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Estimation of quantitative genetic and stability parameters in maize under high and low N levels

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Abstract

It is important to breed maize (*Zea mays* L) cultivars with high performance under variable N levels. We studied the effect of N levels and estimated quantitative genetic parameters for grain yield, quality, and other traits, and examined stability of performance for grain yield in diverse Chinese maize germplasm. From 2006 to 2008, each year 20 and in total 30 maize hybrids, including commercial hybrids currently grown in this region and other experimental hybrids as well as high-oil hybrids, were tested using nine environments (location-year combinations) in North China Plain. In each environment, two replicated trials were grown: one under high N application rate (HN, 225 kg N ha^{-1}) and the other under low N application rate (LN, no N fertilization). Compared to HN, grain yield was significantly reduced (35.6%) under LN level, as well as kernel number per ear, 1000-kernel weight, plant and ear heights, and protein concentration. In the analysis over environments under each N level, genotypic variance was significant and heritability was high for all traits. In the analyses across N levels and environments, genotypic variance was significant for all traits and larger than the genotype × N and/or environment interaction variance components except for protein concentration. In stability analyses across N levels, hybrids differed for their linear response to environments, and some showed dissimilar response under HN and LN levels. Our results indicated that breeding maize adapted to variable N levels is feasible with the Chinese germplasm available in the summer breeding programs in North China Plain. Multi-environment tests are required to identify hybrids with high grain yield under variable N conditions, and examining yield stability separately under HN and LN would be useful.

Keywords: maize, nitrogen, grain yield, quantitative genetic parameters, stability

Abbreviations: EH: Ear height, GE: Genotype × environment, h^2: Heritability (broad-sense), KDMC: kernel dry matter percentage, KNE: kernel number per ear, N: nitrogen, NIRS: near infrared reflectance spectroscopy, PH: plant height, r_G: genotypic correlation, RDN: relative difference between LN and HN levels, SR: shelling percentage, TKW: 1000-kernel weight, σ^2_G: genotypic variance, σ^2GE: genotype × environment interaction variance, σ^2_{GN}: genotype × nitrogen interaction variance, $\sigma2_{GNE}$: genotype × nitrogen × environment interaction variance

Introduction

Combined with genetic improvement, the use of N fertilizer and other cultural improvement have contributed to the increase of maize grain yield (Duvick, 2005). However, besides increasing the cost of cultivation, high N fertilization has adverse effects on the atmosphere, ground water, and other components of the ecosystems (Socolow, 1999; Bowman et al, 2008). Further, in many tropical and subtropical regions, the availability of N fertilizers is often limited. Thus, maize is cultivated under very high to low levels of N fertilizer application. The acreage under maize cultivation in China has rapidly increased during the last 2 decades. Maize now occupies around 30 million ha and has become the most important crop. The North China Plain, located in the north-eastern part of China, is one of the most important areas of maize production. It contributes about one-third of maize to the national production. Intensive winter wheat (*Triticum aestivum* L) - summer maize cropping system (two crops in one year) is practiced in this region and average maize grain yield of 8.5 Mg/ha has been reported across 148 sites in this region (Cui et al, 2008). To obtain high yields, an excessive dose of N fertilizer is being applied (Gao et al, 1999; Ju et al 2009), leading to severe environmental pollutions besides very low N use efficiency (Zhang et al, 1996; Li et al, 1999; Liu and Diamond, 2008). But there are still pockets in this region and large areas in the rest of China and other countries, where maize is grown under conditions that N is applied at below-optimal levels because of high cost relative to economic returns or other reasons (Lafitte and Edmeades, 1994). In view of environmental and economical considerations, it is

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important to breed varieties with high agronomic performance under variable N levels.

The performance and genetic variability for grain yield and other traits under different levels of N has been investigated using diverse maize germplasm that mainly belonged to U.S. Corn belt, European, tropical and subtropical regions (Duvick, 1977, 1984a, b; Castleberry et al, 1984; Brun and Dudley, 1989; Smiciklas and Below, 1990; Russell, 1991; Lafitte and Edmeades, 1994; Bänziger et al, 1997; Akintoye et al, 1999; Bertin and Gallais, 2000; Presterl et al, 2002, 2003; Duvick et al, 2004). With more than 50 years' effort, Chinese breeders have established the heterotic groups (Li, 2009) and there are two distinctive domestic germplams groups, Tangsipingtou and Luda Red Cob, which are intensively used in different breeding programs (Xie et al, 2008). There are many researches in China working on N management, physiological and biochemical basis of N use efficiency, few information is available about genetic analysis using Chinese germplasm (Liu et al, 2008; Wu et al, 2011). And there is little information using different hybrids containing elite germplasm in this region tested under different year-location environments across high and low N levels to explore variance components of different effects, which is important for the breeders to designing the breeding programs.

Maize grains are used mostly as feed for livestock and poultry across the world and also as human food. Thus, nutritional quality traits are also important and it is worthwhile to investigate the influence of N fertilization on these traits. Grain yield has been reported to be negatively correlated with grain oil and protein concentrations, and positively with grain starch concentration (Wassom et al, 2008). Thus, breeding for high yield tended to decrease oil and protein concentrations (Feil et al, 2005). Nevertheless, these relationships were mainly worked out under a given N level. In addition, nutritionally enhanced germplasm, such as high-oil hybrids, have received little attention. Up to now, it is not clear that if it is necessary to evaluate grain oil concentration under different N levels when breeders want to breed high oil hybrid targeting for high and low N levels

Genotype × environment (GE) interaction is a common phenomenon when different genotypes are evaluated across diverse environments. Its presence reduces heritability and complicates the selection of superior genotypes with stable performance across environments. Various approaches have been proposed to define and measure stability of performance, and the most common among these is that of Finlay and Wilkinson (1963) and Eberhart and Russell (1966). In this approach mean performance, linear regression of genotypes on an environmental index, and deviation mean squares are used as stability parameters. Information on the stability of performance under high and low N levels in multi-environment tests is expected to be helpful in better understanding the genotypic performance and identification of desirable genotypes under variable N levels.

The objectives of our study were to (1) investigate the effect of N levels on grain yield, other agronomic, and grain quality traits, (2) estimate variance components, heritability, correlations between performance under high and low N levels, and correlations among various traits, and (3) examine the stability of performance for grain yield under variable N levels.

Materials and Methods

Plant materials

The experiment was conducted during 2006, 2007 and 2008. Each year 20 single-cross hybrids were evaluated. Of the hybrids tested during 2006, 10 hybrids taken at random were replaced in 2007 with an equal number of new hybrids; and these were tested during 2007 and 2008. Thus, in total 30 hybrids were tested (supplementary Table 1). The tested hybrids included 14 experimental and nine commercial hybrids having normal grain oil concentration and seven high oil experimental hybrids. The same set of material has also been used to investigate the N effect on stalk quality (Li et al, 2010).

Experimental design of field trials

The experiments were conducted at four experimental stations (Quzhou, Wuqiao, Shangzhuang, and Tianjin) of the China Agricultural University. During 2007 and 2008, the experiments were laid at all four stations but data could not be recorded at Tianjin in 2008 due to extremely severe lodging. During 2006, the experiments were performed at two locations (Quzhou and Wuqiao) only. Thus, we had nine location-year combination environments (we use the term environment qualified by N level(s) for location-year and location-year-N level combinations, and the term is also explained wherever needed) for each N level. These research stations are representative of North China Plain: Quzhou (36°46'N, 114°57'E, altitude 39.6 m, long-term average rainfall 791.7 mm), Wuqiao (37°18'N, 116°24'E, altitude 18 m, long-term average rainfall 562.5 mm), Shangzhuang (39°59'N, 116°17'E, altitude 43.5 m, long-term average rainfall 576.9 mm) and Tianjin (39°94'N, 117°23'E, altitude 5.0 m, long-term average rainfall 613 mm).

In all nine environments, both high N (HN) and low N (LN) level trials were grown side-by-side on the same field. Each trial was laid out in completely randomized block design with three replications. The LN fields were N-depleted for 2-yr through winter wheat-summer maize cropping system to which no N was applied and crop residues were removed after harvest. No N fertilizer was applied to the LN trial and whereas the HN received 225 kg ha^{-1} N in terms of Urea. N fertilization was splited into 2 times, before sowing and 4-5 weeks after sowing. All other standard crop cultivation practices (such as application of other fertilizers, herbicide and irrigation) in this region were followed. A plot had four rows, each of 5-m length. The row-to-row distance was 0.60 m and plant-to-plant within rows, 0.25 m. Three seeds were sown per hill and the plots were thinned to one seedling/hill at the stage V3 (Ritchie and Hanway, 1982). Final plant density was about 66 666 plants/ha, which is the normal density in this region.

Trait evaluation

Plant height (PH) and ear height (EH) were measured on 10 plants in each plot, 10 days after completion of male flowering. The two middle rows in each plot were hand-harvested at physiological maturity depending on the milk line following the farmers' practice, and fresh ear weight was recorded. A sample of 10 ears in each plot was threshed by hand and the kernels and cobs were weighed separately to determine shelling percentage (SR) as a ratio of kernel fresh weight vs. ear fresh weight. A sample of 400 g kernels was oven dried to a constant weight at 105 C to determine kernel dry matter percentage (KDMC). KDMC was calculated as: KDMC = kernel dry weight without moisture/kernel wet weight*100%. One thousand dried kernels were counted and weighed, and 1,000-kernel weight (TKW) is presented on dry weight basis. Kernel number per ear (KNE) was determined by the average fresh kernel weight per ear measured from 10 fresh ears, TKW and KDMC. Based on fresh ear weight, SR, and KDMC, the grain yield in Mg/ha was computed and adjusted to 15.5% grain moisture content. Relative difference between LN and HN levels (RDN) was used to approximately estimate the responsiveness of each trait of each genotype in each environment from LN to HN levels. RDN was computed as: $RDN = (HN_{mean}-LN_{mean})/LN_{mean}$, where HN_{mean} and LN_{mean} were the average performance under HN and LN levels, respectively, in each environment.

To avoid xenia effects on the grain quality traits, three to five ears in the border rows in each plot were self-pollinated. These ears were harvested at the same time as the two middle rows, and were air-dried. Kernels from the middle part of the each ear were sampled and used to determine the grain quality traits, namely crude protein, oil, and starch concentrations following the method of Wei et al (2005). Each sample was scanned twice with near infrared reflectance spectroscopy (NIRS). By employing this method, Wei et al (2005) had obtained as high correlation coefficients as 0.97, 0.98, and 0.93 between the predicted and actual oil, protein, and starch concentrations, respectively.

Statistical analysis

The tested hybrids included experimental and commercial (a random sample of those which were widely cultivated during the last two decades) hybrids with normal and high grain-oil concentration. The parents of the hybrids belonged to important heterotic groups including Reid Yellow Dent and Lancaster from the USA and the domestic Chinese groups Tangsipingtou and Lvdahonggu, as well as to some tropical germplasm. The hybrids were considered as a random sample of elite germplasm being used in maize breeding programs in North China Plain. This is in accordance with Piepho et al (2008), who discussed the assumption of considering a sample of genotypes, which have undergone substantial selection effort, and concluded that it is reasonable to regard the genotypes as a random sample from some hypothetical population of genotypes.

Plot averages, computed for the traits recorded on individual plants or ears, were used for the statistical analysis. Variance components were computed using PROC MIXED in SAS (Version 9.1, SAS Institute, 2004), with the following model:

$$Y_{ijkl} = \mu + G_i + N_j + E_k + GN_{ij} + NE_{jk} + GE_{ik} + GNE_{ijk} + R(NE)_{jkl} + \sigma_{ijkl}$$

Where, Y_{ijkl} was the performance of i^{th} genotype under j^{th} N level in k^{th} environment (location-year combination) in the l^{th} replication; μ was the overall population mean; G_i, the effect of i^{th} genotype; N_j, the effect of j^{th} N level; E_k the effect of k^{th} environment; GN_{ij}, the effect of genotype × N interaction; NE_{jk}, the effect of N × environment interaction; GE_{ik}, the effect of genotype × environment interaction; GNE_{ijk}, the effect of genotype × N × environment interaction; $R(NE)_{jkl}$ the effect of the l^{th} replication in the k^{th} environment and j^{th} N level; σ_{ijkl} is the error. In the model, G_i, GN_{ij}, GE_{ik}, GNE_{ijk}, and error effects were considered as random and others as fixed effects. Broad-sense heritability (h^2) on plot mean basis under each N level was estimated following Hallauer and Miranda (1981) as:

$$h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_{GE/l} + \sigma^2_{\varepsilon/lr}).$$

Where σ^2_G is the estimate of genotypic variance, σ^2_{GE} is the estimate of genotype × environment interaction variance; $\sigma^2\varepsilon$ is the estimate of error variance; l is the number of environments (location-year combinations), and r is the number of replications in each trial. Phenotypic correlation coefficients (r_P) were computed among traits using entry means across environments under both N levels by applying and adjusting a SAS Macro developed by Holland (2006).

For the stability analysis, a balanced data set of 20 hybrids tested in 14 environments comprising two N levels at seven location-year combinations (four locations during 2007 and three during 2008) were used. The analysis was carried out following Eberhart and Russell (1966) and stability parameters namely mean performance and linear response b_i, were estimated. The analysis was performed using PLABSTAT (Utz, 1993), basing on the mean values of three replications of each genotype in each location.

Results

Mean performances under HN and LN

Average grain yield was significantly ($P = 0.05$) lower under LN than HN level, reduction being 35.6%, and the LN/HN ratio (0.64) was the smallest among the investigated traits (Table 1). In the nine location-year environments, the reduction ranged from 20.9 to

Table 1 - Estimates of mean and variance components (genotypic variance, σ^2_G; genotype × N level interaction variance, σ^2_{GN}; genotype × environment interaction variance, σ^2_{GE}; genotype × N level × environment interaction variance, σ^2_{GNE}; error variance, $\sigma^2\varepsilon$), genotypic coefficient of variation (CV_G), and broad-sense heritability (h^2) of different traits across nine high N (HN) and nine low N (LN), and 18 across N levels environments based on 30 hybrids.

		Traits†									
Analysis/ Estimates	N level	GY (Mg/ha)	SR	KDMC (%)	TKW (g)	KNE (no.)	PH (cm)	EH (cm)	Protein (%)	Oil (%)	Starch (%)
Analysis across nine environments (location-year combinations) under each N level											
Mean	HN	9.71	0.75	66.28	275.2	463.4	269.0	119.6	9.78	4.96	69.99
	LN	6.25	0.74	65.44	246.4	337.7	241.3	102.7	8.66	4.93	70.75
	LN/HN	0.64	0.99	0.99	0.90	0.73	0.90	0.86	0.89	0.99	1.01
σ^2_G	HN	0.35**	7.49**	3.74**	622.5**	1412.5**	298.6**	85.2**	0.07**	1.08**	1.18**
	LN	0.14**	7.93**	4.46**	647.4**	894.9**	279.0**	84.8**	0.07**	1.02**	1.63**
σ^2_{GE}	HN	0.22**	0.74	1.65	216.5**	686.8**	273.8**	47.5**	0.10**	0.31**	0.38**
	LN	0.15**	0.96	2.72	108.0**	290.9**	21.9**	14.5**	0.11**	0.26**	0.41**
σ^2_ε	HN	0.79	1.59	2.75	554.3	2170.8	1029.0	350.1	0.32	0.31	1.37
	LN	0.66	2.08	3.81	510.3	2947.0	281.2	69.6	0.36	0.35	1.29
CV_G (%)	HN	6.09	3.65	2.92	9.07	8.11	6.42	7.72	2.70	20.95	1.55
	LN	5.99	3.80	3.23	10.32	8.86	6.92	8.96	3.06	20.49	1.80
h^2	HN	0.87	0.98	0.93	0.93	0.90	0.81	0.82	0.74	0.96	0.93
	LN	0.77	0.98	0.91	0.95	0.86	0.96	0.95	0.73	0.96	0.95
Analysis across 18 environments (location-year-N level combinations)											
σ^2_G	HN+LN	0.19**	7.61**	4.07**	621.4**	984.6**	351.14**	82.46**	0.06**	1.05**	1.42**
σ^2_{GN}	HN+LN	0.11**	0.57	1.34	60.8**	360.2**	11.10*	17.38**	0.013	0.00	0.00
σ^2_{GE}	HN+LN	0.06**	0.12	0.05	14.9**	191.0**	3.70*	6.72**	0.056**	0.288**	0.258**
$\sigma 2_{GNE}$	HN+LN	0.07**	0.28	0.80	55.5**	132.9*	6.90*	0.00	0.095**	0.00	0.279**

*significance at P< 0.05; **significance at P< 0.01 level; †GY, grain yield; SR, shelling percentage; KDMC, kernel dry matter percentage; TKW, 1000 kernel weight; KNE, kernel number per ear; PH, plant height; EH, ear height; Protein, grain protein concentration; Oil, grain oil concentration; Starch, grain starch concentration

54.5% (data not shown). There was also significant reduction for KNE, TKW, PH, and EH under LN level, whereas KDMC and SR did not differ significantly between the two N levels. For grain quality traits, only crude protein concentration showed significant difference (11.6%) between the two N levels. Grain oil concentration of high-oil hybrids was significantly higher than for normal hybrids under both N levels (Table 2). However, no significant differences were observed for high-oil vs. normal hybrids with respect to their response to N levels for all traits (data not shown).

Variance components analysis

Under both N levels, estimates of σ^2_G and σ^2_{GE} were highly significant ($P < 0.01$) for all traits except that σ^2_{GE} for SR and KDMC were not significant (Table 1). The magnitude of σ^2_G was distinctly larger under HN than LN level for grain yield (2.5 times) and KNE, the two traits showing lowest LN/HN ratios. For other traits, the estimates generally had comparable magnitude. The estimates of σ^2_{GE}, whenever significant, were larger under HN than LN level for grain yield and other agronomic traits but comparable for quality traits. Estimates of CV_G (coefficient of variation of genotypic variance) were of similar magnitude for all traits under both N levels. Estimates of h^2 were higher under HN than LN level for grain yield, lower for PH and EH, and similar for the other traits.

In the combined analysis of variance across 18 environments (location-year-N level combinations), the estimates of σ^2_G were highly significant ($P < 0.01$) for all traits (Table 1). The variance components (σ^2_{GN}, σ^2_{GE}, σ^2_{GNE}) were generally significant ($P < 0.05$) for various traits except SR and KDMC. Estimates of σ^2_G were larger than all interaction variance components for all traits except grain protein concentration for which the estimate of σ^2_{GNE} was the largest. Among

Table 2 - Compararative mean performance of seven high-oil vs. 23 normal hybrids for grain oil concentration, grain protein concentration, and grain yield under high N (HN) and low N (LN) levels across environments.

	Oil (%)		Protein (%)		Grain yield (Mg/ha)	
N level	High-oil(7)†	Normal(23)	High-oil(7)	Normal(23)	High-oil(7)	Normal(23)
HN	6.71^{a‡}	4.44^a	9.94^a	9.80^a	9.52^a	9.71^a
LN	6.67^a	4.42^a	8.91^b	8.60^b	6.08^b	6.30^b

†seven hybrids with high grain oil concentration, and 23 hybrids with normal grain oil concentration; ‡values of each column followed by different letters indicate significance at P<0.05, and same letter indicate no significant difference

Table 3 - Phenotypic correlation coefficients (r_P) among various traits† under nine high N (HN, above diagonal) and nine low N (LN, below diagonal) environments, and r_P between HN and LN (diagonal, underlined with bold face) levels for various traits and between RDN for grain yied vs. other traits based on mean performance of 30 hybrids.

	GY	SR	KDMC	TKW	KNE	PH	EH	Protein	Oil	Starch
GY	0.472**	0.479**	0.399**	0.330**	0.401**	0.197*	0.116*	-0.122	-0.144*	0.410**
SR	0.705**	0.886**	0.303*	0.020	0.317**	-0.055	-0.008	-0.244**	-0.183*	0.336**
KDMC	0.572**	0.411**	0.891**	0.083	-0.076	-0.082	-0.145*	-0.069	-0.296**	0.369**
TKW	0.208*	0.030	0.198*	0.783**	-0.429**	-0.077	-0.188*	-0.069	-0.419**	0.600**
KNE	0.501**	0.344**	0.080	-0.397**	0.566**	0.275**	0.230**	-0.019	0.272*	-0.154*
PH	0.141*	-0.153*	-0.102	-0.121	0.204*	0.848**	0.521**	0.151	0.369**	-0.322**
EH	0.121*	-0.124*	-0.239*	-0.292*	0.300**	0.671**	0.702**	0.042	0.304**	-0.273**
Protein	-0.106	-0.250**	-0.162*	-0.126	0.043	0.163*	0.085	0.554**	0.133*	-0.646**
Oil	-0.185*	-0.220**	-0.340**	-0.359**	0.136	0.443**	0.458**	0.212*	0.952**	-0.605**
Starch	0.630**	0.306*	0.401**	0.357*	-0.159	-0.400*	-0.361**	-0.593**	-0.637**	0.819**
RDN‡										
GY vs. other traits		0.569**	0.185*	0.437**	0.832**	0.613**	0.618**	0.388**	0.131	-0.111

*significance at $P< 0.05$ level; **significance at $P< 0.01$ level;†GY, grain yield; SR, shelling percentage; KDMC, kernel dry matter percentage; TKW, 1000 kernel weight; KNE, kernel number per ear; PH, plant height; EH, ear height; Protein, grain protein concentration; Oil, grain oil concentration; Starch, grain starch concentration; ‡RDN, relative difference between LN and HN levels. $RDN= (HN_{mean}-LN_{mean})/LN_{mean}$, where HN_{mean} and LN_{mean} were the average performance under HN and LN levels in each environment

interactions variance components, estimates of σ^2_{GN}, whenever significant, were the largest. The ratio $\sigma^2_{GN}/\sigma^2_{G}$. was about 0.6 for grain yield and smaller for the other traits.

Correlation analysis among traits under as well as between both N levels

The sign of significant correlation r_P among traits was generally consistent between two N levels (Table 3). The estimates were either positive under both N levels, or negative, though, there were some instances where the correlations were significant under one level but not the other. All such not-significant estimates except one were observed under HN level. These were most prevalent for KDMC and were followed by protein and oil concentrations and then GY. Further, in majority of the cases the magnitude of r_P was numerically larger at LN level than HN. It involved all traits and was less prevalent for GY, TKW, and KNE but most prevalent for KDMC and EH (KDMC and EH vs. all traits except one). Grain yield showed positive correlation (r_P) with yield components (SR, TKWD, KNE) and the component traits were positively correlated among themselves in both N levels (Table 3). Further, grain yield was positively correlated with KDMC under the LN level.

Grain yield was generally negatively correlated with protein and oil, and positively with starch concentration under both N levels. Among quality traits, protein and oil concentrations were positively correlated and showed a strong negative correlation with starch concentration. Plant and ear heights were generally positively correlated with KNE, protein, and oil concentrations, but negatively with TKW and starch concentration. For all traits the r_P between performances under HN and LN levels were highly significant. It was lowest for grain yield (0.47), was followed by KNE and protein (≈0.6), and the highest for Oil concentration (0.95). The RDN of grain yield was significantly ($P< 0.01$) correlated with other traits except for KDMC, grain oil and starch concentrations, and mostly closely correlated with KNE (0.83), followed by PH, EH, SR and TKW.

Stability analysis of grain yield

The hybrids evaluated in 2007 and 2008 were grouped on the basis of their mean performance under LN and HN levels (supplementary Figure 1). Group 1 (Quadrant IV) comprised the hybrids that performed relatively better under LN but poorer under HN level, i.e., efficient but non-responsive for N utilization; group 2 (Quadrant I), the hybrids that performed relatively better under both N levels, i.e., efficient and responsive; group 3 (Quadrant II), the hybrids that performed relatively poorer under LN but better under HN level, i.e. inefficient but responsive; and group 4 (Quadrant III), the hybrids that performed relatively poorer under both N levels, i.e., inefficient and non-responsive. Most of the hybrids belonged to group 2 (efficient and responsive) and group 4 (inefficient and non-responsive). Some high-oil hybrids performed as inefficient but responsive for N utilization (group 3).

Stability analysis across N levels of grain yield showed significant variation due to heterogeneity of linear regression b_i of 20 hybrids across 14 environments (location-year-N level combinations) (analysis of variance not shown). Mean squares due to deviations from regression were not significant. Thus, the response of hybrids to environments was linear. Hybrids H20 and H12 with the highest b_i values (1.31-1.16) also possessed high yield (8.50-8.78 Mg/ha), and four other high yielding (≥8.50 Mg/ha) hybrids, H01, H05, H17, and H18, had average b_is (1.00-1.09) (Table 4). Hybrid H13 had low b_i (0.91) in combination with yield as high as 8.48 Mg/ha. Other hybrids (H02, H03, H04, H06, H11) with low b_is (0.79-0.88), however, had low yields (<8.15 Mg/ha).

Table 4 - Mean performance and linear response b_i for grain yield of selected hybrids showing variation of these parameters in the stability analysis across 14 environments (location-year-N level combinations).

Hybrid	Mean		b_i	
	(Mg/ha)	HN$^{\#}$	HN+LN§	CI‡
H01	8.78	0.96	1.03	0.84-1.23
H02	7.42	0.72	0.82	0.70-0.94
H03	8.15	0.80	0.86	0.67-1.04
H04	7.39	1.11	0.79	0.60-0.98
H05	8.50	1.18	1.09	0.94-1.23
H06	8.11	0.65	0.85	0.63-1.08
H10	7.40	1.18	1.06	0.95-1.16
H11	7.45	0.85	0.88	0.72-1.03
H12	8.50	1.15	1.16	1.03-1.30
H13	8.48	0.71	0.91	0.69-1.12
H17	8.75	1.05	1.09	0.92-1.27
H18	8.85	0.97	1.00	0.89-1.12
H20	8.78	1.32† (0.67)	1.31	1.13-1.48
Range†				
Minimum	7.39 (H04)	0.65 (H06)	0.79 (H04)	0.60-0.98
Maximum	8.86 (H18)	1.37 (H16)	1.31 (V20)	1.13-1.48

$^{\#}b_i$ across the seven high N (HN) level environments; $^{\S}b_i$ across the 14 environments (location-year-N level combinations, HN+LN); †the hybrids showing extreme values of deviation mean square are mentioned in the parentheses; ‡corresponding 95% confidence interval of the bi across the 14 environments

Discussion

Maize (*Zea mays* L) plays an increasingly important role for food, feed and bio-energy production worldwide. To maintain the high grain yield production, high amount of N has been used. However, there is the demand to decrease of N supply in maize production because of the increasing N fertilizer cost as well as the environmental contamination. Therefore, breeding hybrids with high nitrogen use efficiency is the most economical and effective approach to meet this purpose (Below and Uribelarrea, 2006).

To breed for hybrids under variable N levels, especially under LN level, it is very necessary to get the genetic parameters information, such as N responsiveness, variance components and correlations. However, there is little information about the genetic parameters using Chinese germplasm evaluated under different year-location environments across different N levels. Because of this reason, we used different commercial and experimental hybrids adapted in North China Plain and evaluated them under HN and LN levels. In this study, to increase the sample size of hybrids, we randomly changed 10 new hybrids in 2007. Thus, in total 30 maize hybrids were tested under HN and LN levels across 9 environments (year-location combinations) in North China Plain. N level was the only factor responsible for the differences of hybrid performance between HN and LN trials as these trials at each location were, otherwise, identically managed. These hybrids have variable grain yield potentials, their parent lines belonging to different heterotic groups, and include normal hybrids and high-oil hybrids. The hybrids were considered as a random sample of elite germplasm being used in maize breeding programs in North China Plain.

Significant reduction in average grain yield over hybrids in LN vs. HN levels (≈ 36%, LN/HN=0.64) was mainly associated with that in KNE followed by TKW (Table 1). The expression of PH, EH, and protein concentration was also adversely affected by LN level, but to a lesser degree. On the other hand, SR, KDMC, grain oil concentration, and starch concentration were not influenced by N stress. Further, the magnitude of correlation between RDN of grain yield vs. KNE and TKW supported the close association between the expression of grain yield with KNE and TKW. Our results are in accordance with other previous study. Bertin and Gallais (2000) observed similar magnitude of reduction in grain yield under low N, and its association with KNE and TKW in testcrosses of a RIL population. Presterl et al (2003) conducted studies from 1989 to 1999 across various environments using diverse European maize breeding materials and reported similar magnitude of reduction in grain yield under low N level. Among the yield components studied, N stress had an adverse effect mainly on successful fertilization and initiation of kernel formation (KNE), and also grain development (TKW). A reduction in KNE was reported to be due to the abortion of ovules after fertilization (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995). The significant reduction under LN level for protein concentration was in accordance with the biosynthesis pathways that protein synthesis is more dependent on the availability of N.

Breeding for high-oil grain has started getting attention in maize breeding in recent years, because high-oil maize has a high value as both feed and food. However, there is little information in the literature on the effect of N levels on grain oil concentration. In our research, high-oil hybrids showed significantly higher oil concentration than normal hybrids, but no significant differences for grain oil concentration between the two N levels (Table 2). The response of these and normal hybrids to N fertilization was also similar. Thus, to breed high-oil hybrids adapted to variable N levels, this trait need not be determined under different N levels.

Presence of genetic variation and identification of superior genotypes are the basic prerequisites for successful execution of a breeding program. In the present study, the estimates of σ^2_G were significant and those of h^2 were high (> 0.7) for all traits under both N levels. High estimates of h2 indicated relatively greater importance of σ^2_G vs. other variance components. For grain yield, the estimate of σ^2_G was higher under HN than LN level and a similar trend was observed for h^2. Our results are in accordance with those reported in earlier studies performed with US Corn belt, European, subtropical, and tropical germplasm. Generally larger estimates of σ^2_G and/or h^2 have been obtained under HN level (Blum, 1988; Bänziger et al, 1997; Bertin and Gallais, 2000; Sinebo et al, 2002; Betrána et al, 2003; Liu et al, 2008); but opposite trend was also reported (Agrama et al, 1999; Presterl et al, 2000, 2003). Bänziger et al (1997) analyzed 14 tropical maize trials and observed σ^2_G for grain yield under low N to be about one third of that under high level of N; and this compares well with our study. Lower estimate of σ^2_G and h^2 under low N may be expected due to poor expression of genes in stress conditions. In contrast to lower magnitude of σ^2_G under LN level, the estimates of CV_G were similar under two N levels. It was expected as mean grain yield, which was used as a denominator to estimate CV_G, was lower under LN level. Further, in general weaker r_P among traits under HN than LN level and larger frequency of not-signficant r_P under HN level (Table 3) may be result of the selection that the germplasm under study have undergone under HN condition. In fact, the relative magnitude of estimates of σ^2_G and h^2 under different N levels is expected to vary with the germplasm, selection history of the breeding materials, i.e., duration and selection criteria (traits and their relative importance), selection environment, and evaluation environment. The hybrids studied by us were a random sample of the material bred under HN level. It may be expected that selection narrows the genetic base resulting in lower estimate of h^2 in evaluation environments akin to the selection environments, but this did not happen. Further, significant σ^2_G and high h^2 estimates were also obtained under LN level. It may be that alternative physiological mechanisms associated with LN become operative under N stress (Brun and Dudley, 1989; Bänziger et al, 1997). From the practical view point, our results showed that these materials can be used to breed cultivars adapted to variable N levels.

The estimate of σ^2_{GN} has crucial importance in designing the breeding strategy for variable N levels. Significant σ^2_{GN} estimates and their second highest magnitude, next to σ^2_G, for grain yield and most other agronomic traits, underlined the importance of these interactions and indicated that response to N level varied among genotypes, as also reflected for grain yield (supplementary Figure 1). Significant estimates of σ^2_{GN} have also been reported in earlier studies (Bänziger et al, 1997; Betin and Gallais, 2000; Presterl et al, 2003). Yamada (1988) studied genotypic correlation between performance at high and low levels of N to understand the importance of σ^2_{GN}. We obtained very high estimate of r_G (data not shown) as also earlier reported by Presterl et al (2003). Generally significant estimates of the estimate of σ^2_{GE} and σ^2_{GNE}, though, smaller than σ^2_G and σ^2_{GN}, indicated the need for multi-environmental tests to identify desirable material.

The hybrids differed widely for their stability parameters and adaptation to different environments. Hybrids H20 and H12, with the high mean yields and highest bis, were specifically adapted to good environments. On the other hand H13 combined relatively high yield with low b_i, and thereby showed specific adaptation to poor environments. Hybrids H01, H05, H17, and H18, with high yield and average b_is (1.00-1.09) possessed wide adaptation.

The high use of N fertilization is a common feature in North China Plain; yet, the soil and applied N vary substantially in China, India and many other maize growing regions in the developing world (Wang Pu, personal communication; Dhillon BS, personal communication). Hence, there is a need to breed hybrids that perform well under high N as well as under variable N fertilization including N stress. An ideal hybrid should combine high stable performance in poor or stress environments and respond to good management (Verma et al, 1978; Dhillon et al, 1985). Hybrid H5, with high grain yield across N levels (Table 4), had high b_i value under HN level (1.18) and low under LN levels (0.80) meaning that this hybrid has stable grain yield under LN and are responsive to HN conditions, i.e., behave quite like an ideal variety. In contrast H01 with b_i value and mean grain yield across N levels similar to H05, had the linear response under two N levels in the opposite direction (b_i = 0.96 under HN level and 1.35 under LN level). The differential behavior of these and some other hybrids for their linear response at two N levels indicated that there may be different gene(s) controlling the genetic response to contrasting N levels. Such a genotypic response has been suggested to be due to different limiting steps in N-metabolism between high and low levels of N (Bertin and Gallais, 2000, 2001; Coque et al, 2008).

Further, the differential response indicated that the analysis over all the environments may hinder the detection of desirable genotypes.

In conclusion, our study showed that breeding maize adapted to variable N levels is feasible with the germplasm available in the breeding programs in North China Plain. Significant σ^2_G and high h^2 under both N levels in the breeding materials developed under HN conditions showed that breeding maize under HN level may serve to develop hybrids well adapted to high and/or low levels of N. Significant interaction variance components (σ^2_{GN}, σ^2_{GE}, σ^2_{GNE}) indicated the need for multi-environment testing to identify materials with high grain yield performance under variable N conditions. However, unlike grain yield and its components, grain quality traits (protein, oil, and starch concentrations), for which σ^2_{GN} was not significant, need not be determined under different N level. The hybrids differed for stability parameters (mean performance and linear response) across N levels, and there were indications that separate analyses under different N levels may be useful to identify hybrids with adaptation to variable conditions including well managed and stress environments. The next step would be to identify QTL under variable N levels as well as to run model calculations on the allocation of resources including the number of environments under different levels of N that should be employed while breeding hybrids for variable N levels.

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