

Low-nitrogen stress tolerance and nitrogen agronomic efficiency among maize inbreds: comparison of multiple indices and evaluation of genetic variation

Yongshen Wu · Wenguo Liu · Xinhai Li · Mingshun Li ·
Degui Zhang · Zhuanfang Hao · Jianfeng Weng ·
Yunbi Xu · Li Bai · Shihuang Zhang · Chuanxiao Xie

Received: 12 October 2010 / Accepted: 5 March 2011 / Published online: 16 March 2011
© Springer Science+Business Media B.V. 2011

Abstract Limited information is available on genetic variation in low-nitrogen (low-N) stress tolerance and N-use efficiency (NUE) among maize inbreds. To unveil this information, a panel of 189 diverse maize inbred lines was evaluated under contrasting levels of N availability over 2 years.

Electronic supplementary material The online version of this article (doi:10.1007/s10681-011-0409-y) contains supplementary material, which is available to authorized users.

Y. Wu · X. Li · M. Li · D. Zhang · Z. Hao ·
J. Weng · Y. Xu · L. Bai · S. Zhang (✉) · C. Xie (✉)
Institute of Crop Sciences, Chinese Academy of
Agricultural Sciences/National Key Facilities for Crop
Gene Resource and Genetic Improvement, Beijing
100081, China
e-mail: cshzhang2000@yahoo.com.cn

C. Xie
e-mail: cxxie@caas.net.cn

Y. Wu
Guangxi Maize Research Institute of Maize Research,
Guangxi Academy of Agricultural Sciences,
Nanning Guangxi 530227, China

W. Liu
Institute of Maize Research, Jilin Academy
of Agricultural Sciences, Gong-Zhu-Ling,
Jilin 136100, China

Y. Xu
Chinese Academy of Agricultural Sciences and
International Maize and Wheat Improvement Center
(CIMMYT) Joint Research Center, Beijing 100081, China

Low-N agronomic efficiency (LNAE), absolute grain yield (GY) at low-N conditions, and the ratio between GY at low-N and optimum-N conditions were taken into account to represent low-N tolerance. Additionally, N-agronomic efficiency (NAE) along with other agronomic traits was also analyzed. Analysis of variance revealed significant effects of genotype on LNAE, NAE, and GY. The estimated broad-sense heritability was 0.38 for LNAE while it was only 0.11 for NAE, implying that selection based on LNAE should be more effective than NAE. LNAE exhibited highly positive genotypic and phenotypic correlations with GY, ear kernel number (EKN), kernel weight, plant height (PH), and chlorophyll content at low-N conditions, while it was negatively correlated with grain-N content and anthesis-silking interval. Path analysis indicated that the EKN at low-N stress had the highest positive effects on LNAE.

Keywords Genetic variation · Low-N stress tolerance · N-agronomic efficiency · N-use efficiency · Maize

Abbreviations

10 DAP	10 Days after pollination
CI	Confidence intervals
NAE	Nitrogen agronomic efficiency ($\text{kg kg}^{-1} \text{N ha}^{-1}$)
ASI	Anthesis to silking interval (days)
EKN	Ear kernel number
G	Genetic correlation

G_{WC}	Grain yield at no N-fertilizer application control (kg ha ⁻¹)
G_{WF}	Grain yield under optimum N-fertilizer application (kg ha ⁻¹)
GY	Grain yield (kg ha ⁻¹)
h^2	Broad sense heritability
HKW	Hundred kernel weight (g)
N%	N content in dry grain
N	Nitrogen
NDP	Nitrogen-depleted plot
NDVI	Normalized difference vegetation index
N_F	The amount of N fertilizer applied (kg ha ⁻¹)
NP	Normal plot with complete fertilizer application
NUE	N-use efficiency
PH	Plant height (cm)
r	Phenotypic correlation
SPAD	Soil plant analytical development

Introduction

Nitrogen (N) is one of the major essential elements for crop growth and development and is heavily used in modern agriculture to maximize yields. World-wide, N-use efficiency (NUE) for cereal production is approximately 33% (Raun and Johnson 1999). Indeed, only about a third of the applied N is stored in the cereal grain (Raun and Johnson 1999; Tilman et al. 2002). Excessive N fertilization in intensive agricultural areas including major maize production regions has resulted in serious environmental problems because of atmospheric, soil, and water enrichment with reactive N of agricultural origin (Ju et al. 2009). On the other hand, maize cultivation in tropical areas and some other marginal maize production areas occurs at low-N conditions (Monneveux et al. 2005). The efficiency of fertilizer capture and adaptation to low-N stress under conditions of low N supply are important traits for corn production (Tokatlidis and Koutroubas 2004). To reduce the cost of N fertilization and the negative impact of excessive N on the environment, improvement of N-use efficiency (NUE) and low-N tolerance has become a desirable breeding goal in maize. Therefore, improving the NUE and low-N tolerance

is of great importance for both sustainable maize production and environmental benefits.

Breeding for high NUE and low-N stress tolerance requires the availability of adequate genetic variability for the target traits. Genetic variation or variability in N-utilization traits among the accessions of maize, wheat, rice, and spring barley has been investigated as a prerequisite to improve NUE of cereal crops (Moll et al. 1982; Cox et al. 1985; Singh et al. 1998; Anbessa et al. 2009; Cui et al. 2009). In maize, a few single biparental hybrids were used for on-farm measurement of genotypic differences (Moll et al. 1982; Cui et al. 2009). However, genetic variation among maize inbreds is largely unknown. This has significantly impact on both parental choice for breeding hybrids and donor selection for backcross programs.

An efficient measurement of NUE is essential for crop breeding and production. A number of measurements have been developed for evaluation of NUE in crops (Steenbjerg and Jakobsen 1963; Siddiqi and Glass 1981; Moll et al. 1982; Craswell and Godwin 1984) as summarized by Good et al. (2004). Among these, N-agronomic efficiency (NAE), which is defined as the efficiency of converting applied N to grain yield, is much more practicable when a large number of accessions must be evaluated since no labor-intensive or time-consuming chemical analysis is required. This index has been defined as $NAE = \frac{G_{WF} - G_{WC}}{N_F}$, where G_{WF} and G_{WC} are the grain yield with and without N applied, respectively; and N_F is the amount of N fertilizer applied (Craswell and Godwin 1984). Recently, low-N tolerance index (LNTI) is defined as grain yield reduction under low-N stress in comparison with that under optimal condition at the same trial, and is calculated as $(1 - (G_{LN}/G_{OP}) \times 100\%)$, where G_{LN} = grain yield under low-N environment and G_{OP} = grain yield under optimal environmental condition (Francisco et al. 2010). However, the breeding goal for high NUE is to maintain or increase productivity with less N applied (Good et al. 2004). Selection for NAE or LNTI may not necessarily lead to varieties with higher NUE. For example, varieties of a crop that perform worse under unfertilized condition may be selected, because the worse performance at the low N will increase the numerator ($G_{WF} - G_{WC}$), which results in a higher NAE but decrease G_{LN}/G_{OP} , which results in a higher

LNTI. Therefore, it is necessary to develop a novel measurement for N tolerance to ensure an efficient breeding for high maize NUE and low-N tolerance.

In this study, we introduce a formula for measurement of low-N agronomy efficiency (LNAE), which is modified from agronomy efficiency (AE) and expressed as $LNAE (kg\ ha^{-1}) = \frac{G_{WC}}{G_{WF}} G_{WC}$, where G_{WF} and G_{WC} is the grain yield with and without N supplied, respectively. As N application in previous crop seasons will affect the availability of N and the responsibility of maize plants to the N application (Stevens et al. 2005), N-depleted plots (NDP) were used as non-N fertilizer control in this study. It should be mentioned that the G_{WC} used in this study for LNAE and NAE was different from that defined by Craswell and Godwin (1984) as it was the grain yield of the non-N fertilized control at NDP in this study. The objectives of this study were: (i) to develop an applicable evaluation index for low-N tolerance; and (ii) to determine genetic variation for low-N tolerance and NUE in maize inbreds. The results provide some useful information and germplasm donors for breeding low-N tolerance and NUE in maize.

Materials and methods

Plant materials

A total of 189 maize inbred lines were used in this study and their names, pedigree and heterotic group information are provided in Supplementary Table 1. These inbred lines represent a panel of diverse germplasm from both China and North America, including some subtropical and tropical germplasm (Xie et al. 2007).

Field experiments

The field experiments were conducted at Sanya, Hainan, China, during the 2007 and 2008 winter cropping seasons. The soil type at this site is sandy loam, which requires heavy fertilizer input to support plant growth. To exclude the effect of the previous N application on this trial, a N-depleted plot (NDP) was used as non-N fertilizer control in this study. The NDP was developed by removing all biomass of the maize plant from the plot without N-fertilizer application for 2 years. A normal plot (NP) was designed to receive

full fertilization. All the NP and NDP plots received 90 kg $P_2O_5\ ha^{-1}$ as triple superphosphate and 80 kg $K_2O\ ha^{-1}$ as potassium chloride prior to sowing. In the NP, N fertilizer as urea was applied at 100 kg N ha^{-1} before sowing, 100 kg N ha^{-1} at the six-leaf stage, and 50 kg N ha^{-1} before flowering.

Difference in nutrients between N-depleted and normal plots

The difference in the nutrients before the field test in the NDP and NP were evaluated (Supplementary Table 2). The results showed that soil total N was significantly lower in NDP than NP. No significant difference other than N was observed between NDP and NP, indicating that the 2-year-N-depleting effort had been successful in reducing N levels. Soil samples were collected from the 0 to 30 cm soil layer prior to fertilization and planting, and then mixed, air dried, and sieved to remove undecomposed organic materials, after which they were used to determine the organic matter (Walkley 1947), total N (Bremner 1996), total P ($HClO_4-H_2SO_4$ digestion), total K, Olsen-P (0.5 M $NaHCO_3$; Olsen et al. 1954), $NH_4\ OAc-K$ (1 M ammonium acetate at pH 7; van Reeuwijk 1992), and pH (pHS-3C pH meter, Nanjing, water/soil ratio 2.5:1).

Field plot design

All tested maize inbred lines were evaluated in single-row plots in both NP and NDP treatments. The plot size was 3 m² (5-m long with 0.6-m row spacing and 0.2-m plant spacing), with each plot replicated three times in a 14 × 14 alpha-lattice design. To reduce the marginal and neighbor effects, traits were only measured for the plants in each row after discarding the two plants from each end of the row. Originally, the panel of the inbred lines was 196 lines for the 14 × 14 alpha-lattice. However, seven lines out of 196 had data missed due to unexpected reasons during the field tests.

Trait measurements

All phenotypic values shown are the mean for the trait being evaluated as determined from all the scored plants. Days to silking was defined as the interval between the emergence date and the date

when silks emerged in ca. 50% of the plants. Days to pollen shedding was defined as the days from the emergence date to when 50% of the plants in the plot shed pollen. Anthesis-silking interval (ASI) was defined as the interval between the days to pollen shedding and the days to silking. Plant height (PH) was defined as the distance from the ground to the first branch of the tassel at maturity of the score for soil plant analytical development (SPAD) was determined using a portable Minolta chlorophyll meter (SPAD-502, MINOLTA KONICA Japan), while the normalized difference vegetation index (NDVI) was determined using a GreenSeekerTM optical sensor unit (NTech Industries, Inc.) as a measurement of the biomass at the pre-tasseling stage as defined by CIMMYT, at flowering, and 10 days after pollen shedding (10 DAP). Ear kernel numbers (EKN) and 100-kernel weight (HKW) were the average for all the scored plants in each plot. Grain yield (GY) was transformed into kg ha⁻¹. Grain-N content (N%) was determined from kernel samples using the standard Kjeldahl method (Horowitz 1970). N-agronomic efficiency (NAE), which has been calculated as $NAE = \frac{G_{WF} - G_{WC}}{N_F}$, where G_{WF} and G_{WC} are the grain yield with and without N applied, respectively; and N_F is the amount of applied N fertilizer, which was 250 kg N ha⁻¹ in this study. LNAE (kg ha⁻¹) had been calculated as $LNAE = \frac{G_{WC}}{G_{WF}} G_{WC}$, where G_{WF} and G_{WC} is the grain yield with and without N supplied, respectively. Therefore, only one set of data is available for LNAE and NAE since they were calculated using combined data from NDP and NP.

Statistical analyses

Most of the phenotypic data are presented as the mean and range in the Table 1. Analysis of variance (ANOVA) for nutrient traits between NDP and NP was computed with the *t* statistic based on the assumption that the variances of the two treatments are equal. ANOVA was conducted for each test using PROC GLM of the SAS (1999). The genotypes, environments, and blocks were considered as random effects. Estimates of genotypic variance, environmental variance, and genotype × environment (G × E) interaction variance were obtained based on the expected mean squares using data collected in 2 years (Falconer 1989). The broad sense heritability

Table 1 Means and ranges of low-N agronomy efficiency (LNAE), nitrogen agronomic efficiency (NAE), grain yield (GY), and growth traits of 189 maize inbred lines grown in the N-depleted plot (NDP) and normal plot (NP) in 2007 and 2008

Score stage	Pre-tasseling		Flowering		After maturity				
	NDVI	ASI	PH	EKN	HKW	GY	N%	NAE	LNAE
Traits									
NDP	0.57 (0.29–0.79)	2.99 (–1.66–11.17)	104.47 (67.07–158.98)	172.67 (62–273.34)	18.32 (9.22–30.73)	1630.12 (203.93–3112.87)	1.67 (1.28–2.26)	5.29 (1.80–10.17)	1046.60 (86.54–2466.91)
NP	0.69 (0.21–0.87)	2.28 (–1.33–7.30)	126.30 (78.17–173.87)	236.22 (93.32–361.04)	20.47 (12.90–53.27)	2712.04 (685.71–4499.24)	1.85 (1.41–2.85)		
NDP/NP	0.83	1.31	0.83	0.73	0.89	0.60	0.90		

Note: Only one set of data is available for LNAE and NAE since they were calculated using combined data from NDP and NP
NAE nitrogen agronomic efficiency (kg kg⁻¹ N ha⁻¹), ASI anthesis-silking interval (days), EKN ear kernel number, GY grain yield (kg ha⁻¹), HKW hundred kernel weight (g), N nitrogen, N% N content in dry grain, NDVI normalized difference vegetation index, PH plant height (cm), 10 DAP 10 days after pollinating

(h^2) was estimated on a plot basis (Hanson et al. 1956) as $h^2 = \alpha_G^2 / (\alpha_G^2 + \alpha_{GE}^2 + \alpha_r^2)$, where α_G^2 was genotypic variance, α_{GE}^2 was the variance due to G x E interaction, and α_r^2 was the error variance of replication. The CI (confidence interval) were obtained as $CI = \mu \pm T \times \sigma$, where μ was the mean of the h^2 estimates, T was the critical value from the T distribution for a $1 - \alpha$ CI, and σ was the standard deviation of the h^2 estimates (Mallinckrodt et al. 1997). The Proc MIXED of the SAS system to implement restricted maximum likelihood (REML) was used to estimate genotypic and phenotypic correlations (Holland 2006). Path analysis was used to partition the correlation matrix into causal and spurious effects and then the causal effect was partitioned into direct (path coefficient) and indirect effects (correlations) (Ullman 1996).

Results

Genetic variability and heritability of LNAE and NAE

Significant differences were observed among genotypes for low-N agronomic efficiency (LNAE), N-agronomic efficiency (NAE) (Table 1). The G x E interaction was also significant (data not shown). Among the diverse inbred lines, LNAE and NAE along with GY and other physiological and growth traits showed very diverse phenotypic performance. The mean and range of these traits are listed in Table 1. The average LNAE was 1,046.60 kg ha⁻¹, ranging from 86.54 to 2,466.91 kg ha⁻¹. The highest LNAE was nearly 28 times that of the lowest. The average NAE was 5.29 kg ha⁻¹, ranging from 1.80 to 10.17 kg ha⁻¹. The average NAE indicated that 1 kg ha⁻¹ N input in the NP increased grain yield by 5.29 kg ha⁻¹ compared with the NDP. Significant differences were also found for anthesis-silking interval (ASI), chlorophyll content (SPAD read at the pre-tasseling stage), growth and biomass (as measured by NDVI, normalized difference vegetation index), plant height (PH), grain yield (GY), ear kernel number (EKN), kernel weight (HKW, hundred kernel weight), and N content in dry grains (N%) in both NP and NDP plots (Tables 1, 2). All of the scored traits showed great difference among accessions. The ratios of average

Table 2 Estimates of genetic variance and broad-sense heritability for low-N agronomy efficiency (LNAE, kg ha⁻¹) and N-agronomic efficiency (NAE, kg kg⁻¹ N) and growth and yield traits in 189 maize inbred lines across the N-depleted and normal plots in 2007 and 2008

	LNAE (kg ha ⁻¹)	NAE	N%	SPAD	PH (cm)	ASI (days)	EKN	HKW (g)	GY (kg ha ⁻¹)	NDVI
N depleted plot										
F	6.43	2.12	6.64	4.60	10.03	6.59	8.34	14.70	13.95	2.41
$h^2 \pm$ SD	0.38 $\pm$ 0.05	0.11 $\pm$ 0.034	0.45 $\pm$ 0.04	0.38 $\pm$ 0.05	0.43 $\pm$ 0.03	0.4 $\pm$ 0.03	0.47 $\pm$ 0.03	0.56 $\pm$ 0.02	0.53 $\pm$ 0.02	0.32 $\pm$ 0.05
95% CI	29.06–46.94	4.14–17.86	37.56–52.43	28.97–47.03	37.68–48.32	33.16–46.84	40.14–53.86	51.84–60.16	48.33–57.67	22.80–41.20
Normal plot										
F	–	–	9.44	13.76	26.43	3.45	12.05	2.92	15.12	1.74
$h^2 \pm$ SD	–	–	0.74 $\pm$ 0.04	0.53 $\pm$ 0.03	0.6 $\pm$ 0.01	0.27 $\pm$ 0.04	0.49 $\pm$ 0.03	0.23 $\pm$ 0.05	0.48 $\pm$ 0.02	0.2 $\pm$ 0.05
95% CI	–	–	67.14–80.86	47.19–58.81	57.36–62.64	18.27–35.73	43.94–54.06	14.07–31.93	44.22–52.78	10.78–29.22

Note: Only one set data is available for LNAE and NAE since they were calculated using combined data from NDP and NP. $h^2 \pm$ SD presented the mean heritability with stand error (SD)

NAE nitrogen agronomic efficiency (kg kg⁻¹ N ha⁻¹), ASI anthesis-silking interval (days), EKN ear kernel number, GY grain yield (kg ha⁻¹), HKW hundred kernel weight (g), N nitrogen, N% N content in dry grain, NDVI normalized difference vegetation index, PH plant height (cm), 10 DAP 10 days after pollinating, F F value, h^2 broad sense heritability, CI confidence intervals

values for the same trait measured between NDP and NP plots indicated that most of the scored traits were largely affected by low-N stress (Table 1).

The heritability estimate was 0.38 for LNAE, while it was 0.11 for NAE, implying that selection based on LNAE should be more effective than NAE. GY showed heritability estimates of 0.53 for NDP and 0.48 for NP, which indicated no significant difference. However, EKN and HKW showed different patterns of heritability estimates in NDP and NP.

Genotypic and phenotypic correlations of LNAE and NAE

LNAE exhibited positive genotypic and phenotypic correlations with GY, EKN, kernel weight (HKW), plant height (PH), and chlorophyll content (SPAD read), but was negatively correlated with the N content in grain (N%) and ASI in NDP treatments (Table 3). However, the phenotypic correlation of LNAE with GY, EKN, and ASI was much higher in the NDP. NAE exhibited a positive phenotypic correlation with EKN in both the NDP and NP groups, while GY was only positively correlated with EKN in the NDP group. The genetic correlation of NAE with EKN, SPAD, PH, and GY was significant under both conditions. Based on these findings, the EKN was a good visible phenotype for low-N tolerance and NUE among inbreds. These findings support the motion that N supply positively enhanced grain yield in maize hybrids, primarily by increasing the kernel number (Uribealarea et al. 2004). In addition, associations were observed between ASI and GY, and ASI and LNAE under both low-N and normal conditions. In particular, the significance of these associations was higher under low-N conditions.

Path analysis of the effect on LNAE and NAE

Path analysis revealed that the kernel numbers (EKN) and kernel weight (HKW) under low-N stress conditions had significant positive direct effects on LNAE (Fig. 1). These findings imply that a genotype selected due to its high yield under low-N stress conditions may be tolerant to low-N stress and therefore utilize N more efficiently. However, the N content in maize grains had a negative correlation of -0.19 with LNAE and -0.39 with EKN. Possibly,

the nitrogen supply impacted the yield–protein relationship by stimulating protein synthesis rather than inhibiting starch production among maize hybrids. Uribealarea et al. (2004) demonstrated strong genetic control of the grain composition in maize hybrids, which can be modulated by the positive effects of N on the reproductive sink capacity and seed protein synthesis. Our results from the maize inbred lines here also support this conclusion.

Discussion

The comparison of LNAE with NAE and LNTI

The breeding goal for achieving high NUE is to maintain or increase productivity with reduced N application (Good et al. 2004). In LNAE, both the absolute GY at low-N availability and the ratio between low N and optimum N have been taken into account in the formula. Therefore, LNAE meets the breeding goals of selecting varieties capable of maintaining high productivity at low-N availability. It can be used to select materials or varieties that can maintain high GY production under low-nitrogen stress conditions or high-density population conditions with serious competence in soil N availability. However, breeding based on selection for NAE or LNTI may not. Judging from the formula of NAE and LNTI, the genotypes that perform more poorly under unfertilized conditions will be preferentially selected since a low performance at low-N will increase the numerator ($G_{WF} - G_{WC}$) and thus result in a higher NAE while a low performance will decrease GY_{LN}/GY_{OP} and thus result in a higher LNTI. Therefore, the selection based on NAE or LNTI may result in the varieties which have a greater difference between low- and optimum-N conditions. The maize production using these varieties may rely on more N input.

The heritability of LNAE and NAE

In this study, we found the broad-sense heritability of LNAE was much higher than NAE. It represents a better target for selection due to its greater genetic variability. However, the heritability estimate was just 0.38 for LNAE. The heritability might have been underestimated due to the following reasons. First of all, we used a single row with three replicates at NDP

Table 3 Phenotypic (*r*) and genetic (*G*) correlation coefficients for low-N agronomy efficiency (LNAE) and N-agronomic efficiency (NAE) and growth and yield traits among 189 maize inbred lines in the N-depleted plot (NDP) and the normal plot (NP)

	SPAD	PH	ASI	EKN	HKW	N%	LNAE	NAE	GY
NDP									
LNAE									
<i>r</i>	0.37	0.30	−0.40	0.71	0.33	−0.37	–	ns	0.95
<i>G</i>	0.42	0.31	−0.47	0.74	0.36	−0.17	–	0.19	0.98
NAE									
<i>r</i>	ns	ns	ns	0.22	ns	ns	ns	–	ns
<i>G</i>	0.25	0.16	ns	0.39	0.09	ns	0.19	–	0.28
GY									
<i>r</i>	0.40	0.33	−0.45	0.78	0.33	−0.44	0.95	ns	–
<i>G</i>	0.45	0.31	−0.43	0.79	0.34	−0.18	0.98	0.28	–
NP									
LNAE									
<i>r</i>	ns	ns	−0.25	0.41	ns	−0.40	–	ns	0.71
<i>G</i>	0.22	0.27	−0.44	0.49	0.25	−0.44	–	0.19	0.85
NAE									
<i>r</i>	ns	ns	ns	0.42	ns	ns	ns	–	0.46
<i>G</i>	0.24	0.25	−0.16	0.52	0.04	−0.37	0.19	–	0.55
GY									
<i>r</i>	0.25	0.38	−0.27	0.69	0.13	−0.43	0.71	0.46	–
<i>G</i>	0.30	0.38	−0.46	0.70	0.15	−0.46	0.85	0.55	–

LNAE low-N agronomic efficiency (kg ha^{-1}), NAE N-agronomic efficiency ($\text{kg kg}^{-1} \text{N ha}^{-1}$), ASI (days), anthesis-silking interval, SPAD is the flag leaf at the pre-tasseling stage, EKN ear kernel number, N% N content in dry grains, GY grain yield (kg ha^{-1}), HKW (g), hundred-kernel weight, NDVI normalized difference vegetation index, PH (cm) plant height ($P < 0.001$), ns not significant

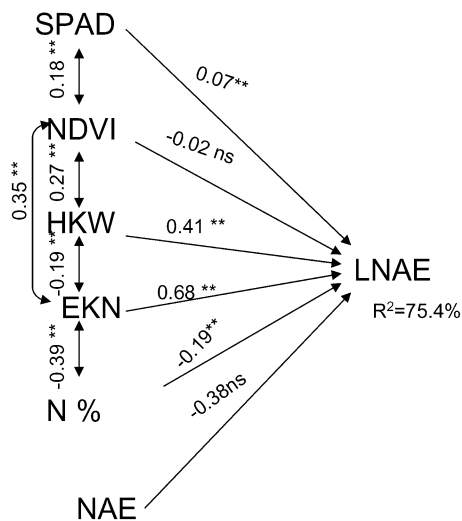


Fig. 1 Path analysis revealed that the kernel numbers (EKN) and kernel weight (HKW) under low-N stress conditions had significant positive direct effects on LNAE (low-N agronomic efficiency)

and NP in the field design in this study; the random effect of the replicates and error variance occupied a large portion of the total variance. We do understand that bigger plot sizes may positively impact the accuracy of estimate. Analyzing plot-size effect among 16 accessions (Supplementary Table 3) suggested that larger plot size was better for evaluating. However, the effect of trial scale with several hectares of field should negatively impact the accuracy. Second, the $G \times E$ variance was also quite large. Third, a lot of the inbred lines used in this study had the same or similar origins and genetic background (Xie et al. 2007). The genetic variance should be underestimated due to a higher degree of freedom from large accessions but less variance among accessions which share the similar background. This is supported by the fact that heritability of other traits like GY was also underestimated compared with other studies. Presterl et al. (2003) found that the heritability estimates for grain yield at high-N ranged from 0.36 to 0.94 and between 0.41 to 0.88 at LN. Most of the experiments had higher heritability estimates than ours.

Response of growth, adaptation, and grain yield to low-N availability

N is one of the most important factors limiting the growth and GY of crops. In the present study,

different levels of N-availability led to altered plant growth and grain yield among diverse lines (Tables 1, 3). Typically, the leaf and canopy chlorophyll levels were strongly affected by N availability. Therefore, these characteristics are often used as a surrogate to direct plant-N status estimation (Schlemmer et al. 2005; Samborski et al. 2009). The scored SPAD values revealed a remarkable difference in the leaves at different positions across growing stages between the NDP and NP. The ratios of mean SPAD reads among 189 lines between NDP and NP ranged from 0.71 to 0.78. Normalized difference vegetation index (NDVI) was correlated with vegetative growth like biomass (Stevens et al. 2005; Flynn et al. 2008) and leaf area index (LAI) (Coyne et al. 2009). Significant genotypic differences were also observed for NDVI and plant height (PH), which were also correlated with biomass and vegetative growth. In the NDP, the NDVI at 10 days after flowering and the PH at maturity were reduced by ca. 20 and 17%, respectively (Table 1). The average anthesis to silking interval (ASI) was 2.99 days and ranged from -1.66 to 11.17 days in the NDP, which was higher than in the NP. Environmental conditions such as shade (Moss and Stinson 1961), density (Woolley et al. 1962; Monneveux et al. 2005), drought (Hall et al. 1982; Fischer et al. 1989; Bolaños and Edmeades 1996), and low-N availability (Monneveux et al. 2005) affect the ASI due to changes in the time to silking in maize hybrids or populations. Reports have shown that the shorter ASI under stressful conditions is an indirect index for greater tolerance and biomass partitioning to the developing ear (Edmeades et al. 1993, 1999; Chapman and Edmeades 1999; Monneveux et al. 2005). Therefore, the ASI should reflect adaptation to low-N stress. In the present study, GY was significantly reduced by an average of 40%, while the ear kernel number (EKN) was reduced by 27%, and the hundred kernel weight (HKW) was reduced by 11%. These findings suggested that the N supply positively enhanced grain yield, by increasing primarily the kernel number and secondarily the kernel weight. However, the ratios of the means between NDP and NP for EKN and HKW were 0.73 and 0.89, respectively. These findings, suggest that low-N stress may affect the grain yield in inbreds through a reduction in kernel numbers more than a reduction in kernel weight (or size). The effect of N supply on GY and grain composition among a large set of diverse inbred lines was consistent with

the results observed in the hybrids (Uribelarrea et al. 2004).

The selection of genotypes with adaptation to low N and high N input levels requires an interaction between genotypes and N levels ($G \times N$). In the present study, differences in grain yields between N levels were approximately 40%, resulting in significant $G \times N$ interactions in the experiments (data not shown). Presterl et al. (2002) reported in the experiment that the of $G \times N$ interactions, line development at LN resulted in hybrids being better adapted to LN but less well adapted to HN. In total, unlike previous studies on genetic variability in NUE among maize hybrids, a large set of inbred lines was evaluated under contrasting N-availability conditions in this study. The LNAE, both the absolute GY at low-N availability and the ratio between low N and normal N were taken into account in order to evaluate the low-N tolerance among accessions. The results of this study provide evidence of genetic variation for low-N tolerance and NUE among a large number of inbred lines subjected to various levels of N availability and suggest the feasibility of applying LNAE for evaluating accessions in field experiments. The results presented herein also document the information required for parental choice and will help initiate the process of designing breeding programs to improve maize NUE for breeders who use these materials.

Acknowledgments Assistance provided by Drs. Huihui Li and Jiankang Wang for data analysis is greatly appreciated. We acknowledge the valuable comments and suggestion from anonymous reviewers that improved this paper. Funding for this research was provided by the National Natural Sciences Foundation of China (#30871535), the National High Technology Research and Development Program of China (“863” #2006AA10Z191), and the National Basic Research Program (“973” #2009CB118404).

References

- Anbessa Y, Juskiw P, Good A, Nyachiro J, Helm J (2009) Genetic variability in nitrogen use efficiency of spring barley. *Crop Sci* 49:1259–1269
- Bolaños J, Edmeades GO (1996) The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Res* 48:65–80
- Bremner JM (1996) Nitrogen-total. In: Sparks DL (ed) *Methods of soil analysis*. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA and ASA, Madison, pp 1085–1112
- Chapman SC, Edmeades GO (1999) Selection improves drought tolerance in tropical maize populations. II. Direct and correlated responses among secondary traits. *Crop Sci* 39:1315–1324
- Cox MC, Qualset CO, Rains DW (1985) Genetic variation for nitrogen assimilation and translocation in wheat. I. Dry matter and nitrogen accumulation. *Crop Sci* 25:430–435
- Coyne PI, Aiken RM, Maas SJ, Lamm FR (2009) Evaluating YieldTracker forecasts for maize in western Kansas. *Agron J* 101:671–680
- Craswell ET, Godwin DC (1984) The efficiency of nitrogen fertilizers applied to cereals grown in different climates. In: Tinker PB, Lauchli A (eds) *Advances in plant nutrition*, vol 1. Praeger Publishers, New York, pp 1–55
- Cui ZL, Chen XP, Mi GH, Chen FJ, Li F, Zhang FS (2009) Interaction between genotypic difference and nitrogen management strategy in determining nitrogen use efficiency of summer maize. *Plant Soil* 317:267–276
- Edmeades GO, Bolaños J, Hernández M, Bello S (1993) Causes for silk delay in a lowland tropical maize population. *Crop Sci* 33:1029–1035
- Edmeades GO, Bolaños J, Chapman SC, Lafitte HR, Banzinger M (1999) Selection improves drought tolerance in tropical maize populations. I. Gains in biomass, grain yield, and harvest index. *Crop Sci* 39:1306–1315
- Falconer DS (1989) *Introduction to quantitative genetics*, 3rd edn. Longman, London
- Fischer KS, Edmeades GO, Johnson EC (1989) Selection for the improvement of maize yield under moisture deficits. *Field Crops Res* 22:227–243
- Flynn ES, Dougherty CT, Wendroth O (2008) Assessment of pasture biomass with the normalized difference vegetation index from active ground-based sensors. *Agron J* 100: 114–121
- Francisco M, Pangirayi T, John D (2010) S_1 selection of local maize landraces for low soil nitrogen tolerance in Zambia. *Afr J Plant Sci* 4:67–81
- Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Sci* 9:597–605
- Hall AJ, Vilella F, Trapani N, Chimenti C (1982) The effects of water stress and genotype on the dynamics of pollen-shedding and silking in maize. *Field Crops Res* 5:349–363
- Hanson CH, Robinson HF, Comstock RE (1956) Biometrical studies of yield in segregating populations of Korean lespechea. *Agron J* 48:268–272
- Holland JB (2006) Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. *Crop Sci* 46:642–654
- Horowitz W (1970) *Official methods of analysis*, 11th edn. AOAC, Washington, pp 17–18
- Ju XT, Xing GX, Chen XP, Zhang SL, Zhang LJ, Liu XJ, Cui ZL, Christie P, Zhang FS (2009) Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proc Natl Acad Sci USA* 106:3041–3046
- Mallinckrodt CH, Golden BL, Reverter A (1997) Approximate confidence intervals for heritability from method R estimates. *J Anim Sci* 75:2041–2046

- Moll RH, Kamprath EJ, Jackson WA (1982) Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agron J* 74:562–564
- Monneveux P, Zaidi PH, Sanchez C (2005) Population density and low nitrogen affects yield-associated traits in tropical maize. *Crop Sci* 45:535–545
- Moss DN, Stinson HT (1961) Differential response of corn hybrids to shade. *Crop Sci* 1:416–418
- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939. U.S. Govt. Print. Office, Washington
- Presterl T, Groh S, Landbeck M, Seitz G, Schmidt W, Geiger HH (2002) Nitrogen uptake and utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. *Plant Breed* 121:480–486
- Presterl T, Seitz G, Landbeck M, Thiemt EM, Schmidt W, Geiger HH (2003) Improving nitrogen-use efficiency in European maize: estimation of quantitative genetic parameters. *Crop Sci* 43:1259–1265
- Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production. *Agron J* 91:357–363
- Samborski SM, Tremblay N, Fallon E (2009) Strategies to make use of plant sensors-based diagnostic information for nitrogen recommendations. *Agron J* 101:800–816
- SAS Institute Inc (1999) SAS/STAT user's guide, Vers. 8. SAS Institute Inc., Cary, NC
- Schlemmer MR, Francis DD, Shanahan JF, Schepers JS (2005) Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agron J* 97:106–112
- Siddiqi MY, Glass DM (1981) Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *J Plant Nutr* 4:289–302
- Singh U, Ladha JK, Castillo EG, Punzalan G, Tirol-Padre A, Duqueza M (1998) Genotypic variation in nitrogen use efficiency in medium- and long-duration rice. *Field Crops Res* 58:35–53
- Steenbjerg F, Jakobsen ST (1963) Plant nutrition and yield curves. *Soil Sci* 95:69–90
- Stevens WB, Hoeft RG, Mulvaney RL (2005) Fate of nitrogen-15 in a long-term nitrogen rate study: I. Interactions with soil nitrogen. *Agron J* 97:1037–1045
- Tilman DG, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. *Nature* 418:671–677
- Tokatlidis IS, Koutroubas SD (2004) A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. *Field Crops Res* 88: 103–114
- Ullman JB (1996) Structural equation modelling. In: Tabachnick BG, Fidell LS (eds) Using multivariate statistics, 3rd edn. HarperCollins, New York, pp 709–811
- Uribealarea M, Below FE, Moose SP (2004) Grain composition and productivity of maize hybrids derived from the Illinois protein strains in response to variable nitrogen supply. *Crop Sci* 44:1593–1600
- Van Reeuwijk LP (1992) Procedures for soil analysis, 3rd edn. ISRIC, Wageningen
- Walkley A (1947) A critical examination of a rapid method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci* 63:251–263
- Woolley DG, Baracco NP, Russell WA (1962) Performance of four corn inbreds in single-cross hybrids as influenced by plant density and spacing patterns. *Crop Sci* 2:951–956
- Xie CX, Zhang SH, Li MS, Li XH, Hao ZF, Bai L, Zhang DG, Liang YH (2007) Inferring genome ancestry and estimating molecular relatedness among 187 Chinese maize inbred lines. *J Genet Genomics* 34(8):738–748