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RESEARCH

Yield Stability Differs in Commercial Maize Hybrids in Response to Changes in Plant Density, Nitrogen Fertility, and Environment

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ABSTRACT

Continued yield increases in modern commercial maize (*Zea mays* L.) hybrids will require increased plant density, improved nitrogen-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 nitrogen (N) fertilizer levels to assist breeding programs to select 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}) and three N rates (0, 67, and 252 kg N ha^{-1}). 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, whereas the yield response to increased N ranged from +4.47 to +5.64 Mg ha^{-1}. Hybrids that combined above-average yield under unfertilized and low-N conditions exhibited greater-than-average yield stability across environments under high-N conditions. Hybrid yield stability variance was larger under high-N than under low-N conditions because of greater genotype × environment interaction. Hybrids that were adaptable to high plant density and N conditions exhibited greater-than-average yield potential and yield variation across environments. Selecting hybrids with both high yield and yield stability may be difficult, as yield under lower N levels and yield increases with high N fertilization were negatively correlated.

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Abbreviations: BLUP, best linear unbiased predictor; CH, Champaign, IL; CRM, corn relative maturity; DK, DeKalb, IL; HB, Harrisburg, IL; KN, kernel number; KW, kernel weight; NUE, nitrogen-use efficiency; V2–V4, between the two- and four-leaf growth stages.

Maize yield increases since the 1930s have been attributable to a combination of genetic improvement and improved 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 maximize yield potential of a hybrid using a corresponding recommended agricultural management system. In addition, continued increases in maize yield will depend on a hybrid's ability to utilize resources more efficiently when grown under greater plant 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, to minimize the current maize yield gap that exists in the U.S. Corn Belt (Ruffo et al., 2015).

For maximum yield, N is the nutrient required in the largest amount for maize production (286 kg N to produce 14.4 Mg ha^{-1} of grain) accompanied by a high N harvest index (58%) (Bender et al., 2013). Increased N fertilizer rate was one of the major crop management practices that contributed to

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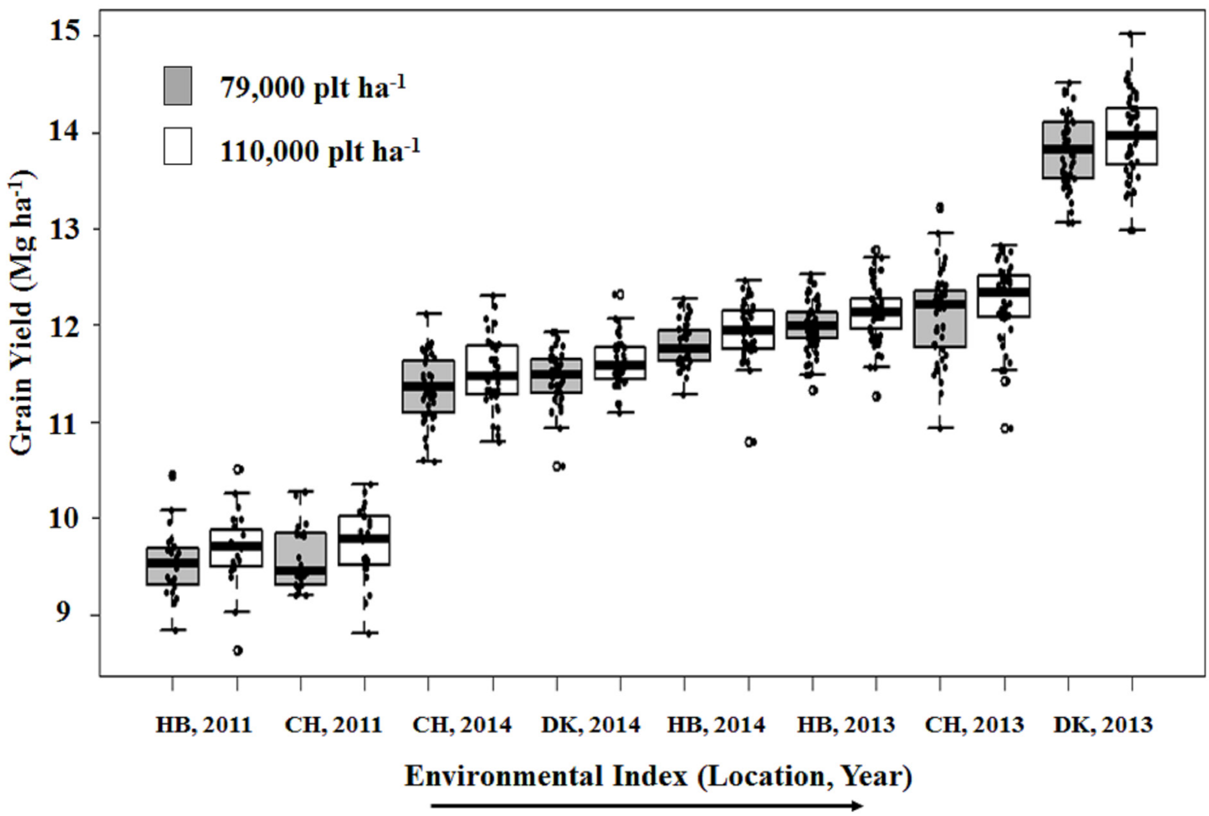

Fig. 1. Influence of location, year, and plant population on grain yield, arranged by increasing average yield (Environmental index) of the normal and high population plots for 101 maize hybrids grown at DeKalb, IL (DK), Champaign, IL (CH), and Harrisburg, IL (HB) under high N conditions (252 kg N ha^{-1}) 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 extend to the 10th and 90th percentiles.

increased maize yield during the past 20 yr (Egli, 2008). In comparisons of the genetic gain of maize hybrids under different levels of N fertility, newer hybrids (developed in 1990s) exhibited greater yield than older hybrids (developed in 1970s) under low- and high-N conditions (Tollenaar et al., 1997; O'Neill et al., 2004). Current maize hybrids have greater total N uptake and N utilization (the ratio of yield increase to the difference in plant N content of a fertilized compared to an unfertilized crop); and approximately 70% of the genetic gain in maize yield under high-N conditions is attributable 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 the contemporary average plant density of producers in the area), only evaluating a hybrid's yield responses to different crop-management practices at the precommercial stage. Therefore, there are limited reports on genetic variability of elite hybrids for different N use efficiency (NUE) traits (Bertin and Gallais, 2001). 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 NUE, tolerance to increased plant density is one of the most valuable agronomic advances since the development of the maize hybrid (Duvick, 1977). Maize yield increases across different N fertilizer and plant density conditions were associated with greater N uptake and greater ear sink strength (kernel weight and number) during reproductive development (Ciampitti et al., 2013). The kernel weight component has been found to be more related to the yield increases than to kernel number, and was associated with a longer grain-filling period, improved biomass remobilization during reproductive development, enhanced stress tolerance to N loss, and higher plant densities (Chen et al., 2016).

The most recent maize yield record in the United States (31.5 Mg ha^{-1}) was achieved with a plant density (128,000 plants ha^{-1}) much greater than that commonly used (National Corn Growers Association, 2015), indicating that the average yield of maize is still far from reaching 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 attributable to the fact that the yield potential of individual plants has not increased in the past 80 yr, rather maize hybrids have better stress tolerance, including the ability to tolerate increased 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 unit area, thereby increasing the area-wide source-sink ratio (Borrás et al., 2003). In turn, the greater number of potential kernels produced per unit area in density-tolerant hybrids may minimize the yield decreases caused by environmental stresses.

Genotypes that are tolerant to abiotic and biotic stresses are expected to have improved yield stability, that is, improved 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 considered adaptable genotypes and are expected to show improved 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 in relation to the corresponding environmental indices (average performance of multiple hybrids in a particular environment). Hybrids with high yield stability have been further characterized as "work-horse" hybrids, whereas hybrids with high adaptability are dubbed as "race-horse" hybrids (Tollenaar and Lee, 2002).

One of the future challenges of maize breeding will be selecting genotypes with higher yields in response to higher plant density, with concurrent improvement in yield stability across environments (Tokatlidis and Koutroubas, 2004). Genetic gain for yield was 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, grown at 79,000 plants ha^{-1} or greater, are single-eared plants and are better adapted to increased plant densities than older hybrids (Tollenaar et al., 1992). Breeding programs targeting for maize hybrids with improved tolerance to high plant density and utilization of N fertilizer could benefit from a comprehensive evaluation of a representative number of commercial maize hybrids, N rates, plant densities, and environments to develop future selection strategies. Therefore, the objective of this research was to measure the genotype × environment × management interaction by evaluating an extensive assortment of current maize hybrids for yields and classify them for yield stability and crop-management adaptability to improve future breeding programs.

MATERIALS AND METHODS

Cultural Practices

Research sites were planted for two years at DeKalb, IL (DK; 41°47′ N lat., 88°50′ W long.; 15 May 2013 and 20 May 2014), three years at Champaign, IL (CH; 40°03′ N lat., 88°14′ W long.; 18 May 2011, 19 May 2013, and 8 May 2014), and three years at Harrisburg, IL (HB; 37°43′ N lat., 88°27′ W long.; 1 June 2011, 29 May 2013, and 23 May 2014). Soil types at the research sites were predominantly 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 at each location 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. Preemergence 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. Postemergence herbicide Roundup [N-(phosphonomethyl)glycine; Monsanto, St. Louis, MO] was applied at a rate of 1.75 L ha^{-1} when necessary.

Field evaluations used 101 representative elite single-cross maize hybrids; all were commercially available at the time and adapted to the state of Illinois. These 101 commercial maize hybrids had a variety of biotechnological traits and seed treatments (i.e., fungicides, insecticides, and/or nematicides), were from 11 different seed brands, and had relative maturities ranging from 101 to 117 d (Supplementary Table S1). Due to the latitudinal diversity of the experimental sites, the nature of corn hybrid relative maturities, and the availability of seed, not all hybrids were planted in each site or year. However, on average, 42 hybrids were planted in 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. The standard plant density is based on contemporary farmer practices in the state of Illinois. Final plant stands were recorded prior to harvest. Unfertilized (0 kg N ha^{-1}) check plot yields were used to estimate hybrid N stress tolerance, whereas 67 and 252 kg N ha^{-1} were used to estimate the yield response to low and high N fertilizer, respectively. Nitrogen treatments were broadcast applied as urea

(46–0–0) between the V2 and V4 developmental stages in each environment (Ritchie et al., 1997). Nitrogen application dates were 18 June 2013 and 17 June 2014 at DeKalb, IL; 3 June 2011, 13 June 2013, and 29 May 2014 at Champaign, IL; and 01 July 2011, 25 June 2013, and 13 June 2014 at Harrisburg, IL. The experimental design was a randomized complete block with four replications within each environment in a split–split-plot arrangement. The main plot was hybrid, the split plot was N fertilizer rate, and the split–split plot was plant density level.

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 sample from each plot was collected during harvest, from which 300 random kernels were selected and weighed to estimate mean kernel weight (KW). Kernel number (KN) per unit area was estimated from the total plot grain weight, mean 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 sample that was used for measuring yield components.

Statistical Analysis and Derived Measurements

Statistical analysis was performed using PROC MIXED statement in SAS version 9.4 (SAS Institute, 2013). Nitrogen fertilizer rates and plant density levels were included in the model as fixed effects, whereas environment, block, and hybrid were considered random effects. The designation of hybrid as a random effect was to allow an inference about the distribution of current maize hybrid yield performances due to the other sources of variation and not examine specific hybrids. 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.

As 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 (BLUPs) were calculated within each N fertilizer by plant density treatment using restricted maximum likelihood estimation. Therefore, the phenotypic observations (y_{ijk}) were modeled according to Eq. [1]:

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

where Y_{ijk} is the phenotypic observation of ith environment within jth block, for kth hybrid, μ is the overall mean, E_i is the random effect of ith environment (i = 1, 2,…, and 8), $B_{(i)j}$ is the random effect of jth block (j = 1, 2, 3, and 4) nested within ith environment, G_k is the genetic random effect of kth hybrid (k = 1, 2, …, and 101), $(G \times E)_{ik}$ is random effect of the interaction between kth hybrid and ith 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; the ratio between the genotypic and phenotypic variances) 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 to grain yield from the unfertilized control treatment (Moll et al., 1982). In addition, yield stability and adaptability were calculated using 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 yields of an individual hybrid across environments when the latter are arranged in increasing average yield order) for 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 $b < 1$ ("work-horse"), whereas 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 comparison of their yield performance to the mean distribution across environments for four phenotypic traits. The phenotypic traits considered in this study were: (i) unfertilized check plot yield (yield at 0 kg N ha^{-1} and 79,000 plants ha^{-1}), (ii) low N yield response (yield change between 0 and 67 kg N ha^{-1} at 79,000 plants ha^{-1}), (iii) yield response to high N (yield change between 0 and 252 kg N ha^{-1} at 79,000 plants ha^{-1}), and (iv), yield response to plant density (yield change between 79,000 and 110,000 plants ha^{-1} with 252 kg N ha^{-1}). Mean separations for different hybrid groups were analyzed in PROC MIXED using hybrid group as a fixed effect at the 5% significance level. The test for equal stability variances across hybrid groups was performed using the Brown–Forsythe method via PROC GLM (Brown and Forsythe, 1974).

RESULTS AND DISCUSSION

Weather Conditions

Air temperature and rainfall amounts from all environments were obtained from the National Oceanic and Atmospheric Administration (NOAA) and are presented in Supplementary Table S2. July 2011 was hot and dry; at the Champaign, IL site, precipitation was 79 mm below the 10-yr average, whereas both minimum and maximum average daily temperatures were greater than the 10-yr average at Champaign (by +3°C) and Harrisburg (by +2°C)

(Supplementary Table S2). The late vegetative to early reproductive developmental stages of maize usually occur during July to August in the U.S. Corn Belt; these are important growth stages in determining maize yield. Data from 2012 of the trial were excluded from the analysis because of severe drought stress. In contrast, the weather of 2013 provided ideal environmental conditions for maize development and yield at all three sites, with daily temperatures similar to or cooler than the 10-yr average, and above-average precipitation during June (+83, +49, and +22 mm at DeKalb, Champaign, and Harrisburg, respectively). In 2014, above-average precipitation also occurred during June (+117, +98, and +20 mm at DeKalb, Champaign, and Harrisburg, respectively, compared with the 10-yr average), and below-average temperature occurred in July, with both minimum and maximum average daily temperatures deviating from the 10-yr average by -2 to -3°C for all sites. The environmental conditions in 2014 led to a statewide record corn yield of 12.5 Mg ha^{-1} (USDA National Agricultural Statistics Service, 2017).

Hybrid Yields and Variance Components

Overall, maize grain yield was affected by the environment, hybrid, nitrogen rate, and their interactions (Table 1). In contrast, the population change was only significant in combination with environment ($P = 0.012$). Averaged across environments, N fertilizer increased maize yield, but plant density did not alter the overall average hybrid yield within an N rate (Table 2). The environments used in this study provided different crop-growing conditions (Supplementary Table S2). Consequently, across 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 by -1.6 to +2.4 Mg ha^{-1} at the standard plant density (79,000 plants ha^{-1}) and by -1.9 to +2.3 Mg ha^{-1} at the higher plant density (110,000 plants ha^{-1}) (Fig. 1). Although hybrids exhibited similar yields at both plant densities, the highest-yielding hybrids within each environment usually resulted from the higher plant density and the highest rate of 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 2). Low N (67 kg N ha^{-1}) increased yield by +2.8 and +2.9 Mg ha^{-1} over the unfertilized control, and the yield response to high fertility (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 at the low

Table 1. Analysis of variance for yield of 101 maize hybrids (Hyb.) grown at eight environments (Env.; covering three locations and three years), two plant density populations (Pop.), three N fertilizer rates (Nrate), and four replications (Rep.).

Source of variation	df	SS	MS	*F* value	*P* > *F*
Environment	7	7085	1012.1	602.1	≤0.001
Hybrid	100	1705	17.1	10.1	≤0.001
Nrate	1	33780	33780.0	20094.1	≤0.001
Population	1	4	4.0	2.1	0.148
Rep (Env.)	24	1061	44.2	26.3	≤0.001
Env. × Hyb.	221	1825	8.3	4.9	≤0.001
Env. × Nrate	7	837	119.6	71.1	≤0.001
Env. × Pop.	7	30	4.3	2.6	0.012
Hyb. × Nrate	100	316	3.2	1.9	≤0.001
Hyb. × Pop.	100	139	1.4	0.8	0.896
Hyb. × Nrate × Pop.	101	131	1.3	0.8	0.958
Residuals	7096	11929			

Table 2. Plant density and N fertilizer effects on yield from BLUP analyses, variance components from Eq. [1], 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) for 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	41.8 ± 4.1
	252	11.4 ± 0.4	1.62	0.16	0.23	0.75	0.52 ± 0.09	20.2 ± 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	43.3 ± 3.9
	252	11.6 ± 0.5	1.94	0.27	0.26	0.83	0.61 ± 0.06	22.2 ± 2.2

† Yield, *H*^{2}, and NUE average values are shown with 95% confidence interval.

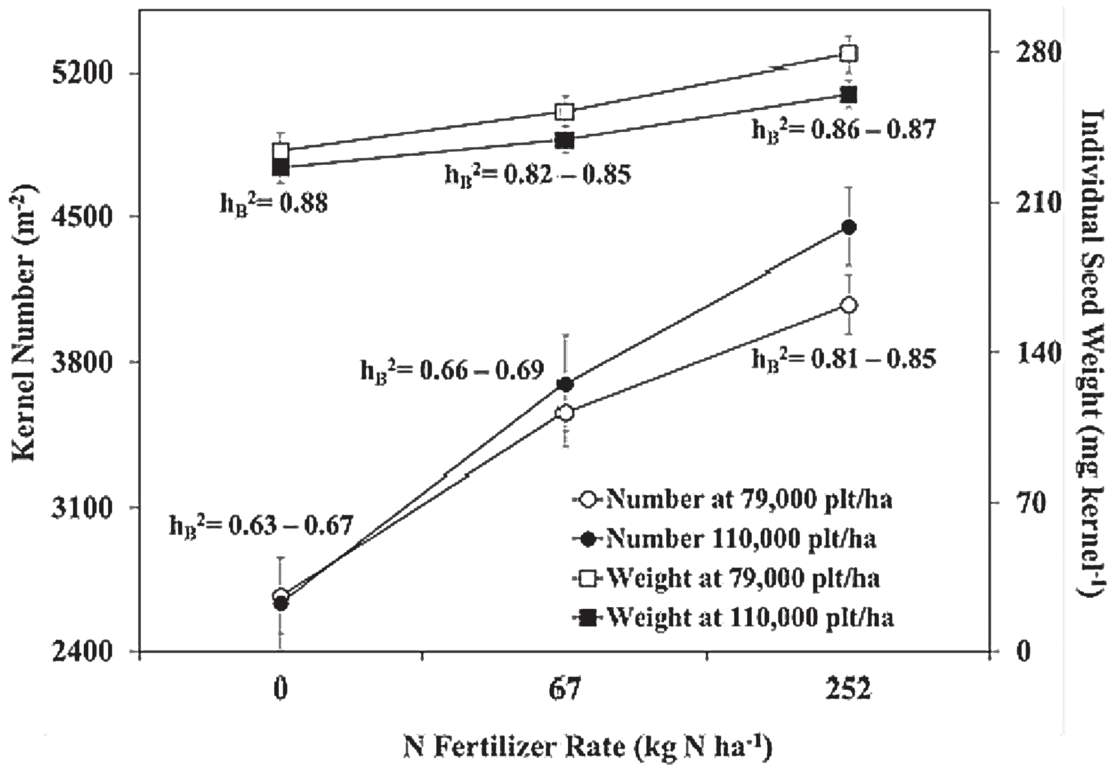

Fig. 2. Plant density and N fertilizer rate effects on average kernel number, kernel weight, and broad-sense heritability (estimated on hybrid-mean basis) for 101 maize hybrids grown at three locations (DeKalb, Champaign, and Harrisburg, IL) for three years (2011, 2013, and 2014). Bars extending from data points indicate ± 1 standard error for 95% significance level.

N rate was greater than the NUE at high N. Increased plant density may increase NUE under greater N fertilizer rates (165 and 330 kg N ha^{-1}) (Boomsma et al., 2009). However, the data in Table 2 show no change in NUE with increasing plant population. While other studies found maize hybrids showing improved tolerance to crowding stress (Tollenaar and Lee, 2002) in combination with NUE improvement (Tollenaar and Wu, 1999), possibly the diverse range of hybrids in this study might have concealed the improvements in these parameters.

Environmental (σ^2_E), genetic (σ^2_G) and the genotype × environment interaction ($\sigma^2_{G \times E}$) variances for yield differed across N rates and plant densities (Table 2). While the environmental variance for yield decreased under higher N fertility conditions, the genetic variance tended to increase. Other investigators have also reported a reduction in environmental variance for yield because of better agronomic conditions (Bänziger and Cooper, 2001). Also, high N conditions may have reduced the soil heterogeneity and allowed for an increased genetic effect (Bertin and Gallais, 2001). 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 fertility conditions can increase heritability (Brun and Dudley, 1989; Bänziger et al., 1997; Bertin and Gallais, 2001) or decrease heritability (Agrama et al., 1999) depending on the germplasm and the agronomic conditions used for evaluation. Higher heritability under high N conditions may 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 because of reduced stand uniformity and individual plant yield (Fasoula and Tollenaar, 2005). In this study, genetic variance for yield increased by 41% and broad-sense heritability increased by 15% with increased plant density (Table 2). Therefore, current maize hybrids are more tolerant of crowding stress as a group, and under these conditions, the greater variability in 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 (Fig. 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 conditions, KW and KN were not affected by plant density. However, the highest 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 controls. Kernel number often exhibits greater

Table 3. 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) for three years (2011, 2013, and 2014).

Kernel parameter	N fertilizer rate (kg N ha^{-1}) 0	67	252
	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 $P \leq 0.001$.

plasticity than KW when maize hybrids are exposed to different agronomic conditions (Sadras and Slafer, 2012; Boomsma et al., 2009). Similarly, KN was more correlated to yield than KW across all N and plant density treatments (Table 3). 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 when unfertilized and KN at both plant densities. Correlations between KW and KN with 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 2) and with specific hybrid grain characteristics. Individual mean 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, both N fertilizer and plant density had an effect on grain quality (Table 4). Grain oil concentration was stable across plant density treatments. Therefore, because of increased yield, the highest N fertilizer rate increased oil content by approximately 35% over the unfertilized control, regardless of the plant density. In addition, the highest N fertilizer rate increased grain protein content by 60% at both plant densities over the unfertilized control because of 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 unit area of both traits, whereas no changes in grain quality were observed with increased plant density. Nitrogen fertility affected protein more than oil content, most likely because of the inherent composition of proteins. These results using 101 commercial hybrids revealed less genetic variation in grain protein and oil concentration than previous studies (Below, et al., 2004, Uribelarrea et al., 2007).

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 between different seed brands among maize hybrids' responses to N fertilizer and plant density (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 (Table 5); indicating that hybrids with tolerance to crowding stress under high N conditions can produce high yields, while hybrids that are susceptible to crowding stress have a more limited yield, regardless of N fertilizer availability. While full-season hybrids can exhibit greater biomass plasticity and partitioning to the grain in response to plant

Table 4. 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) for three years (2011, 2013, and 2014).

Kernel parameter	N fertilizer rate (kg N ha^{-1}) 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 ± 95% confidence interval.

Table 5. 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 the average of three locations (DeKalb, Champaign, and Harrisburg, IL) and three years (2011, 2013, and 2014). Low and high N for NUE indicate the values with N fertilizer supplied at 67 and 252 kg N ha^{-1}, respectively. Low and high N for 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		ΔKN		ΔKW	
				Low N	High N	Low N	High N	Low N	High N
		plant ha^{-1}	Mg ha^{-1}	kg kg N^{-1}		kernel m^{-2}		mg kernel^{-1}	
1	113	110,000	12.5	45.4	24.5	1235	2027	6.6	35.5
2	115	110,000	12.2	46.7	25.4	1252	2021	15.8	51.5
3	110	110,000	12.2	38.9	24.1	1040	1909	-0.3	41.5
4	112	110,000	12.2	40.8	23.0	945	1765	8.8	29.3
5	113	110,000	12.2	47.7	24.1	1169	1968	13.7	33.0
6	111	110,000	12.2	44.3	23.8	1199	2075	8.8	33.4
7	111	110,000	12.2	45.9	24.3	1353	2599	9.2	17.9
8	113	110,000	12.1	44.4	23.5	1041	1838	23.8	44.8
9	114	110,000	12.1	42.1	21.7	1039	1714	17.5	39.4
10	109	110,000	12.1	45.5	24.6	979	1540	19.0	58.9
193	107	110,000	10.9	38.8	19.0	1001	636	2.7	15.4
194	102	79,000	10.9	41.6	18.6	906	493	15.5	29.3
195	115	110,000	10.9	41.9	22.5	1194	973	-3.7	16.9
196	109	110,000	10.9	39.3	19.9	888	804	14.2	21.7
197	111	110,000	10.9	39.9	20.7	1003	824	14.5	32.3
198	113	79,000	10.8	40.0	18.5	665	562	22.8	45.3
199	109	110,000	10.7	43.6	21.1	1185	960	12.7	23.9
200	113	110,000	10.6	41.2	20.7	968	797	7.7	20.3
201	114	110,000	10.4	45.2	19.6	1141	440	16.2	34.9
202	112	110,000	10.3	41.7	19.6	923	545	11.4	25.6
Average from 10 highest-yielding hybrids			12.2	44.2	23.9	1125	1946	12.3	38.5
Average from 10 lowest-yielding hybrids			10.7	41.3	20.0	987	703	11.4	26.6
Least significant difference† ($P \leq 0.01$)			0.1	3.0	1.2	161	299	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}).

density compared with short-season hybrids (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 differential NUE and yield component proportions across N rates and plant densities (Table 5). The 10 highest-yielding hybrids exhibited, on average, higher NUE ($P \leq 0.01$) under high N (252 kg N ha^{-1}) than the 10 lowest-yielding hybrids. Similarly, the 10 highest-yielding hybrids had greater KN at high N fertility compared to the 10 lowest-yielding hybrids, but there were no significant differences in KN at low N, or in KW between the groups. Differences in NUE between hybrids when grown under high N are attributed to both the difference in a hybrid's yield performance when unfertilized and its yield response to N fertility.

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), check-plot (0 kg N ha^{-1} fertilization) yields were compared with the response to low N (Fig. 3A). Yields without fertilizer ranged from 5.87 to 6.78 Mg ha^{-1} and the yield response to low N 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 when unfertilized but had an above-average yield response to low N; group 2A (9% of the hybrids) included those with above-average yield both when unfertilized and in response to low N; group 3A (4% of the hybrids) included those with below-average yield when unfertilized and in response to low N; and group 4A (45% of the hybrids) included those with above-average yield when unfertilized but below-average yield response to low N. Newer cultivars with transgenic traits have been found to have greater NUE and yield at low N, with a corresponding lower N rate to maximize yield (Haegele et al., 2013) and are more represented in groups 1A and

2A, possibly because of greater root mass (Head et al., 2017)(Fig. 3A). Because yield from unfertilized plots and the yield response to low N were negatively correlated ($r = -0.35$, $P < 0.05$), 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 high N fertility and increased plant density (Fig. 3B). The yield response of hybrids, averaged across environments, to high N fertilizer (252 kg N ha^{-1}) 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 with results from previous studies under similar agronomic conditions, the findings reported here identified substantially greater yield ranges for both response to high 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 included 25% of the hybrids with below-average yield response to high N but above-average response to increased plant density; group 2B included 24% of the hybrids with above-average yield responses to both high N and increased plant density; group 3B contained 25% of the hybrids with below-average yield response to both high N and plant density; and group 4B consisted of 26% of the hybrids with above-average yield response to high 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 of 11.64 Mg ha^{-1}, which was significantly higher ($P < 0.001$) than those of groups 1B (11.32 Mg ha^{-1}) and 4B (11.19 Mg ha^{-1}). However, under high N and high plant density, group 2B (11.99 Mg ha^{-1}) had a significantly higher yield ($P < 0.001$) than groups 3B (11.15 Mg ha^{-1}), 4B (11.59 Mg ha^{-1}), and 1B (11.67 Mg ha^{-1}). Maize hybrids that were adapted to increased N fertilizer and plant density exhibited higher than average yield and would be more suitable for intensive crop management practices.

Hybrid Stability Analysis

Yield stability was evaluated across different N rates and plant densities for 61 maize hybrids that were grown in at least three environments. Hybrids exhibited similar yield stability correlations between N levels across different plant densities (Table 6). Overall, phenotypic correlations for hybrid stability (b-values) across N treatments and plant densities for yield ranged from nonsignificant to 0.85 ($P \leq 0.001$). Correlation coefficients tended to be larger at standard plant density compared with high plant density. In addition, hybrid yield stability appeared to be more associated with a hybrid's tolerance to N stress when unfertilized than with a hybrid's response to N fertilizer

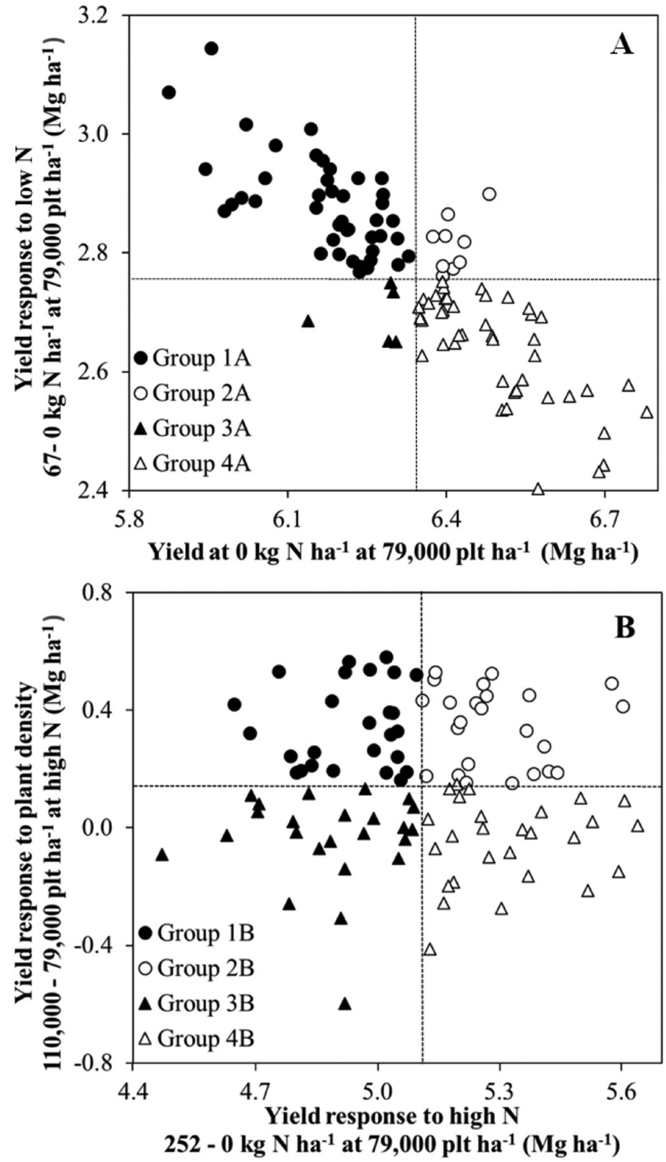

Fig. 3. Characterization of 101 elite maize hybrids under different agronomic conditions: a) relationship between yield when unfertilized (0 kg N ha^{-1}) and in response to low N (67–0 kg N ha^{-1}) and b) relationship between yield response to greater plant density (110,000–79,000 plt ha^{-1}) at high N conditions and yield response to high 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.

or tolerance to crowding stress. Increased $\sigma^2_{G \times E}$ at high N and high plant density may have contributed to 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 7). Group 2A (hybrids with above-average yield when unfertilized and in response to 67 kg N ha^{-1}) had better

Table 6. 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).

Plant density	N rate	79,000 plant ha^{-1} 0	79,000 plant ha^{-1} 67	79,000 plant ha^{-1} 252	110,000 plant ha^{-1} 0	110,000 plant ha^{-1} 67
plant ha^{-1}	kg N 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*

* Significant at $P \leq 0.10$.
**Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† NS, not significant

Table 7. Plant density and N fertilizer effects on yield stability (*b*-value) of 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	No.	0	67	252	0	67	252
		Yield response to low N vs. 0 N at 79,000 plants ha^{-1}					
1A†	26	0.98	1.03	0.97	0.96	1.06	1.03
2A	4	1.01	0.80	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 ($P \leq 0.05$)‡		NS	0.18	0.68	NS	0.25	0.56
		Yield response to increased density vs. to high N					
1B	17	1.05	1.08	1.36	1.10	1.10	1.14
2B	17	0.99	1.02	1.05	0.96	1.04	1.10
3B	12	0.94	0.99	0.97	0.91	1.00	0.84
4B	16	1.00	0.98	0.77	0.96	0.95	0.93
LSD ($P \leq 0.05$)‡		0.06	NS	0.45	0.10	NS	NS
Variance§		0.01b	0.03b	0.41a	0.02b	0.06b	0.27a

† Hybrid groups: 1A (hybrids with below average yield under unfertilized conditions and above average yield response to low N), 2A (hybrids with above average yield both unfertilized conditions and in response to low N), 3A (hybrids with below average yield under unfertilized conditions and in response to low N), 4A (hybrids with above average yield under unfertilized conditions and below average yield response to low N), 1B (hybrids with below average yield response to high N and above average response to increased plant density), 2B (hybrids with above average yield responses to both high N and increased plant density), 3B (hybrids with below average yield response to both high N and plant density), and 4B (hybrids with above average yield response to high N and below average response to increased plant density).

‡ Least square difference for yield stability between hybrids within N and plant density treatments ($P \leq 0.05$); NS, Nonsignificant.

§ Within a plant density level, variances followed by the same letter are not significant different according to the Brown–Forsythe method ($P \leq 0.05$).

yield stability (smaller b-value) than groups 1A, 3A and 4A at 67 kg N ha^{-1} under both plant densities and higher yield stability than group 4A at 252 kg N ha^{-1} at standard plant density. Yield stability of 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 that this stability is plant density-independent. On the other hand, hybrid group 2B (hybrids with above-average yield response to both high N and increased plant density) exhibited average yield stability across N and plant density treatments. Hybrids with above-average response to high N and below-average response to increased plant density (group 4B) had higher yield stability than did hybrids with below-average yield response to high N and above-average response to increased plant density (group 1B).

In previous studies, reduced response to selection for yield at high plant density has been attributed 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 across time has increased stand uniformity and reduced plant-to-plant variability. In addition, current maize hybrids appeared to be more density-dependent, exhibit reduced barrenness, and are mostly single-ear hybrids. Hybrids with a large yield response to high N fertilizer rates and that are also plant density-independent (produce less-than-average yield decreases from 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 7). Under high N conditions, yield stability variance was notably higher under standard plant density than under the high plant density condition. Possibly, the plasticity of the typically greater number of kernels per plant and kernel weight produced at lower densities led to this higher yield stability variance (Boomsma et al., 2009).

CONCLUSIONS

Current elite maize hybrids expressed large genetic variation for yield in tolerance to N deficiency, 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 with a hybrid's ability to tolerate high plant density conditions. Selecting hybrids with above-average yield performance under both unfertilized and low N conditions may be challenging in a maize breeding program, as less than 10% of the hybrids evaluated in this study were characterized as such. These hybrids achieved greater than average yield stability under high-yield environments. Although hybrids with an above-average yield response to high N fertilizer supply and increased plant density produced higher yields, these hybrids also had larger-than-average 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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References

Agrama, H.A.S., A.G. Zakaria, F.B. Said, and M. Tuinstra. 1999. Identification of quantitative trait loci for nitrogen use efficiency in maize. Mol. Breed. 5:187–195. doi:10.1023/A:1009669507144

Bänziger, M., F.J. Betrán, and H.R. Lafitte. 1997. Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Sci. 37:1103–1109. doi:10.2135/cropsci1997.0011183X003700040012x

Bänziger, M., and M. Cooper. 2001. Breeding for low input conditions and consequences for participatory plant breeding: Examples from tropical maize and wheat. Euphytica 122:503–519. doi:10.1023/A:1017510928038

Below, F.E., L.E. Christensen, A.J. Reed, and R.H. Hageman. 1981. Availability of reduced-N and carbohydrates for ear development of maize. Plant Physiol. 68:1186–1190. doi:10.1104/pp.68.5.1186

Below, F.E., J.R. Seebauer, M. Uribelarrea, M.C. Schneerman, and S.P. Moose. 2004. Physiological changes accompanying long-term selection for grain protein in maize. In: J. Janick, editor, Plant breeding reviews. Vol. 1. John Wiley & Sons Inc., Hoboken, NJ. p. 133–151.

Bender, R.R., J.W. Haegele, M.L. Ruffo, and F.E. Below. 2013. Nutrient uptake, partitioning, and remobilization in modern, transgenic insect-protected maize hybrids. Agron. J. 105:161–170. doi:10.2134/agronj2012.0352

Bertin, P., and A. Gallais. 2001. Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines. II. QTL detection and coincidences. Maydica 46:53–68.

Boomsma, C.R., J.B. Santini, M. Tollenaar, and T.J. Vyn. 2009. Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review. Agron. J. 101:1426–1452. doi:10.2134/agronj2009.0082

Borrás, L., G.A. Maddonni, and M.E. Otegui. 2003. Leaf senescence in maize hybrids: Plant population, row spacing and kernel set effects. Field Crops Res. 82:13–26. doi:10.1016/S0378-4290(03)00002-9

Brown, M.B., and A.B. Forsythe. 1974. Robust tests for the equality of variances. J. Am. Stat. Assoc. 69:364–367. doi:10.1080/01621459.1974.10482955

Brun, E.L., and J.W. Dudley. 1989. Nitrogen responses in the USA and Argentina of corn populations with different proportions of ñint and dent germplasm. Crop Sci. 29:565–569. doi:10.2135/cropsci1989.0011183X002900030003x

Chen, K., J.J. Camberato, M.R. Tuinstra, S.V. Kumudini, M. Tollenaar, and T.J. Vyn. 2016. Genetic improvement in density and nitrogen stress tolerance traits over 38 years of commercial maize hybrids release. Field Crops Res. 196:438–451. doi:10.1016/j.fcr.2016.07.025

Ciampitti, I.A., S.T. Murrell, J.J. Camberato, M. Tuinstra, Y. Xia, P. Friedemann, and T.J. Vyn. 2013. Physiological dynamics of maize nitrogen uptake and partitioning in response to plant density and nitrogen stress factors: II. Reproductive phase. Crop Sci. 53:2588–2602. doi:10.2135/cropsci2013.01.0041

Coque, M., and A. Gallais. 2007. Genetic variation among European maize varieties for nitrogen use efficiency under low and high nitrogen fertilization. Maydica 52:383–397.

de Leon, N., and J.G. Coors. 2002. Twenty-four cycles of mass selection for prolificacy in the Golden Glow maize population. Crop Sci. 42:325–333. doi:10.2135/cropsci2002.0325

Ding, L., K.J. Wang, G.M. Jiang, D.K. Biswas, H. Xu, L.F. Li, and Y.H. Li. 2005. Effects of nitrogen deficiency on photosynthetic traits of maize hybrids released in different years. Ann. Bot. (Lond.) 96:925–930. doi:10.1093/aob/mci244

Duvick, D.N. 1977. Genetic rates of gain in hybrid maize yields during past 40 years. Maydica 22:187–196.

Duvick, D.N. 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). In: D.L. Sparks, editor, Advances in agronomy. Vol. 86. Elsevier Academic Press, Inc., San Diego, CA. p. 83–145. doi:10.1016/S0065-2113(05)86002-X

Egli, D.B. 2008. Comparison of corn and soybean yields in the United States: Historical trends and future prospects. Agron. J. 100:S79–S88. doi:10.2134/agronj2006.0286c

Fasoula, V.A., and M. Tollenaar. 2005. The impact of plant population density on crop yield and response to selection in maize. Maydica 50:39–48.

Finlay, K.W., and G.N. Wilkinson. 1963. Analysis of adaptation in a plant breeding programme. Aust. J. Agric. Res. 14:742–754. doi:10.1071/AR9630742

Gallais, A., M. Coque, and P. Bertin. 2008. Response to selection of a maize population for adaptation to high or low nitrogen fertilization. Maydica 53:21–28.

Haegele, J.W., K.A. Cook, D.M. Nichols, and F.E. Below. 2013. Changes in nitrogen use traits associated with genetic improvement for grain yield of maize hybrids released in different decades. Crop Sci. 53:1256–1268. doi:10.2135/cropsci2012.07.0429

Hallauer, A.R., and J.H. Sears. 1969. Mass selection for yield in 2 varieties of maize. Crop Sci. 9:47–50. doi:10.2135/cropsci1969.0011183X000900010016x

Head, G.P., M.W. Carroll, S.P. Evans, D.M. Rule, A.R. Willse, T.L. Clark, N.P. Storer, R.D. Flannagan, L.W. Samuel, and L.J. Meinke. 2017. Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm: Efficacy and resistance management. Pest Manag. Sci. 73:1883–1899. doi:10.1002/ps.4554

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martínez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breed. Rev. 22:9–112.

Moll, R.H., E.J. Kamprath, and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 74:562–564. doi:10.2134/agronj1982.00021962007400030037x

National Corn Growers Association. 2015. National corn yield contest. NCGA, Chesterfeld, MO. www.ncga.com/for-farmers/national-corn-yield-contest (accessed 01 Aug. 2016).

O'Neill, P.M., J.F. Shanahan, J.S. Schepers, and B. Caldwell. 2004. Agronomic responses of corn hybrids from different eras to deficit and adequate levels of water and nitrogen. Agron. J. 96:1660–1667. doi:10.2134/agronj2004.1660

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1997. How a corn plant develops. Spec. Rep. No. 48. Iowa State University of Science and Technology Cooperate Extension Service, Ames, IA.

Ruffo, M.L., L.F. Gentry, A.S. Henninger, J.R. Seebauer, and F.E. Below. 2015. Evaluating management factor contributions to reduce corn yield gaps. Agron. J. 107:495–505. doi:10.2134/agronj14.0355

Sadras, V.O., and G.A. Slafer. 2012. Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. Field Crops Res. 127:215–224. doi:10.1016/j.fcr.2011.11.014

Sarlangue, T., F.H. Andrade, P.A. Calviño, and L.C. Purcell. 2007. Why do maize hybrids respond differently to variations in plant density? Agron. J. 99:984–991. doi:10.2134/agronj2006.0205

SAS Institute. 2013. SAS 9.4 user's guide. SAS Institute, Cary, NC.

Simmonds, N.W. 1995. The relation between yield and protein in cereal grains. J. Sci. Food Agric. 67:309–315. doi:10.1002/jsfa.2740670306

Tokatlidis, I.S., and S.D. Koutroubas. 2004. A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. Field Crops Res. 88:103–114. doi:10.1016/j.fcr.2003.11.013

Tollenaar, M., A. Aguilera, and S.P. Nissanka. 1997. Grain yield is reduced more by weed interference in an old than in a new maize hybrid. Agron. J. 89:239–246. doi:10.2134/agronj1997.00021962008900020014x

Tollenaar, M., L.M. Dwyer, and D.W. Stewart. 1992. Ear and kernel formation in maize hybrids representing three decades of grain yield improvement in Ontario. Agron. J. 32:432–438.

Tollenaar, M., and E.A. Lee. 2002. Yield potential, yield stability and stress tolerance in maize. Field Crops Res. 75:161–169. doi:10.1016/S0378-4290(02)00024-2

Tollenaar, M., and J. Wu. 1999. Yield improvement in temperate maize is attributable to greater stress tolerance. Crop Sci. 39:1597–1604. doi:10.2135/cropsci1999.3961597x

Uribelarrea, M., S.P. Moose, and F.E. Below. 2007. Divergent selection for grain protein affects nitrogen use in maize hybrids. Field Crops Res. 100:82–90. doi:10.1016/j.fcr.2006.05.008

USDA National Agricultural Statistics Service. 2017. Quickstats. USDA-NASS, Washington, DC. https://quickstats.nass.usda.gov/results/6AC0CC3C–B523–3E72–B299–BDB1AA1981DE (accessed 2 May 2017).