitN	-0.50		-	-	-	-
RTN	-	-		-0.11	0.53	-0.16
IntRTD	-	-	-0.30		0.42	-0.17
MaxRTD	-	-	0.09	-0.35		-0.56
RTR	-	-	-0.04	-0.10	-0.37	

†Check and InitN parameters were used to calculate ‘Workhorse’ indices (Eq. [4.4]). RTN, IntRTD, MaxRTD, and RTR parameters were used to calculate ‘Racehorse’ indices (Eq. [4.5]).

Table 4.6. Relative weights (b_n) for yield under low N (Check, b_1), initial yield increase with N fertilizer (InitN, b_2), maximum yield response to N fertilizer (RTN, b_3), yield response to intermediate increased plant population (IntRTD, b_4), yield response to maximum increased plant population (MaxRTD, b_5), and yield response to narrower row spacing (RTR, b_6). Relative weights were generated across different number of environments (E) and hybrids (N). A total of 67 hybrids were evaluated at DeKalb, Yorkville, Champaign, and Harrisburg in 2015 and 2016.

Year	**E**	**N**	**Relative weights**					
			Check (b_1)	**InitN (b_2)**	**RTN (b_3)**	**IntRTD (b_4)**	**MaxRTD (b_5)**	**RTR (b_6)**
2015	3	43	0.355	0.645	0.485	0.026	0.004	0.486
2016	3	42	0.846	0.154	0.616	0.085	0.013	0.286
2015 + 2016	6	67	0.735	0.260	0.615	0.104	0.039	0.242

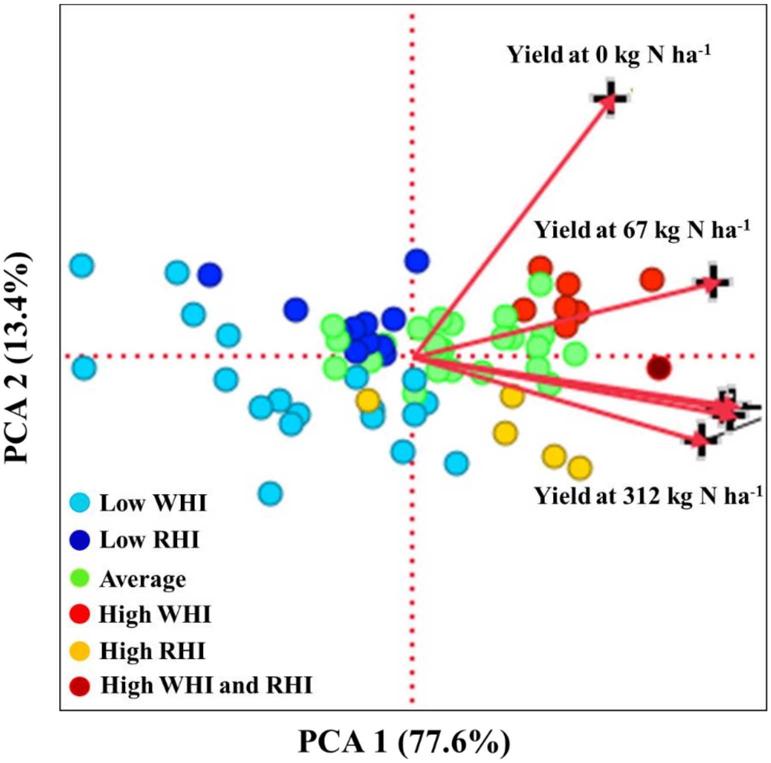

Figure 4.1. Biplot derived from principal component analysis using average yield of 67 hybrids grown at different agronomic conditions: (i) 79,000 plants ha^{-1} at 76-cm row space and 0 kg N ha^{-1}, (ii) 79,000 plants ha^{-1} at 76-cm row space and 67 kg N ha^{-1}, (iii) 79,000 plants ha^{-1} at 76-cm row space and 312 kg N ha^{-1}, (iv) 94,000 plants ha^{-1} at 76-cm row space and 312 kg N ha^{-1}, (v) 108,000 plants ha^{-1} at 76-cm row space and 312 kg N ha^{-1}, and (vi) 108,000 plants ha^{-1} at 50-cm row space and 312 kg N ha^{-1}. Vectors represent average yield at different agronomic conditions, with high-N vectors in the order of iv, v, and vi from top to bottom on the figure. Hybrids were categorized within low 'Workhorse' index (WHI < 4), low 'Racehorse' index (RHI < 4), average 'Workhorse' and 'Racehorse' indices (4 < WHI < 7 and 4 < RHI < 7), high 'Workhorse' index (WHI > 7), high 'Racehorse' index (RHI > 7), and high 'Workhorse' and 'Racehorse' indices (WHI > 7 and RHI > 7). Data points for each hybrid were averaged over their evaluations at DeKalb, Yorkville, Champaign, and Harrisburg in 2015 and 2016.

Table 4.7. Pearson's pairwise correlation coefficients between 'Workhorse' (lower triangle) and 'Racehorse' (upper triangle) indices for the different locations and years. Correlation coefficients were calculated using common hybrids planted at Champaign (CH), DeKalb (DK), Yorkville (YV), and Harrisburg (HB), in 2015 and 2016.

'Racehorse index'

'Workhorse index'	CH, 2015	DK, 2015	HB, 2015	CH, 2016	YV, 2016	HB, 2016
CH, 2015	-	0.38 **	0.70***	0.47*	0.52**	0.44*
DK, 2015	0.26*	-	0.48***	NS	NS	NS
HB, 2015	0.47***	NS	-	NS	0.44*	NS
CH, 2016	NS	NS	NS	-	0.52***	0.54***
YV, 2016	NS	NS	NS	0.30*	-	0.53***
HB, 2016	NS	NS	NS	0.42***	0.38*	-

'Workhorse index'

'Racehorse index'

NS, non- significant.
* Significant at $P \leq 0.10$.
**Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

CHAPTER 5

CONCLUSIONS AND DISCUSSION

Genetic improvement and hybrid performance under different N conditions

Maize is an important staple crop in many developing countries and the primary energy source for animal production worldwide. Increases in world demand for food will require greater maize yield and more agricultural inputs. Therefore, intensive agronomic management practices and genetic improvement of nitrogen use efficiency (NUE) need to be combined in order to obtain a more sustainable maize yield increase worldwide. This research evaluated the genetic variation of N-responsive traits using ex-PVP and commercial maize hybrids and identified novel research strategies for the genetic improvement of maize NUE.

The genetic improvement of maize NUE has received considerable attention in the scientific community and was one of the major focuses of this dissertation. Experiments conducted from chapters 1 and 3 were planted in adjacent fields with similar planting dates and agronomic practices. Therefore, similar environmental conditions between experiments from chapters 1 and 3 allow an indirect comparison between ex-PVP and commercial maize hybrids for NUE performance. Averaged across different years and environments, commercial maize hybrids exhibited 28% and 25% greater yield than ex-PVP hybrids under low and high N conditions (0 and 252 kg N ha^{-1} at 79,000 plants ha^{-1}, respectively) (Tables 1.1 and 3.1). Yield differences between ex-PVP and commercial hybrids suggests that maize breeding may have simultaneously improved N stress tolerance and the yield response to N fertilizer. As a result, a similar average NUE was observed between commercial and ex-PVP maize hybrids (16.5 kg $kg_{Nfert.}^{-1}$).

In addition to the yield performance under different N fertilizer rates, the variance components observed between commercial and ex-PVP germplasms provides a better understanding of NUE breeding. When averaged across different years and environments, the genetic variance of both commercial and ex-PVP hybrids increased and the residual variance decreased with additional N fertilizer (Tables 1.1 and 3.1). This comparison indicates that hybrids may express more of their genetic yield potential under high N and lower soil heterogeneity conditions, thereby increasing the power of selection. In addition, N fertilizer supplementation increased the environmental variance for ex-PVP hybrids but decreased it for commercial hybrids. These differences in environmental influences found between ex-PVP and commercial hybrids suggest that commercial hybrids exhibited more yield stability across high N environments than ex-PVP hybrids, highlighting the genetic improvement in current commercial maize genotypes.

Breeding strategies for maize NUE improvement

Phenotypic characterization of maize NUE in field conditions requires complex measurements and experimental procedures. Having a better understanding of the genetic basis of different N use traits and their contribution to NUE will assist breeding programs in accurate field phenotyping. In addition, the integration of genomic selection using desirable phenotypic traits may increase the genetic gain of maize NUE breeding programs. One of the major objectives of this project was to identify N use traits associated with N stress tolerance and/or high N conditions that have high genomic prediction accuracy and to evaluate their application in a NUE breeding program. While substantial genetic variation was found across different N use traits, breeding for improved N stress tolerance or for a greater yield response to N fertilizer will require different phenotypic selection traits.

Phenotypic traits associated with N utilization under low N (GU, genetic utilization) or biomass partitioning (HI, harvest index) were highly correlated to yield under N stress conditions (Table 1.2). Genetic utilization and HI also exhibited high heritability (Tables 1.1 and 1.2). Moreover, GU was the most stable trait measured across low N environments (Figure 1.2A). In addition to the phenotypic selection, HI and GU were the secondary traits that provided the highest genomic prediction accuracy under N stress conditions (Table 2.3). However, HI under low N provided higher prediction accuracy than GU across different training composition and training sizes. Therefore, under N stress environments, the most effective phenotypic trait to select for is HI for the following reasons: i) HI is highly genetically controlled and associated with yield under N stress conditions, ii) HI requires less genetic information in the training population than GU within the same prediction accuracy value, iii) HI requires less number of hybrids to be phenotyped than GU within the same prediction accuracy, and iv) HI is easier and cheaper to measure than GU.

Nitrogen use efficiency (NUE), N-uptake efficiency (NUpE), and N-utilization efficiency (NUtE) were highly correlated to yield under high N conditions (Table 1.2). However, NUE exhibited greater broad-sense heritability (H^2) than NUpE and NUtE (Table 1.1). The derivation of NUpE and NUtE from multiple plant measurements (individual plant biomass, seed weight, and N concentration) may have contributed to increased residual error of these factors. On the other hand, NUE is derived from only two component measurements (yield at low and high N). In addition to higher H^2, NUE was more stable than NUpE and NUtE across high N environments (Figure 1.2B). As such, among secondary traits associated with yield at high N conditions, NUE exhibited the greatest genomic prediction accuracy.

The large genotypic variation of N use traits found among the ex-PVP germplasm highlights the opportunity that exists for selecting maize genotypes with desirable NUE performance. Inbred lines of ex-PVP germplasm were identified with high general combining ability for N stress tolerance combined with a high yield potential under high N conditions (Chapter 1). Moreover, genomic prediction can be integrated into NUE breeding programs using specific phenotypic traits depending on the target environment for N condition (low or high N). Since H^2 and prediction accuracy for yield at low N is less than for yield at high N, breeding for low N tolerance may benefit more by the use of secondary traits in genomic selection than when breeding for increased yields at high N. Furthermore, HI at low N exhibited higher H^2 and higher prediction accuracy than yield at low N.

Maximizing yield potential using hybrid agronomic characterization

The integration between innovative agronomic practices and novel breeding strategies has become a common research effort in the seed industry. Therefore, it is of critical importance that research agronomists and breeders understand the so called genotype × environment × management interaction (G×E×M) for better hybrid selection and placement. In contrast to the traditional maize breeding scheme, current breeding efforts are selecting and characterizing maize genotypes for specific agronomic traits (e.g., tolerance to N deficiency, yield response to N fertilizer, and tolerance to crowding stress) earlier in the breeding pipeline. As a result, coordinated improvement between hybrid adaptation to crop management and yield stability requires knowledge of agronomic factors affecting hybrid performance.

Current maize hybrids exhibit a large genotypic variation for their yield response to different agronomic factors (Chapters 3 and 4). Notably, the diverse yield responses found among the commercial hybrids to additional N fertilizer and increased plant density highlights the importance of accurate agronomic characterization of maize hybrids (Figure 3.3). Hybrids that combined an above average tolerance to N stress and an above average yield response to initial N fertilizer supply accounted for less than 10% of all hybrids evaluated during 2011 and 2014 (9 of 101 hybrids). These hybrids exhibited greater yield stability with high N fertilizer regardless of the concurrent plant density conditions (Table 3.6). On the other hand, hybrids with high yield responses to maximum N fertilizer and increased plant density exhibited greater yield potential overall.

The Smith-Hazel index was used to better characterize hybrids based on their yield response to different crop management conditions (Chapter 4). Across the different agronomic management settings studied, N fertilizer was the most important factor impacting maize yield, followed by narrower row spacing, and then increased plant density (Table 4.3). As a result, the agronomic parameter associated with N stress tolerance exhibited the highest relative weight for the 'Workhorse' index (index selection for hybrids with high yield stability) when estimated across years and environments (Table 4.5). Similarly, the agronomic parameter associated with the yield response to maximum N fertilizer contributed the highest relative weight for the 'Racehorse' index (index selection for hybrids with increased yield adaptability to intensified crop management).

Selection indices using the yield response to key agronomic factors have categorized hybrids according to their yield performance across different N fertilizer rates, plant density, and row arrangement conditions (Figure 4.1). The previous chapter reported the first attempt to categorize hybrids based on their agronomic features using selection indices. However, the low correlations found between hybrid indices across environments suggest that more research is needed for generating selection indices based on hybrid agronomic features (Table 4.6). The integration of genomic marker data and crop growth models into hybrid characterization efforts may be possible by using innovative statistical model approaches.

In addition to categorizing maize hybrid's responses to agronomic factors, this research evaluated the effect of different crop management practices on maize yield. Averaged across all hybrids and environments, increased plant density (Chapters 3 and 4) and narrower row spacing (Chapters 4) did not increase maize yield. Yet all the top-yielding hybrids within each environment were planted at the highest plant density (Chapters 3 and 4) and the narrower row spacing conditions (Chapters 4). Increased plant density imparted more plant-to-plant competition for abiotic resources (e.g., radiation, water, and soil nutrients) increasing the genotypic variance. These increases in genotypic variance associated with increased plant density suggest that there exists different crowding stress tolerances among commercial hybrids. Continued advances in maize yield potential will require more intensive agronomic management practices using maize hybrids that combine a high yield response to N fertilizer, tolerance to crowding stress, and adaptation to narrower row spacing.