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Abstract

Genotypes with better root development have good nutrient acquisition capacity and may yield better under limited nitrogen (N) conditions and consequently can help reduce the N fertilization rate and hence mitigate some economic and ecological problems. This study focused on the genotypic variation among diverse maize inbred lines for seedling and adult plant traits under contrasting N levels. Seventy four lines were screened under high and low N levels in a climate chamber and in the field. High phenotypic diversity was observed for seedling and adult plant traits together with moderate to high broad-sense heritability estimates. Seedling total root length and root dry weight were significantly correlated with other root traits in maize. Of the adult plant traits evaluated in the field, the anthesis-silking interval and the leaf chlorophyll contents were significantly correlated with grain yield under both low and high N levels. In one location, the seminal root length was correlated with grain yield both under low and high N levels and the root dry weight was correlated with grain yield under high N. Selection indices based on secondary root traits along with grain yield could lead to an increase in selection efficiency for grain yield under N stress condition. By identifying lines with better root development, particularly lines with longer SRL, it may be possible to select inbred lines with higher grain yield particularly under low N condition.

Disciplines

Agricultural Science | Agronomy and Crop Sciences | Plant Breeding and Genetics

Comments

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Genotypic variation and relationships between seedling and adult plant traits in maize

(*Zea mays* L.) inbred lines grown under contrasting nitrogen levels

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Abstract

Genotypes with better root development have good nutrient acquisition capacity and may yield better under limited nitrogen (N) conditions and consequently can help reduce the N fertilization rate and hence mitigate some economic and ecological problems. This study focused on the genotypic variation among diverse maize inbred lines for seedling and adult plant traits under contrasting N levels. Seventy four lines were screened under high and low N levels in a climate chamber and in the field. High phenotypic diversity was observed for seedling and adult plant traits together with moderate to high broad-sense heritability estimates. Seedling total root length and root dry weight were significantly correlated with other root traits in maize. Of the adult plant traits evaluated in the field, the anthesis-silking interval and the leaf chlorophyll contents were significantly correlated with grain yield under both low and high N levels. In one location, the seminal root length was correlated with grain yield both under low and high N levels and the root dry weight was correlated with grain yield under high N. Selection indices based on secondary root traits along with grain yield could lead to an increase in selection efficiency for grain yield under N stress condition. By identifying lines with better root development, particularly lines with longer SRL, it may be possible to select inbred lines with higher grain yield particularly under low N condition.

Introduction

Nitrogen (N) is a key macro-element in maize production. Maize breeders aim to improve the nitrogen use efficiency (NUE) to reduce extensive N application which in turn reduces the costs to farmers, and minimizes N losses into the environment (Zhang et al. 1995; Zhang et al. 1997). One of the ways of reducing N fertilization is by developing cultivars that have better NUE. Considerable variation for N uptake and NUE were reported among maize lines based on grain yield data (Presterl et al. 2002 a, b; Presterl et al. 2003; O'Neil et al. 2004; Uribebarrea et al. 2004). However, little attention was given to the root related traits as a selection criteria to improve NUE (Tuberosa and Salvi 2007).

Root growth and development are critical for N uptake since N is a mobile element which can easily be lost due to leaching, run off and volatilization (Sigunga et al. 2002; Gehl et al. 2005; Al-Rawashdeh and Abdel-Ghani 2008). A larger root system should improve N uptake and consequently, plant growth (Mackay and Barber, 1986; Eghball and Maranville 1993; Marschner, 1998). In grasses, N deficiency leads to a higher root to shoot ratio (R:S) (Durieux et al. 1994; Monaco et al. 2003; Bonifas et al. 2005; Li et al. 2009). However, contradictory results were found

regarding the consequence of N starvation on root growth. Under low N levels, plants adapt by rapidly increasing their root systems to exploit nutrients from larger soil volumes. However, reduced root biomass was also detected in a N deficient soil (Wang et al. 2004). Under N deficiency t an increase was observed (Maizlisch et al. 1980) in the root length, the number of primary roots, and the elongation rate of the first order laterals. Consistent with these observations, enhanced primary and secondary root elongation rates were reported under P and K deficiency (e.g., Narayanan and Reddy 1982; Anuradha and Narayanan 1991). In contrast, N fertilization enhanced root growth, root dry weight total length of lateral roots (Barber and Mackay 1986; Wang et al. 2004). In view of these contradictory results, there is a need for more comprehensive studies before an attempt to model root growth under N deficiency.

Our current knowledge on variation for NUE in maize is predominantly based on grain yield data obtained from field trials. Genotypic differences for yield at different N levels were reported (e.g., Presterl et al. 2002 a, b and 2003; O'Neil et al. 2004; Uribelarrea et al. 2004), indicating the presence of potentially useful genetic variation for tolerance to N deficiency. However, there are only few reports describing variation in root morphology of cereals in response to different levels of N supply (Kondo et al. 2003; Wang et al. 2004), often using a very limited number of lines. Also, association between root development and grain yield was not tested widely due to the difficulty in evaluating root development in plants. In this study, we evaluated a diverse set of 74 maize inbred lines for response to N by studying the seedling root and shoot characteristics, and adult plant traits grown under contrasting N levels in the field. Our objectives were to (i) study the phenotypic variation of maize lines at seedling and adult plant stage under high N (HN) and low N (LN) conditions , (ii) quantify the broad-sense heritability and correlation coefficients for various seedling and adult plant traits under LN and HN levels, and (iii) to determine the relationship of seedling traits with adult plant traits. To our knowledge, this is the first comprehensive study aimed at studying the response of seedling and adult plant traits of a diverse set of maize inbred lines to N.

Materials and Methods

Plant materials

Seventy four maize inbred lines were used in this study including 44 expired Plant Variety Protection (PVP) lines and 30 public inbred lines (Table 1). Lines used in this study were obtained from the North Central Regional Plant

Introduction Station in Ames, Iowa. All lines were selfed at Agronomy farm, Iowa State University, in the summer of 2009 for multiplication and subsequently weighed for 100 kernels. All lines received the same agricultural practices including fertilization and weeding.

Paper roll culture conditions for seedling trait measurements

Maize lines were tested in two independent experiments under contrasting levels of N in Hogland nutrient solution (Hogland and Arnon 1950) contained HN (15 mM of NO_3^-) and LN (1.5 mM NO_3^-). Other elements remained constant in both N treatments (Supplementary Table 1). The experimental design was a Randomized Complete Block Design (RCBD) with split-plot arrangement (N levels as main plots and lines as sub-plots) with two replicates. Each line within a replicate was represented by three healthy and homogenous seedlings.

Seeds used in paper roll culture were surface sterilized with 6% sodium hypochlorite for 15 minutes and subsequently washed three times with sterile water. Seeds were then placed on brown germination paper (Anchor Paper, St. Paul, MN, USA) pre-moisturized with fungicide solution Captan® (2.5g/l), and afterwards rolled up vertically (Woll et al. 2005). Rolls were then kept in 2 l glass beakers with two N levels (Hershey 1994). Experiments were carried out in a growth chamber under a photoperiod of 16/8 h at 25/22 °C (day/night) and relative humidity of 65% with photosynthetically active radiation of 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Seedling root measurements

After 14 days (at two leaf stage), seedlings were preserved in 30% ethanol to prevent additional growth. Primary root length (PRL), total length of seminal roots (SRL), total length of crown roots (CRL) and shoot length (SL) were measured manually using a ruler. Total length of lateral roots (LRL) was measured using image analysis software (WinRhizo Pro 2009, Regent Instruments, Quebec, Canada). Total length of roots (TRL) was estimated by summing PRL, SRL, CRL and LRL. Dry weight of seedlings shoots (SDW) and roots (RDW) were recorded after drying at 80 °C for at least 48 hrs.

Field study

In summer 2010, lines were planted at two locations: Agronomy research station, Iowa State University, Ames, IA (Ames) and at Pioneer Hi-Bred research station, Marion, IA (Marion) in a RCBD design in two row plots. Because

of the wide range in the flowering time of the lines, lines were divided into seven maturity groups. Entries within each group were randomized and maturity groups were planted in the order of their flowering time to prevent shading effect. For the LN treatment, no N was applied at Ames and 56 kg N ha⁻¹ was applied at Marion. For the HN treatment, 250 kg N ha⁻¹ was applied at Ames and 269 kg N ha⁻¹ at Marion. Planting density was 69,187 plants ha⁻¹ and each line was planted in two-row plots. Rows were 5.64 and 5.31 m long at Ames and Marion respectively and spaced 0.76 m apart. Anthesis to silking interval (ASI), leaf chlorophyll content (CHLMET) and plant height (PLTHT) were recorded at both locations. ASI was measured by calculating the difference in growing degree units (GDU's) between anthesis and silking time (McMaster and Wilhelm 1997). Days to anthesis (DA) and days to silking (DS) were recorded as the number of days from sowing to the day when 50% of anthers extruded outside the glumes and when silk becomes visible, respectively. Chlorophyll content was measured from the flag leaf after 15 days of silking using a chlorophyll meter SPAD-502 (Minolta Camera Co., Osaka, Japan). PLTHT was estimated as the distance between the ground surface and the tip of the central tassel spike. Grain yield was recorded on plot basis using hand and machine harvest at Ames and Marion, respectively.

Statistical analysis

For phenotypic traits, range, mean, and standard deviation (s.d.) were calculated under LN and HN treatments. The percent reduction in response to N stress were estimated as follows [(HN-LN)/(HN)]. Ranges and mean values were based on best linear unbiased prediction (BLUP) (Piepho et al. 2008). These estimates were then used to classify lines into three different categories (n) based on their performance (Zar 1996): (i) low performing lines [$\leq \bar{x} - s.d.$], (ii) medium performing lines [$\geq \bar{x} - s.d.$] to [$\leq \bar{x} + s.d.$], and (iii) high performing lines [$\geq \bar{x} + s.d.$]. For each trait, the percentage of lines belonging to each category was calculated. Thereafter, Shannon-Weaver polymorphic diversity index (H') as described by Hutchenson (1970) was estimated: $H' = \sum_{i=1}^n P_i \ln P_i$, where P_i is the proportion of individuals in the ith category and n is the number of phenotypic classes.

A mixed model implemented in PROC MIXED in SAS (Statistical Analysis Software, 2002) was used to perform the analysis of variance and to estimate variance components and broad-sense heritability (h_c^2) for all traits. Pearson correlation coefficients were calculated using PROC CORR in SAS at HN and LN treatments.

Results

Comparison of means and ranges under high and low nitrogen treatment

Seedling traits

Seedling traits were significantly ($P=0.01$) affected by lines and N levels (Table 2) and their interaction. Root development was more pronounced under LN compared to HN, which is evident from the increase in RDW (9.2%) and TRL (10.8%). PRL, SRL, and LRL are the main contributors for root biomass increase under LN (the increase ranged from 12.1% to 27.8%). In contrast, CRL was higher under HN compared to LN. The increase in SL, SDW and seedling biomass under HN were 19.5, 21.2% and 12%, respectively..

Most lines fell in medium performing category (frequency ranged from 0.62 to 0.91) (Table 3). H' ranged from 0.64 to 0.92 for most traits except for PRL ($H' = 0.38$). BLUP estimates of seedling traits across different N levels identified lines HP301, PHG84, FR19, I29, and LH74 as having low SDW, ranging from 42.0 to 52.8 mg/seedling, whereas lines such as GEMN-0192, PHG71, PHZ51, GEMN-0187, and PHR36 had the highest SDW values ranging from 118.6 to 144.9 mg/seedling. Large root was more pronounced in inbreds GEMN-0193, PHG71, A554, PHT77, and PHZ51 with TRL values ranging from 242.7 to 264.9 cm, whereas PHG84, PHV63, LH51, HP301, and B97 had TRL values ranging from 74.3 to 153.1 cm. Overall, lines PHG71, B47, LH1, GEMN-0187, and PHR36 displayed higher TSB (range= 189.4 to 218.7 mg/seedling), whereas lower TSB was observed in PHG74, HP301, FR19, I29, and SG18 (range=62.3 to 89.7 mg/seedling).

Adult plant traits

All adult plant traits were significantly ($P=0.01$) affected by lines and N levels and their interactive effect (Table 2). Most lines responded to N stress by reduced chlorophyll content, plant height, and increased ASI (Table 2). Average grain yield under LN was 3.2 and 2.2 times lower compared to HN at Ames and Marion locations, respectively. In Ames, PHR36, LH59, FR19, PHG69, and A554 had the lowest grain yield values under LN (0.14 to 0.23 t/ha), while inbreds PHW65, PHW17, PHG72, LP5, and PHG50 displayed the highest grain yield (1.24 to 1.74 t/ha). Under HN, LH57, N7A, PHR36, H49, and B97 had the lowest grain yield (0.97 to 1.29 t/ha), while lines PHG72, NS701, GEMN-0190, LP5, and B47 had the highest grain yield (3.20 to 3.6 t/ha). In Marion, LH146Ht, LH123HT, B37, PHG35, and A554 had the lowest grain yield (0.31 to 0.5 t/ha) under LN, while lines LH38, PHW65, NS701, P39, and NQ508 displayed the highest grain yield (1.35 to 1.52 t/ha). Under HN, lines A554, LH146Ht, B97, CO255, and B37 had the lowest grain yield (0.43 to 0.74 t/ha), while lines A632, Q381, GEMN-0193, LH1, and P39 had the highest grain yield (2.8 to 3.4 t/ha).

Unpaired t-test for different phenotypic traits revealed no significant differences between PVP and public inbred lines as well as between lines grouped based on their genetic background (Stiff Stalk Synthetic, Non-Stiff Stalk

Synthetic, Iodent, Temperate Stiff Stalk etc.). Correlation between kernel weight and seedling and adult plant traits were non-significant (data not shown).

Variance components and narrow-sense heritability estimates

Variance components for lines were significant ($P = 0.01$) for seedling and adult plant traits (Table 4). Variance components due to Line×N level interaction were not significant, except for PRL. However, there was significant line × experiment interaction both in seedling and adult plant traits except for PRL and RDW. h_c^2 values, based on the entry mean basis calculated across experiments and N levels, were higher in seedling traits compared to adult plant traits. Due to high genotype×environment interactions for adult plant traits, statistical analyses were performed separately for each environment. In seedling traits, h_c^2 values across N levels ranged from 0.71 to 0.89, whereas h_c^2 of adult plant traits ranged from 0.39 to 0.90. The lowest h_c^2 values were found in ASI ($h_c^2 = 0.39$ and 0.54 at Ames and Marion, respectively), while the highest values were obtained in PLTHT ($h_c^2 = 0.82, 0.90$ at Ames and Marion, respectively).

Correlations within seedling and adult plant traits

Due to significant interactions between lines and environments, correlations between traits were performed for individual environments. For Ames, correlations ($P=0.01$) between ASI and grain yield was weak and negative ($r = -0.34$) under LN, and positive between CHLMET and grain yield ($r = 0.51$) (Table 5). Correlations between ASI, CHLMET, and grain yield were weak under HN ($r = -0.36$ and 0.38 , respectively). For Marion, associations between ASI, CHLMET, and grain yield were not significant both at HN and LN treatments (data not shown).

TRL was significantly ($P=0.01$) and positively correlated with PRL, CRL, SRL, and LRL both under LN ($r = 0.48$ to 0.67) and HN conditions ($r = 0.62$ to 0.70). RDW was significantly and closely correlated with TRL both under LN and HN conditions ($r = 0.76$ and 0.79 , respectively).

Correlations between seedling and adult plant traits

Grain yield measured at Ames was significantly ($P = 0.05, 0.01$ under LN and HN, respectively) and positively correlated with SRL both under LN and HN conditions ($r = 0.24$ and 0.36 , respectively) (Table 5). Also, grain yield and RDW were positively and significantly ($P = 0.01$) correlated ($r = 0.23$) under HN. Other adult plant traits ASI, CHLMET, and PLTHT were not correlated with seedling traits under both N treatments. For Marion, non-significant correlations were found between adult plant traits and seedling traits, both under LN and HN conditions.

Discussion

Substantial genetic variation was observed for seedling and adult plant traits among maize lines evaluated for response to different N treatments. Similarly, genetic variability in root morphology (Kondo et al. 2003; Wang et al. 2004) and grain yield (Gallais and Coque 2005; Coque and Gallais 2006) were reported in mapping populations in response to N. Kernel size was not correlated with other traits evaluated in our study indicates that seed size has no effect on plant performance. Similarly, Hund et al. (2007) and Manavalan et al. (2011) found weak correlations between kernel weight and both TRL and RDW across diverse sets of maize lines.. In our study TRL and RDW under HN and LN conditions were closely and significantly correlated ($r = 0.79, 0.76$ respectively), consistent with a study performed in distilled water (Kumar et al. 2011, in press, $r = 0.75$). The typical response of maize plants to N starvation is an increased R: S ratio by enhancing assimilates translocation from shoot to root by increasing root surface area and decreases shoot growth (Maizlisch et al. 1980; Chun et al. 2005; Tian et al. 2005; Wang et al. 2003). The findings of our study are in agreement with these earlier studies, as most lines showed a higher R: S ratio under LN as compared with HN treatment. The response of individual lines in terms of SL and SDW was higher under HN compared to LN treatment. Under field conditions, ASI, CHLMET, PLTHT and grain yield were shown to be particularly sensitive to LN (Bänziger et al. 2000). ASI was 2.6 and 2 times higher under LN compared to HN conditions in Ames and Marion, respectively, indicating that both anthesis and silking time were affected in maize lines due to N deficiency. Lines exhibited severe senescence due to N stress and hence CHLMET was 1.6 and 1.2 times lower compared to HN in Ames and Marion, respectively. Under LN grain yield was about 31.5% and 46.2% of those obtained under HN treatment. Lower ASI, grain yield and higher CHLMET under LN indicates that severe N stress was induced at these sites and that there is significant genetic variation among the lines which could facilitate the identification of genetic loci controlling these traits through genetic mapping studies (Bänziger et al. 1997).

Presence of high H' for seedling and adult plant traits with moderate to high h_c^2 indicates that maize lines used in this study are a rich source to improve seedling and adult plant performance under LN conditions (Medici et al. 2005). Lines with contrasting performance in response to N might be useful to establish QTL mapping populations to identify genomic regions associated with root development and grain yield under N stress. For example, among the nine NAM parents used in this study, HP301 was found to be a good contrast to B73 in terms of TRL and TSB both under LN and HN conditions. Respective genotyped recombinant inbred line families are already available (Yu

et al., 2008) and could be used in QTL detection. These lines could be used also in gene based association and/or genome wide association studies to identify the genomic regions/genes associated with seedling and adult plant traits.

The existence of line×N interactions for adult plant traits indicate that the best genotypes under LN are not necessary performing well at a HN level (Medici et al. 2005). Significant genetic variability and line×N interactions found in our field study are consistent with other field studies in maize (Moll et al. 1987; Bertin and Gallais, 2000). In case of seedlings traits, non-significant line×N interactions indicate that the ranking of the lines under LN and HN condition did not change (except for PRL). This might be due to the genetic architecture of seedling traits in these lines, and fairly stable environment conditions in the hydroponic system compared to variable conditions in the field.

The challenge of breeding for LN environments is to develop a suitable framework for selection. Because of the low h_c^2 of grain yield under LN conditions, selection for high heritable secondary traits along with grain yield was proposed (Lafitte et al. 2003). Bänziger et al. (2000) showed that selection gain for grain yield increased by 20% when secondary traits were used for selection under LN. In accordance, ASI and CHLMET were significantly correlated with grain yield both under LN ($r = -0.34$ and 0.51 , respectively) and HN ($r = -0.36$ and 0.38 , respectively) conditions. Thus, developing selection indices based on these secondary traits along with grain yield could lead to the increase in NUE (Bänziger and Lafitte 1997). Similar to our results, respective positive correlations between seedlings traits were reported elsewhere (Zuber 1968; Nass and Zuber 1971; Andrew and Solanki, 1996). In view of these significant correlations for TRL and RDW with other root traits, combined with high h_c^2 values, selection for TRL and RDW may be sufficient to effectively screen large numbers of entries, in order to identify lines with vigorous root development at seedling stage.

Paper roll assays have several advantages such as quick and precise measurements of root traits under controlled environmental conditions, and ability to screen large number of lines. However, main disadvantages are the artificial screening conditions, which might not properly represent field conditions. Results indicate that significant and positive correlations between seedling root traits (SRL and RDW) and adult plant traits for the Ames location indicating that increased seedling root growth might contribute to a higher grain yield. The non-significant correlations between seedling and adult plant traits at Marion might be due to higher N level in LN treatment. Therefore, additional field multi-locational experiments are required to validate associations between seedling root

traits and grain yield. Due to low correlation between line *per se* and test cross performance (Presterl et al. 2002 a), it is crucial to establish, whether root – grain yield relationships at per se level are transferable to hybrid conditions.

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Table 1: Name, genetic background, origin and category of lines studied.

Inbred line	Background	Origin	Category
I1430	Non Stiff Stalk Synthetic	US, Colorado	PVP ⁺
A632	Temperate Stiff Stalk	US, Minnesota	PVP
B47	Stiff Stalk Synthetic	US, Iowa	PVP
DJ7	Unknown	US	PVP
FR19	Stiff Stalk Synthetic	US, Illinois	PVP
LH1	Stiff Stalk Synthetic	US, Iowa	PVP
LH119	Stiff Stalk Synthetic	US, Iowa	PVP
LH123HT	Unknown	US, Iowa	PVP
LH145	Stiff Stalk Synthetic	US, Iowa	PVP
LH146Ht	Stiff Stalk Synthetic	US	PVP
LH150	Unknown	US	PVP
LH156	Unknown	US	PVP
LH38	Unknown	Unknown	PVP
LH51	Non Stiff Stalk Synthetic	US, Iowa	PVP
LH54	Non Stiff Stalk Synthetic	US	PVP
LH57	Non Stiff Stalk Synthetic	US	PVP
LH59	Non Stiff Stalk Synthetic	US	PVP
LH60	Non Stiff Stalk Synthetic	US	PVP
LH74	Stiff Stalk Synthetic	US, Iowa	PVP
LH82	Unknown	US, Iowa	PVP
LH85	Unknown	US	PVP
LP1 NR HT	Temperate Stiff Stalk	US	PVP
LP5	Stiff Stalk Synthetic	France	PVP
ML606	Non Stiff Stalk Synthetic	US, Illinois	PVP
NQ508	Unknown	US, Illinois	PVP
NS501	Stiff Stalk Synthetic	US, Illinois	PVP
NS701	Stiff Stalk Synthetic	US, Illinois	PVP
PHG35	OH07-Midland	US, Iowa	PVP
PHG39	Unknown	US, Iowa	PVP
PHG47	Iodent	US, Iowa	PVP
PHG50	Iodent	US, Iowa	PVP
PHG71	Stiff Stalk Synthetic	US, Iowa	PVP
PHG72	Iodent	US, Iowa	PVP
PHG83	Iodent	US, Iowa	PVP
PHG84	OH07-Midland	US, Iowa	PVP
PHJ40	Stiff Stalk Synthetic	US, Iowa	PVP
PHR36	Unknown	US, Iowa	PVP
PHT55	Unknown	US, Iowa	PVP
PHT77	Non Stiff Stalk Synthetic	US, Iowa	PVP
PHV63	Unknown	US, Iowa	PVP
PHW17	Stiff Stalk Synthetic	US, Iowa	PVP
PHW65	OH07-Midland	US, Iowa	PVP
PHZ51	Non Stiff Stalk Synthetic	US, Iowa	PVP
Q381	Unknown	US	PVP
A554	Non Stiff Stalk Synthetic	US, Minnesota	Public* ^α
B37	Stiff Stalk Synthetic	US, Iowa	Public ^α
B57	Non Stiff Stalk Synthetic	US, Iowa	Public ^α
B73	Stiff Stalk Synthetic	US, Iowa	Public ^β
B97	Non Stiff Stalk Synthetic	US, Iowa	Public ^β
CMV3	Unknown	Canada, Manitoba	Public ^α
SG 18	Popcorn	US, Iowa	Public ^α
CO255	Unknown	Canada, Ontario	Public ^α
F2	Unknown	France, Puy-deDome	Public ^α
GEMN-0187	GEM	US, Iowa	Public ^δ
GEMN-0190	GEM	US, Iowa	Public ^δ
GEMN-0191	GEM	US, Iowa	Public ^δ
GEMN-0192	GEM	US, Iowa	Public ^δ
GEMN-0193	GEM	US, Iowa	Public ^δ
GEMS-0188	GEM	US, Iowa	Public ^δ
GEMS-0189	GEM	US, Iowa	Public ^δ
H49	Non Stiff Stalk Synthetic	US, Indiana	Public ^α

Continued Table 1

Inbred line	Background	Origin	Category
H99	Non Stiff Stalk Synthetic	US, Indiana	Public ^α
HP301	Popcorn	US, Indiana	Public ^β
I 205	Unknown	US, Iowa	Public ^α
I29	Popcorn	US, North Carolina	Public ^α
II14H	Sweet corn	US, Illinois	Public ^β
KI11	Tropical lines	Thailand	Public ^β
Mo17	Non Stiff Stalk Synthetic	US, Missouri	Public ^α
Ms71	Non Stiff Stalk Synthetic	US, Michigan	Public ^β
N28	Stiff Stalk Synthetic	US, Nebraska	Public ^α
N7A	Stiff Stalk Synthetic	US, Nebraska	Public ^α
NC358	Tropical lines	US, North Carolina	Public ^β
Oh43	Non Stiff Stalk Synthetic	US, Ohio	Public ^β
P39	Sweet corn	US, Indiana	Public ^β

^αPVP = Expired plant variety protection line, ^{*}Public= Public inbred line, ^αSNP = lines used in the maize diversity study using Single Nucleotide Polymorphism markers, ^βNAM = Nested Association Mapping founder lines, ^δGEM = Germplasm Enhancement of Maize (GEM) lines

Table 2: Best Linear Unbiased Prediction estimates of Ranges and Means of seedling and adult plant traits measured under High and low N condition for 74 maize lines

Trait		Low N			High N			% of Reduction	Analysis of Variance		
		Max	Min	Mean	Max	Min	Mean		Mean	Lines	Lines × N level
Shoot length (cm)		25.7	13.6	21.3	31.9	16.8	26.5	19.45	**	ns	**
Primary root length (cm)		31.0	9.2	26.5	26.8	7.7	23.7	-12.09	**	*	ns
Crown root length (cm)		46.7	22.3	33.1	77.5	20.3	39.5	16.35	**	ns	**
Seminal root length (cm)		98.7	23.9	59.3	90.1	20.0	53.2	-11.55	**	ns	**
Lateral root length (cm)		134.7	34.4	76.3	109.7	22.0	59.7	-27.84	**	ns	*
Total root length (cm)		246.9	89.6	193.3	248.8	71.3	174.6	-10.75	**	ns	**
Shoot dry weight (mg)		128.59	44.05	86.71	169.62	69.41	110.02	21.19	**	ns	**
Root dry weight (mg)		78.41	16.98	51.74	69.19	17.49	47.39	-9.18	**	ns	ns
Total plant biomass (mg)		200.38	70.54	138.44	236.83	87.29	157.36	12.02	**	ns	**
Anthesis-Silking Interval (GDUs)	Ames – IA, 2010	276.8	20.6	113.4	206.7	-17.3	43.0	-163.67	**	**	na
Chlorophyll Measurement		36.4	16.3	27.5	55.1	34.7	43.6	37.01	**	**	na
Plant Height (cm)		172.0	112.3	138.6	231.4	131.9	178.9	22.50	**	**	na
Yield (MT/ha)		3.589	0.285	1.444	7.418	1.991	4.579	68.47	**	**	na
Anthesis-Silking Interval (GDUs)	Marion – IA, 2010	137.2	46.7	86.3	90.3	2.4	43.6	-98.02	**	ns	na
Chlorophyll Measurement		47.4	34.8	41.9	53.9	44.7	50.4	16.86	**	ns	na
Plant Height (cm)		222.2	145.5	185.9	255.5	170.1	218.4	14.85	**	**	na
Yield (MT/ha)		3.142	0.651	1.945	7.002	0.882	4.205	53.75	**	**	na

* significant at $P < 0.05$; **significant at $P < 0.01$; ns - non significant; na - not applicable; GDUs - Growing Degree Units

Table 3: Frequency distribution and Shannon-Weaver diversity index (H^{*}) for seedling and adult plant traits measured across Nitrogen levels.

Trait			Frequency [†]			H [*]
			Small	Medium	Large	
Shoot Length (cm)			0.14	0.73	0.14	0.77
Primary root length (cm)			0.05	0.91	0.04	0.38
Crown root length (cm)			0.19	0.66	0.15	0.87
Seminal root length (cm)			0.15	0.70	0.15	0.81
Lateral root length (cm)			0.14	0.73	0.14	0.77
Total root length (cm)			0.14	0.73	0.14	0.77
Shoot dry weight (mg)			0.15	0.66	0.19	0.87
Root dry weight (mg)			0.15	0.66	0.19	0.87
Total plant biomass (mg)			0.15	0.70	0.15	0.81
Anthesis-Silking Interval (GDUs)	Ames-IA, 2010		0.14	0.73	0.14	0.77
Chlorophyll Measurement			0.20	0.65	0.15	0.89
Plant Height (cm)			0.16	0.68	0.16	0.85
Yield (MT/ha)			0.16	0.69	0.15	0.83
NUE			0.16	0.70	0.14	0.81
Anthesis-Silking Interval (GDUs)			0.18	0.62	0.20	0.92
Chlorophyll Measurement	Marion-IA, 2010		0.16	0.73	0.11	0.77
Plant Height (cm)			0.18	0.65	0.18	0.89
Yield (MT/ha)			0.15	0.72	0.14	0.79
NUE			0.09	0.80	0.11	0.64

[†]:Small, medium and large are the percentage of low performing lines with non desirable root characteristics [$\leq \bar{x} - s.d.$], lines with medium performance [$\geq \bar{x} - s.d.$] to [$\leq \bar{x} + s.d.$]; high performing lines with desirable traits [$\geq \bar{x} + s.d.$], respectively.

Table 4: Estimates of variance components and broad sense heritabilities (h_c^2), for seedling and adult plant traits of 74 maize lines combined over experiments and nitrogen levels.

		Variance Components				h_c^2
		Lines	Lines×Experiment	Lines × Nitrogen	Error	
Shoot length (cm)		8.72**	1.28**	0.0 ^{ns}	10.07	0.80
Primary root length (cm)		7.35**	0.40 ^{ns}	0.61*	4.53	0.87
Crown root length (cm)		139.81**	34.25**	0.0 ^{ns}	208.23	0.71
Seminal root length (cm)		405.92**	99.74**	0.0 ^{ns}	503.64	0.78
Lateral root length (cm)		544.49**	0.0*	0.0 ^{ns}	589.25	0.76
Total root length (cm)		1323.**	221.33**	0.0 ^{ns}	1551.6	0.80
Shoot dry weight (mg)		600.77**	32.26**	0.0 ^{ns}	578.62	0.83
Root dry weight (mg)		187.94**	0.0 ^{ns}	0.0 ^{ns}	135.24	0.89
Total plant biomass (mg)		1366.5**	7.25**	0.0 ^{ns}	1104.8	0.87
Anthesis-Silking Interval (GDUs)	Ames – IA, 2010	1028.1**	na	1343.7**	3802.0	0.39
Chlorophyll Measurement		13.05**	na	9.43**	11.21	0.63
Plant Height (cm)		277.87**	na	79.99**	78.04	0.82
Yield (MT/ha)		0.52**	na	0.75**	0.94	0.46
Anthesis-Silking Interval (GDUs)	Marion – IA, 2010	597.57**	na	217.16 ^{ns}	1574.6	0.54
Chlorophyll Measurement		11.49**	na	0.11 ^{ns}	26.11	0.64
Plant Height (cm)		433.61**	na	39.05**	116.62	0.90
Yield (MT/ha)		0.72**	na	0.44**	0.39	0.70

* significant at $P=0.05$; **significant at $P=0.01$; ns - non significant; na - not applicable

Table 5: Phenotypic correlation coefficients among various pairs of the seedling and adult plant traits (Ames-IA location) measured in 74 maize inbred lines grown in low nitrogen (above diagonal) and high nitrogen (below diagonal) treatment.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Anthesis-Silking Interval		-0.32**	0.00 ^{ns}	-0.34**	-0.01 ^{ns}	-0.01 ^{ns}	0.19 ^{ns}	0.00 ^{ns}	-0.02 ^{ns}	0.04 ^{ns}	0.02 ^{ns}	0.14 ^{ns}	0.07 ^{ns}
2	Chlorophyll Measurement	-0.28*		0.09 ^{ns}	0.51**	0.10 ^{ns}	0.00 ^{ns}	-0.02 ^{ns}	0.08 ^{ns}	-0.09 ^{ns}	0.01 ^{ns}	0.17 ^{ns}	0.12 ^{ns}	0.16 ^{ns}
3	Plant Height (cm)	-0.24*	0.18 ^{ns}		-0.08 ^{ns}	-0.02 ^{ns}	-0.14 ^{ns}	-0.06 ^{ns}	0.11 ^{ns}	-0.18 ^{ns}	-0.05 ^{ns}	0.14 ^{ns}	0.08 ^{ns}	0.13 ^{ns}
4	Yield (MT/ha)	-0.36**	0.38**	0.24*		0.05 ^{ns}	0.13 ^{ns}	-0.01 ^{ns}	0.24*	-0.13 ^{ns}	0.08 ^{ns}	0.05 ^{ns}	0.07 ^{ns}	0.06 ^{ns}
5	Shoot length (cm)	-0.10 ^{ns}	0.13 ^{ns}	0.11 ^{ns}	0.11 ^{ns}		0.53**	0.18 ^{ns}	0.47**	0.39**	0.64**	0.71**	0.66**	0.73**
6	Primary root length (cm)	-0.13 ^{ns}	0.02 ^{ns}	-0.02 ^{ns}	0.18 ^{ns}	0.67**		0.19 ^{ns}	0.30**	0.42**	0.58**	0.34**	0.48**	0.42**
7	Crown root length (cm)	0.08 ^{ns}	0.19 ^{ns}	-0.02 ^{ns}	0.09 ^{ns}	0.45**	0.30*		0.38**	0.01 ^{ns}	0.48**	0.27*	0.41**	0.35**
8	Seminal root length (cm)	-0.03 ^{ns}	0.02 ^{ns}	0.09 ^{ns}	0.36**	0.45**	0.39**	0.42**		-0.08 ^{ns}	0.62**	0.42**	0.65**	0.54**
9	Lateral root length (cm)	-0.14 ^{ns}	-0.08 ^{ns}	0.07 ^{ns}	-0.13 ^{ns}	0.48**	0.54**	0.09 ^{ns}	-0.08 ^{ns}		0.67**	0.32**	0.33**	0.35**
10	Total root length (cm)	-0.06 ^{ns}	0.03 ^{ns}	0.09 ^{ns}	0.15 ^{ns}	0.73**	0.70**	0.66**	0.62**	0.64**		0.59**	0.76**	0.70**
11	Shoot dry weight (mg)	0.05 ^{ns}	0.19 ^{ns}	0.09 ^{ns}	0.13 ^{ns}	0.82**	0.53**	0.43**	0.43**	0.40**	0.66**		0.77**	0.96**
12	Root dry weight (mg)	0.06 ^{ns}	0.20 ^{ns}	0.10 ^{ns}	0.23*	0.75**	0.56**	0.55**	0.59**	0.37**	0.79**	0.85**		0.91**
13	Total seedling biomass (mg)	0.05 ^{ns}	0.20 ^{ns}	0.10 ^{ns}	0.16 ^{ns}	0.82**	0.56**	0.48**	0.49**	0.40**	0.72**	0.99**	0.93**	

* significant at $P<0.05$; **significant at $P<0.01$; ns-non significant.

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Supplementary table1: Hoagland solution recipe used in the study

	Formula	Mol. Wt	Per liter of nutrient solution
Potassium nitrate	KNO_3	101.1	5 ml of 1 M
Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	236.15	5 ml of 1 M
Monopotassium phosphate	KH_2PO_4	136.09	1 ml of 1 M
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	246.47	2 ml of 1 M
Micronutrient stock solution			1 ml of stock solution
Iron chelate	Fe-EDTA		1-5 ml of 1000 mg/l
Minus nitrogen			
10% nitrogen	$Ca(NO_3)_2 \cdot 4H_2O$	236.15	0.75 ml of 1 M
Monocalcium phosphate	$Ca(H_2PO_4)_2 \cdot H_2O$	252	10 ml of 0.05 M
Calcium sulfate dihydrate	$CaSO_4 \cdot 2H_2O$	172.17	200 ml of 0.01 M
Potassium sulfate	K_2SO_4	174.26	5 ml of 0.5 M
Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	246.47	2 ml of 1 M
Micronutrient stock solution			1 ml of stock solution
Iron chelate	Fe-EDTA		1-5 ml of 1000 mg/l
Micronutrient stock solution			Per litre
Boric acid	H_3BO_3		2.86 g
Manganese chloride – 4 hydrate	$MnCl_2 \cdot 4H_2O$		1.81 g
Zinc sulfate – 7 hydrate	$ZnSO_4 \cdot 7H_2O$		0.22 g
Copper sulfate – 5 hydrate	$CuSO_4 \cdot 5H_2O$		0.08 g
85% Molybdic acid	MoO_3		0.02 g

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