Research Article

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Lateral root elongation enhances nitrogen-use efficiency in maize genotypes at the seedling stage

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Abstract

BACKGROUND: Maize plants show great variation in root morphological response to nitrogen (N) deficit, and such alterations often determine N-use efficiency (NUE) plants. This study assessed genotypic variation in root morphology and NUE in selected 20 maize genotypes with contrasting root system size grown in a semi-hydroponic phenotyping system for 38 days under control (4 mmol L^{-1} NO_3^-) and low N (LN) (40 µmol L^{-1}) for 38 days after transplanting.

RESULTS: Maize genotypes exhibited different responses to LN stress in each of the 28 measured shoot and root traits. The 20 genotypes were assigned into one of the three groups: N-efficient (eight genotypes), medium (four genotypes), and N-inefficient (eight genotypes), based on shoot dry weight ratio (the ratio of shoot dry weight at LN and control) ± one standard error. In response to LN stress, the N-inefficient genotypes had significant reduction in biomass production by ~58% in shoots and ~64% in roots, while the N-efficient genotypes maintained their biomass. Under LN supply N-efficient genotypes showed a plasticity response that would result in both sparse lateral branching and increased root elongation as a whole or at each growth strata, and N efficiency positively correlated with lateral or axial root elongation and root elongation at different depths.

CONCLUTSION: The total lateral root length was the main contributor to the improved N foraging and utilization in maize under LN conditions, followed by axial root length. Total lateral root length can be considered in breeding programs for producing maize cultivars with high NUE at the early seedling stage.

Supporting information may be found in the online version of this article.

Keywords: biomass; root system architecture; N utilization; root elongation; genotypic variation

INTRODUCTION

Nitrogen (N) is the quantitatively most important nutrient for crop plants. Of around 100 million tons of N applied annually in agricultural fields on earth, only 30–40% can be utilized by crops.[1,2] Given the loss of most N fertilizers leading to environmental pollution as well as increase of cost, breeding crop cultivars with efficient N uptake under less N supply is of pressing concern in sustainable agriculture.

Root plasticity is critical for increasing nutrient uptake. Maize root system comprises both embryonic (a primary root and seminal roots) and post-embryonic shoot borne-crown crown roots or nodal roots. The primary, seminal, and nodal roots are also called 'axial', which all develop higher order lateral branches, namely, lateral roots (LRs).[3,4] Maize changes its root system architecture when cultivated under low N (LN) stress. For example, LN stress was extensively documented to increase average length of axial or nodal roots,[5-8] while decreasing the number of nodal roots.[5,7-10]

LR growth contributes largely to nutrient uptake and root system architecture.[11,12] The response of LR growth to LN stress are inconsistent in maize. LN stress was shown to decrease the total length of LRs in a solution culture,[5,9,13] while other researchers found LR elongation was increased by LN stress in aeroponic or sand culture,[7,8] and under extended LN supply the first-order LR density was decreased,[5] or increased.[8,14]

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Root growth angle is crucial for its spatial distribution, and is a major determinant of root foraging depth in the soil.[15,16] In response to N deficiency, root growth angle of maize becomes steeper presumably for enhanced foraging of N in deeper soil horizons.[17]

Phenotypic variation in root systems exists for the capture and utilization of plant nutrients including N, and provides genetic resources for developing genotypes with improved performance cultivating in suboptimal nutrient conditions.[18,19] Root growth of maize, particularly at early seedling stage, is able to cope with various stressors like N deficiency through the efficient foraging of nutrients accessible via water mass flow.[20,21] Screening for genotypic variability in root traits have proven essential in assessing efficiency for N utilization in maize under control or field conditions.[10,17,22-27] Few studies, however, have investigated the correlation between variation in root-related traits (except root biomass and total root length) and N status (N-use efficiency, NUE) in maize, and particularly root traits for potential use as screening criteria for breeding N-efficient maize cultivars under conditions of limited N availability. In this study, we employed 20 maize genotypes possessing varying root morphologies that were selected from a root phenotyping study of 174 genotypes[28] using an established semi-hydroponic system.[29] Our objectives were to assess the morphological changes in root growth responding to LN stress and NUE in maize at the seedling stage, and to identify specific root traits that may contribute to their NUE.

MATERIALS AND METHODS

Plant materials

Twenty maize genotypes with contrasting root morphology (root biomass and total root length mainly) and shoot biomass were selected from our recent study of characterizing variability in root traits among 174 genotypes.[28] The selected genotypes were Dongke301, Dongyue116, Huayu11, Jindan52, Liyu39, NS2020, NS3314, NS590b, NS770, Pinyu3, Shandan2002, Shengrui999, Shenyu26, Xinyu106, Xundan29, Zhengdan958, Zhongke11, 923×911, 926×911, and ay120-113.

Plant culture and N treatment

The semi-hydroponic phenotyping system[29] was used in this study. The system was recently used for studying maize root system, such as phenotyping root trait variability,[28] root response to salinity stress,[30] low phosphorus and cadmium stress (unpublished). Each bin system comprised a 240 L container (wheelie bin, length × width × depth: 60 cm × 40 cm × 100 cm) and 20 growth pouches. The growth pouch contained a 5 mm thick acrylic panel (24 cm × 50 cm) enveloped with black calico cloth (51 cm × 60 cm). Each bin was supplied with 25 L low concentration nutrient solution (in μmol L^{-1}): 600 K_2SO_4, 200 $MgSO_4$, 600 $CaCl_2$, 20 KH_2PO_4, 5 H_3BO_3, 0.75 $ZnSO_4$, 0.75 $MnSO_4$, 0.2 $CoSO_4$, 0.2 $CuSO_4$, 0.03 Na_2MoO_4, and 20 EDTA-Fe. An automatic pumping and irrigation system provide water and nutrients to the growth pouches for plant growth during the experiment.

Seeds of each maize genotype were surface sterilized with 10% hydrogen peroxide (H_2O_2) for 10 min, rinsed thoroughly with tap water, and sown in washed sand at room temperature. Water was applied daily to maintain optimum sand moisture for seed germination. When the roots reach approximately 2 cm long (around 4 days after sowing), eight uniform seedlings of each genotype were carefully transplanted into the growth pouches of bins. Each growth pouch within a single bin system accommodated one plant of the same genotype with 20 plants of 20 genotypes per bin.

There were two N treatments applied in nutrient solution as calcium nitrate ($Ca(NO_3)_2$) at 2 mmol L^{-1} (sufficient N, control) and 20 μmol L^{-1} (LN). For LN treatment, calcium chloride ($CaCl_2$) was supplemented to maintain the same calcium ion (Ca^{2+}) concentrations as in the control.[5] A randomized factorial design was used consisting of two N treatments (factor one) and 20 maize genotypes (factor two), with four replicates per treatment (eight bin systems in total). The bin positions were changed weekly to reduce possible environmental effects.

Measurements of plant morphological traits

Plants were assessed at 38 days after transplanting (DAT). At harvest, stem width was manually recorded. Plants with root systems were photographed after removing the wrapped cloth. Shoots were separated and oven-dried at 70 °C for 72 h to determine shoot dry weight (in mg $plant^{-1}$). Primary root length, and maximal root width (the maximal horizontal width of a root system) were measured with a ruler. Number of seminal and crown roots was counted and their lengths were measured. The length of the apical unbranched zone in the primary roots was recorded by the distance from root tip to the point where visible first-order laterals appear. All first-order laterals along the axial roots were counted at 2 cm intervals, then the lateral density (in cm^{-1}) on primary, seminal and nodal roots were calculated. Nodal root angle from the horizontal (degree from the horizontal nodal root follows) was manually measured from the root images with a protractor.

Root subsamples in 20 cm sections were excised from the root base in sequence and stored at 4 °C until scanning. Samples of root sections were floated and spread in a transparent tray (21 cm × 28 cm), and scanned in grayscale at 300 dpi with a scanner (Epson Perfection V800, Long Beech, CA, USA). The root images were analyzed using WinRHIZO Pro (2012b, Regent Instruments, Quebec City, Canada) to generate morphology data including root length, root average diameter, root tips, root surface area and root volume for each section. The root sections from the same plant were combined and oven-dried to obtain root dry weight (in mg $plant^{-1}$). The following root traits were calculated from the earlier measured data:

Total length of LRs = total root length – total length of axial roots.

Average seminal root length = total seminal root length/seminal root number.

Average nodal root length = total nodal root length/nodal root number.

Specific root length = total root length/root dry mass.

Root-to-shoot ratio = root dry mass/shoot dry mass.

Shoot N content

To measure shoot N content, oven-dried leaf samples were milled. Then 0.8–1.2 g milled samples and 1.9 g catalyzer ($K_2SO_4/CuSO_4/Se$ = 100:10:1) were added into a digestion tube, and digested with 5 mL sulfuric acid at 365 °C for 60 min until the solution was translucent. After cooling to room temperature, N content (in mg g^{-1} dry weight) was determined by the standard macro-Kjeldahl procedure using Kjeltec 8400 analyzer (Foss Tecator AB, Hoganan, Sweden).

Table 1. Analysis of variance (ANOVA) for 27 shoot and root morphological traits and shoot nitrogen (N) content in 20 maize genotypes cultivated for 38 days in a semi-hydroponic system under sufficient N (control) and low N (LN) treatments. The sources of variation were N, genotype, and their interactions (ns, not significant)

	Unit	N	Genotype	N × Genotype
Shoot dry weight	mg plant^{-1}	<0.001	<0.001	0.624 ns
Stem width	mm	<0.001	<0.001	0.596 ns
Root dry weight	mg plant^{-1}	0.001	<0.001	0.679 ns
Root-to-shoot ratio		0.217 ns	<0.001	0.889 ns
Root width	cm	0.444 ns	<0.001	0.042
Nodal root angle	°	<0.001	0.219 ns	0.796 ns
Primary root length	cm	0.989 ns	<0.001	0.033
Average seminal root length	cm	0.046	<0.001	0.791 ns
Average nodal root length	cm	0.78 ns	<0.001	0.558 ns
Total axial root length	cm	0.075 ns	<0.001	0.368 ns
Total root length	cm	0.853 ns	<0.001	0.084 ns
Total lateral root length	cm	0.362 ns	<0.001	0.121 ns
Seminal root number		0.047	<0.001	0.455 ns
Nodal root number		0.006	<0.001	0.107 ns
Lateral density on primary roots	cm^{-1}	0.706 ns	0.009	0.605 ns
Lateral density on seminal roots	cm^{-1}	<0.001	0.132 ns	0.261 ns
Lateral density on nodal roots	cm^{-1}	<0.001	0.011	0.204 ns
Apical unbranched zone of primary roots	cm	<0.001	<0.001	0.62 ns
Specific root length	cm mg^{-1}	<0.001	<0.001	0.696 ns
Root diameter	µm	0.027	<0.001	0.652 ns
Root surface area	cm^2	0.085 ns	<0.001	0.171 ns
Root volume	cm^3	0.003	<0.001	0.373 ns
Root length at 0–20 cm depth	cm	0.879 ns	<0.001	0.17 ns
Root length at 20–40 cm depth	cm	0.777 ns	<0.001	0.225 ns
Root length at 40–60 cm depth	cm	0.898 ns	<0.001	0.251 ns
Root length below 60 cm depth	cm	0.596 ns	<0.001	0.39 ns
Shoot N content	mg g^{-1}	<0.001	0.002	0.111 ns

Evaluation of relatively N-efficient and N-inefficient genotypes

The N efficiency in 20 genotypes was evaluated using the ratio of shoot dry weight under LN and the control treatments: genotypes with a mean shoot dry weight ratio greater than one standard error above or below their median value were grouped as N-efficient and N-inefficient, respectively. The calculated N efficiency quantifies the relative N-deficiency tolerance or NUE in maize genotypes.[31,32] The grouped genotypes were ranked for N efficiency, together with their increment/reduction percentages in measured growth traits (shoot or biomass, stem width, axial root length, etc.) and shoot N contents under LN supply relative to the N sufficient controls.

Statistical analyses

Data were analyzed by the analysis of variance (ANOVA) procedure of SPSS 17.0 (IBM, Armonk, NY, USA). A two-factor ANOVA in general linear model multivariate analysis was conducted to analyze the effects of N treatments and genotypes on shoot and root morphological traits, and shoot N content. Pearson's correlation was applied to examine the relationships between the variables.

RESULTS

The multivariate analyses revealed significant differences in seedling traits among maize genotypes and between N treatments ($P \leq 0.05$, Table 1). Significant interactions between genotypes and N treatments were only observed in root width and primary root length.

The N efficiency among genotypes

The N efficiency, assessed using the ratio of shoot dry weight at deficient N supply to shoot dry weight under the control, varied significantly between the 20 genotypes, ranging from 0.42 to 0.99 (Fig. 1(A,B)). Based on the significance interval separated at one standard error above or below the median genotype value, eight, four and eight genotypes were assigned into N-efficient, medium, and N-inefficient genotype groups, respectively. The N-efficient genotypes comprised Shengrui999, Xundan29, Zhongke11, NS2020, Shandan2002, Shenyu26, Dongke301 and Dongyue116; the N-inefficient genotypes comprised NS3314, 923×911, Zhengdan958, NS770, 926×911, NS590b, Xinyu106 and Liyu39; and the medium genotypes comprised Jindan52, Huayu11, ay120-113 and Pinyu3 (Supporting Information Fig. S1).

Shoot traits

Shoot dry weight across the 20 genotypes ranged from 198 to 481 mg plant^{-1} under control and 157 to 461 mg plant^{-1} under LN conditions (Supporting Information Table S1). LN treatment decreased shoot dry weight the most (by ~58%) in N-inefficient genotypes, more so (by ~16%) in medium N-efficient genotypes, and less (by ~9%) in N-efficient genotypes (Table 2). Stem width across the 20 genotypes ranged from 3.3 to 5.2 mm under control

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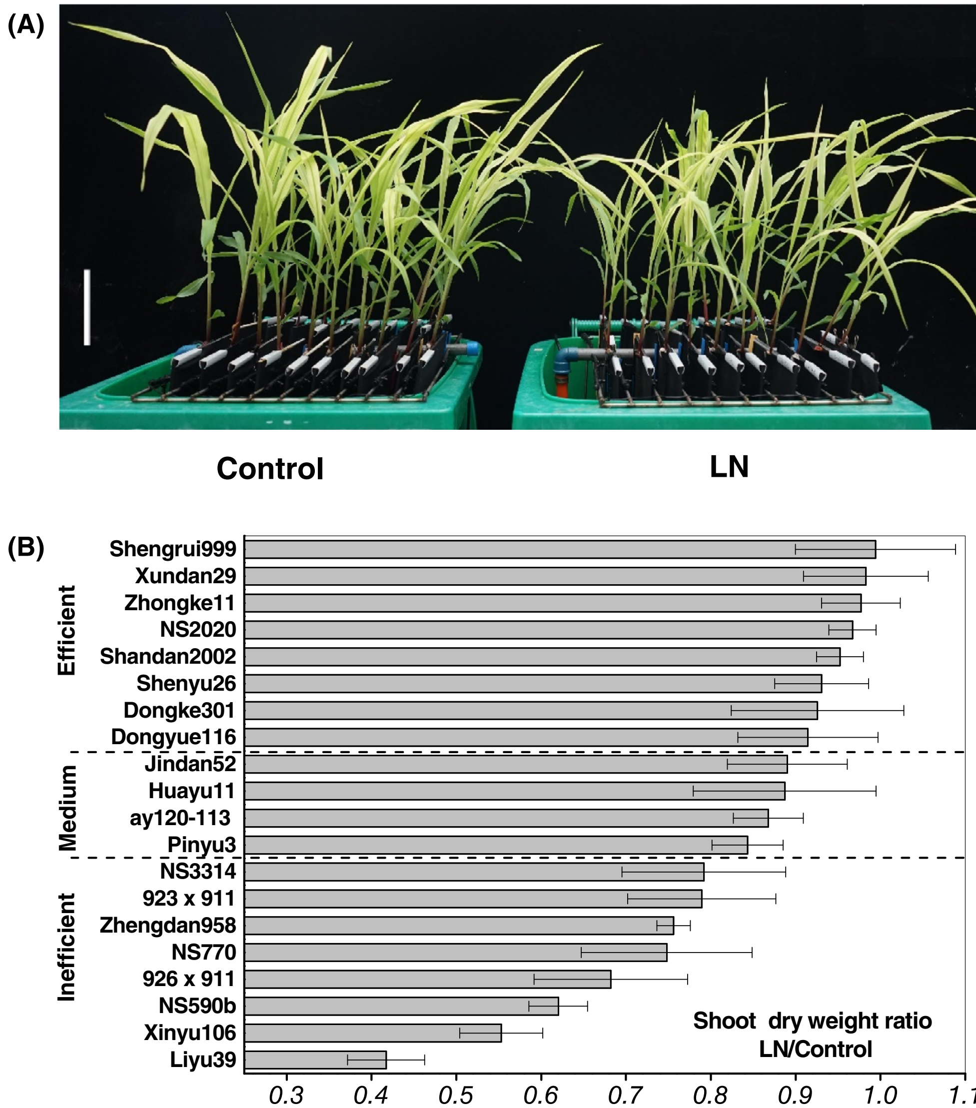

Figure 1. (A) Plant shoot responses to low nitrogen (LN) stress (right) of 20 maize genotypes compared to those under sufficient nitrogen (N) (control, left) 38 days after transplanting in a semi-hydroponic system in a glasshouse. Bar = 20 cm. (B) Nitrogen (N) efficiency (relative shoot dry weight under LN and the control treatments) for 20 maize genotypes cultivated for 38 days in a semi-hydroponic system. The horizontal dashed lines separated N efficiency interval at one standard error above or below the median genotype value. Data are means ± standard error of four replicate plants. Control: sufficient N treatment; LN, low N treatment.

and 3.1 to 5.1 mm under LN conditions (Table S1). Under LN treatment, stem width decreased the most (by ~30%) in N-inefficient genotypes, and the least (by ~10%) in N-efficient genotypes, or remained unchanged in some N-efficient or medium genotypes including Xundan29, Zhongke11 and ay120-113 (Table 2).

Root biomass production and root-to-shoot ratio

Root dry weight across the 20 genotypes ranged from 80.6 to 238 mg plant^{-1} under control and 64.1 to 222 mg plant^{-1} under LN conditions (Table S1). Under LN treatment, N-inefficient genotypes showed most reduction (by ~64%) in root dry weight, whereas most N-efficient or medium genotypes maintained their root dry weight (Table 2). The root-to-shoot ratio was differently affected in the 20 genotypes when exposed to N deficiency (Table S1). Compared to the corresponding control genotypes, in some genotypes such as Jindan52, 926×911 and ay120-113, the root-to-shoot ratio increased, whereas in other genotypes such as Liyu39 and Zhongke11, the root-to-shoot ratio decreased or remained unchanged (Table 2). The large variation in shoot and root dry weight between N treatments and among the genotypes, may explain the fluctuating pattern in root-to-shoot ratio of genotypes under LN treatment.

Root width and nodal root angle

Root width varied across the 20 genotypes from 8.4 to 20.4 cm under control and 7.3 to 20.3 cm under LN conditions (Table S1). LN treatment generally decreased root width the most in N-inefficient genotypes such as Liyu39 (39%), 926×911 (29%) and Xinyu106 (20%), but increased it the most in N-efficient or medium genotypes such as Jindan52 (72%), Huayu11 (41%) and Shandan2002 (27%), although genotypic variation existed within each group (Table 2). Similar results were obtained in root surface area and root volume (Tables S2 and S3).

Nodal root angle across the 20 genotypes ranged from 57.3° to 67.7° under control and 62.3° to 74.3° under LN conditions (Table S1). Under LN treatment, nodal root angle increased

Table 2. Percentage changes of shoot dry weight, stem width, root dry weight, root-to-shoot ratio, root width, nodal root angle, primary root length, average seminal root length and average nodal root length in 20 maize genotypes cultivated for 38 days in a semi-hydroponic system under low nitrogen (LN) relative to sufficient nitrogen (N) (control) treatments. The grouped genotypes with contrasting N efficiency were ranked from up to down based on the value of N efficiency from high to low among the 20 genotypes

Genotype	Shoot dry weight	Stem width	Root dry weight	Root-to-shoot ratio	Root width	Nodal root angle	Primary root length	Average seminal root length	Average nodal root length
Shengrui999	*−1*	−6	*−4*	**−4**	−3	14	−4	10	4
Xundan29	*−2*	*0*	*−4*	**−2**	*18*	**4**	*28*	*38*	1
Zhongke11	*−2*	*0*	*−3*	0	17	**8**	−4	6	*31*
NS2020	*−3*	*−7*	−10	**−5**	−13	**2**	*16*	22	*25*
Shandan2002	*−5*	*−2*	0	5	*27*	*25*	0	*59*	8
Shenyu26	−7	−4	*−1*	*8*	1	**8**	1	*24*	**−18**
Dongke301	−7	*−2*	−7	**−2**	5	*15*	3	22	−12
Dongyue116	−9	−10	−9	0	−7	*22*	*11*	23	0
Jindan52	−11	**−15**	*−1*	*16*	*72*	12	−6	15	*18*
Huayu11	−11	−6	−7	5	*41*	**0**	**−10**	*47*	*68*
ay120-113	−13	*0*	−4	*9*	*22*	11	*33*	13	**−21**
Pinyu3	−16	−5	−13	5	**−19**	**7**	*15*	*24*	−9
NS3314	−21	−6	−16	4	−15	*17*	*11*	15	−11
923×911	−21	−10	**−22**	**−2**	−1	*16*	**−19**	6	4
Zhengdan958	−24	−12	−19	*8*	−10	9	3	**−17**	*19*
NS770	**−25**	−13	**−22**	4	**−19**	9	**−14**	11	−5
926×911	**−32**	**−14**	**−25**	*12*	**−29**	*15*	−1	**−15**	**−25**
NS590b	**−38**	**−26**	**−36**	7	**−19**	9	−1	**−5**	−13
Xinyu106	**−45**	**−24**	**−46**	0	**−20**	11	**−17**	**−39**	**−17**
Liyu39	**−58**	**−30**	**−64**	**−13**	**−39**	14	**−30**	**−19**	**−40**

Note: For each trait, the top five percentage changes appear in bold typeface form and the bottom five are in italic typeface.

the most in some N-efficient genotypes such as Shandan2002 (25%) and Dongyue116 (22%), followed by some N-inefficient genotypes such as NS3314 (17%) and 923 × 911 (16%), and the least in some N-efficient genotypes such as NS2020 (2%) and Xundan29 (4%), or remained unchanged in the medium N-efficient genotype Huayu11, compared to the corresponding control (Table 2).

Axial and LR length

Primary root length across the 20 genotypes ranged from 45.5 to 84 cm under control and 41 to 86.8 cm under LN conditions (Table S4). In general, N deficiency decreased primary root length the most in N-inefficient genotypes such as Liyu39 (30%), 923×911 (19%) and Xinyu106 (17%), but increased it the most in N-efficient and medium genotypes such as ay120-113 (33%), Xundan29 (28%) and NS2020 (16%), and there existed a great genotypic variation within different groups (Table 2). The effect of LN treatment on the average seminal and nodal root length was similar (Tables 2 and S4), although large genotypic variation existed in seminal and nodal root number (Tables S2 and S3). As a result, under LN conditions, the total axial root length generally decreased the most in N-inefficient genotypes, but increased the most in N-efficient or medium genotypes (Tables 3 and S4). A similar result was observed in the total root length, and the total LR length mirrored by the results of the total root length as well (Tables 3 and S4).

LR density along axial roots

Across the 20 genotypes, lateral density on primary roots varied from 4.9 to 6.3 under control and 4.6 to 6.1 under LN conditions (Table S5). Compared to the corresponding control, under N deficiency lateral density on primary roots decreased the most in some N-inefficient genotypes including Liyu39 (10%) and 923×911 (10%) but also increased the most in some other N-inefficient genotypes including NS3314 (13%) and Zhengdan958 (12%) (Table 3). A similar pattern was observed in the N-efficient genotype groups likewise. The similar result was obtained for the root diameter (Tables S2 and S3).

Across the 20 genotypes, lateral density on seminal roots ranged from 4.3 to 5.3 under control and 2.8 to 4.2 under LN conditions (Table S5). LN treatment decreased lateral density on seminal roots the most in N-inefficient or medium genotypes such as Zhengdan958 (39%) 923×911 (38%) and Pinyu3 (38%), and the least in N-efficient genotypes such as NS2020 (7%) and Zhongke11 (19%), although genotypic differences existed within different groups (Table 3).

Across the 20 genotypes, lateral density on nodal roots ranged from 4.3 to 5.9 under control and 3.6 to 4.6 under LN conditions (Table S5). LN treatment decreased lateral density on nodal roots the most in some N-inefficient genotypes like Xinyu106 (32%) and NS590b (26%) but also the least in some other N-inefficient genotypes like NS3314 (13%) and NS770 (15%) (Table 3). A similar pattern was likewise observed in the N-efficient genotype groups. Similar results were found for the seminal and nodal root number (Tables S2 and S3).

Length of apical unbranched zone of primary roots and specific root length

Across the 20 genotypes, length of apical unbranched zone on primary roots ranged from 7.9 to 15.1 cm under control and 9.4 to 16.6 cm under LN conditions (Table S5). Under LN treatment,

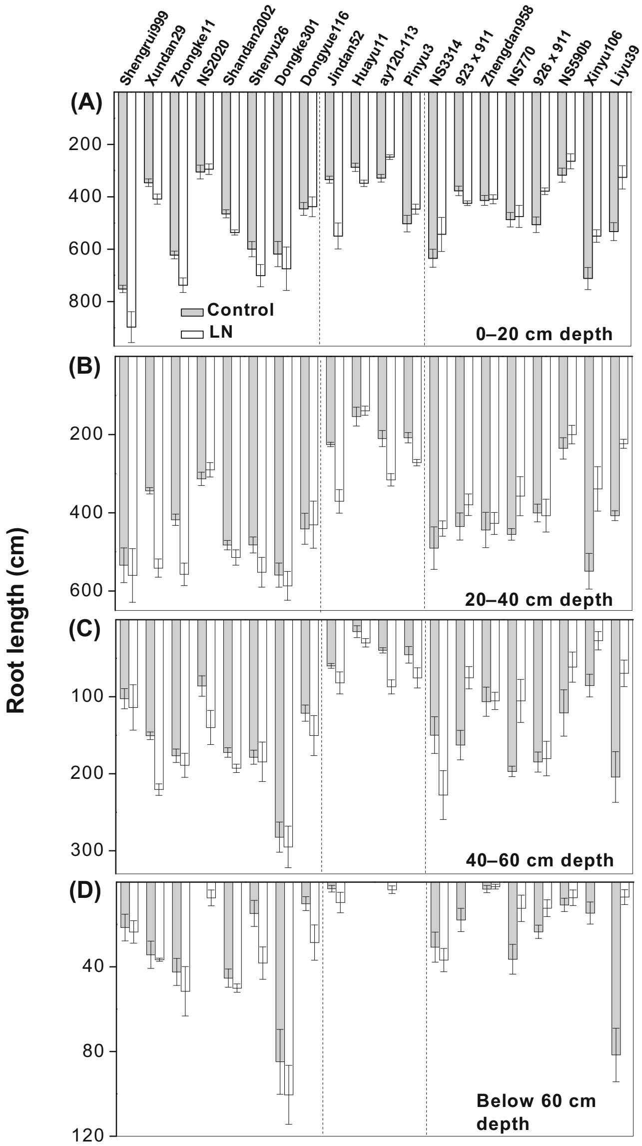

Figure 3. Root length variation at each 20-cm depth in upper 0–60 cm layer and below 60 cm depth for 20 maize genotypes cultivated for 38 days in a semi-hydroponic system under sufficient nitrogen (N) (control) and low N (LN) treatments. Data are means ± standard error of four replicate plants.

length of apical unbranched zone of primary roots increased the most in some N-efficient or medium genotypes such as Huayu11 (43%), Jindan52 (39%) and Dongke301 (37%), and the least in some N-efficient or inefficient genotypes such as Shengrui999 (1%) and Liyu39 (2%), compared to their corresponding control, although genotypic variation existed within different groups (Table 3).

Specific root length varied from 4.5 to 6.9 cm mg^{-1} across the 20 genotypes under control and 6.4 to 9.8 cm mg^{-1} under LN conditions (Table S5). LN treatment increased specific root length the most in some N-efficient or medium genotypes including Jindan52 (60%) and Xundan29 (47%) or the N-inefficient genotype Liyu39 (44%), the least in some N-efficient or inefficient genotypes including NS770 (5%) and Shandan2002 (11%), and genotypic variation existed within different groups (Table 3).

Shoot N status

Nitrogen deficiency caused decreases in shoot N accumulation in the maize genotypes, and different genotypes had different responses (Fig. 2). Across the 20 genotypes, shoot N content ranged from 35.2 to 42.9 mg g^{-1} under control and 29.2 to 34.6 mg g^{-1} under LN conditions. LN treatment decreased shoot N content the most in some N-inefficient genotypes including 923×911 (26%) and NS770 (22%) or the medium genotype ay120-113 (25%), the least in some N-efficient or inefficient genotypes including Liyu39 (7%) and Shenyu26 (15%), and there existed genotypic variation within different groups.

Root elongation in different rooting depths

Most maize genotypes tended to decrease in root length at lower rooting depth regardless of N treatment (Fig. 3). After subjected to N deficiency, root length in the upper layer, 0–20 cm and 20–40 cm, was decreased in N-inefficient genotypes (except 923×911 at 0–20 cm depth and 926×911 at 20–40 cm depth), but increased in all N-efficient genotypes except NS2020 and Dongyue116 and most medium N-efficient genotypes. The root length ranged from 248 to 897 cm in the 0–20 cm layer and 139 to 587 cm in the 20–40 cm layer across the 20 genotypes under LN conditions (Fig. 3). However, in the deeper layer (40–60 cm and below 60 cm), N-inefficient genotypes (except NS3314) had less root length under LN treatment than the same genotypes grown under control condition, whereas N-efficient and medium genotypes had more root length under LN treatment than under the control. The root length ranged from 27 to 295 cm in the 40–60 cm layer and 0–100 cm in the layer below 60 cm depth across the 20 genotypes under LN conditions (Fig. 3).

Correlation analysis

The relationships among N-efficiency, morphological traits and shoot N contents were analyzed (Table 4). In the N sufficient control, N-efficiency was positively correlated with shoot N content, and negatively correlated with shoot dry weight, root dry weight, stem width, root width, average nodal root length, total axial root length, total LR length, total root length, root surface area, root volume, and root length in different rooting depth layers (except the layer below 60 cm depth), respectively.

However, in the LN treatment, N-efficiency was positively correlated with shoot dry weight, root dry weight, stem width, root width, primary root length, average seminal root length, total axial root length, total LR length, total root length, root surface area, root volume, and root length in different rooting depth layers, but not significantly correlated with average nodal root length and shoot N content.

DISCUSSION

As one of the essential mineral elements, N limitation has a strong effect on plant growth and development.[33,34] In the current study, when exposed to LN stress, shoot dry weight was decreased more in N-inefficient genotypes, such as Liyu39 and Xinyu106, than in N-efficient or medium genotypes, such as Shengrui999, Xundan29 and Jindan52 (Tables 2 and S1). As a consequence, N-efficient or medium genotypes maintained their shoot growth and thus stem width under LN conditions. Similarly, root dry weight was more inhibited by LN supply in N-inefficient genotypes, but less affected in most N-efficient and medium genotypes (Tables 2 and S1). Thus, during LN stress, N-efficient and medium genotypes mostly had increased or maintained root

Table 3. Percentage changes of total axial root length, total root length, total lateral root length, lateral density on primary, seminal and nodal roots, length of apical unbranched zone of primary roots and specific root length in 20 maize genotypes cultivated for 38 days in a semi-hydroponic system under low nitrogen (LN) relative to sufficient nitrogen (N) (control) treatments. The grouped genotypes with contrasting N efficiency were ranked from up to down based on the value of N efficiency from high to low among the 20 genotypes

Genotype	Total axial root length	Total root length	Total lateral root length	Lateral density on primary roots	Lateral density on seminal roots	Lateral density on nodal roots	Length of apical unbranched zone of primary roots	Specific root length
Shengrui999	*9*	13	14	*9*	−31	−17	**1**	17
Xundan29	−1	*39*	*62*	0	−30	−18	19	*47*
Zhongke11	*22*	*23*	*23*	−6	*−19*	*−15*	4	*26*
NS2020	−15	4	13	2	*−7*	*−9*	**1**	**16**
Shandan2002	−2	11	15	**−9**	*−22*	**−26**	14	**11**
Shenyu26	*14*	*16*	16	−2	−24	−23	20	19
Dongke301	**−19**	7	*17*	*12*	*−22*	*−15*	*37*	18
Dongyue116	−14	3	7	**−8**	−32	**−29**	*29*	17
Jindan52	*44*	*62*	*68*	−2	*−22*	*−13*	*39*	*60*
Huayu11	*17*	12	9	−4	−23	*−7*	*43*	21
ay120-113	−9	*24*	*54*	6	**−34**	−22	7	*32*
Pinyu3	**−22**	5	16	2	**−38**	−21	*26*	22
NS3314	−9	−4	−3	*13*	*−20*	*−13*	**2**	17
923×911	8	**−12**	**−19**	**−10**	**−38**	−22	13	**15**
Zhengdan958	**−24**	−2	11	*12*	**−39**	**−25**	13	23
NS770	−11	**−19**	**−22**	*−7*	−24	*−15*	20	**5**
926×911	−17	**−12**	−6	*7*	−33	−23	4	**14**
NS590b	−15	**−22**	**−25**	−6	**−34**	**−26**	12	19
Xinyu106	**−18**	**−33**	**−36**	**−9**	−28	**−32**	**2**	23
Liyu39	**−66**	**−49**	**−43**	**−10**	−28	−17	**2**	*44*

Note: For each trait, the top five percentage changes appear in bold typeface form and the bottom five are in italic typeface.

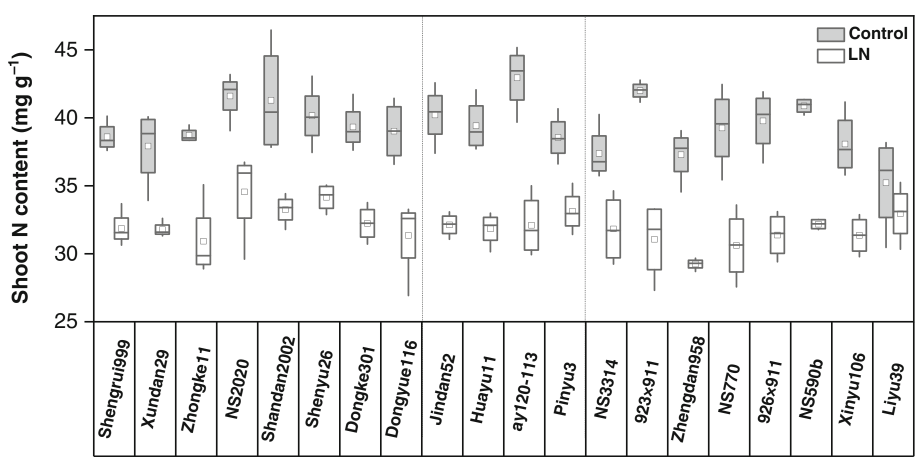

Figure 2. Shoot nitrogen (N) content in 20 maize genotypes cultivated for 38 days in a semi-hydroponic system under sufficient N (control) and low N (LN) treatments. □ represents the mean ($n = 4$).

width, surface area and volume, compared with the corresponding control genotypes. The architectural response of roots allows plants to optimize N acquisition and utilization in conditions of LN availability.[19,20,35] Under LN stress, the positive and differential relationships found between the seedling N efficiency and stem width ($r = 0.48$), root width ($r = 0.24$), surface area ($r = 0.52$) and volume ($r = 0.49$) (Table 4), suggest the varying effects of these traits on the foraging and utilization of N for maize plants under suboptimal N availability. The nodal root angle became steeper in most of the tested genotypes under LN supply, as compared to the corresponding genotypes under control (Tables 2 and S1). It follows that maize plants subjected to N deficiency tend to develop a steep root growth angle to promote deep rooting and thus N foraging.[15,17]

As reported by Wang *et al*., when grown at LN supply, maize plants may possess longer or shorter axial roots.[9] In general, N-efficient and medium genotypes in our study had increased or maintained total axial root length, while N-inefficient genotypes had reduced total axial root length under LN stress, relative to the corresponding control (Tables 3 and S4). The reason for this

Table 4. Pearson correlation coefficients for nitrogen (N) efficiency, shoot and root morphological traits, and shoot N content of maize genotypes in the sufficient N (control) and low N (LN) treatments

	N efficiency		Shoot N content	
	Control	LN	Control	LN
Shoot N content	0.23*	0.04		
Shoot dry weight	−0.29**	0.57**	−0.42**	−0.23*
Stem width	−0.30**	0.48**	−0.28*	−0.22
Root dry weight	−0.29*	0.51**	−0.42**	−0.27*
Root-to-shoot ratio	−0.08	−0.07	−0.08	−0.15
Root width	−0.36**	0.24*	−0.35**	−0.09
Nodal root angle	0.14	−0.06	0.06	0.05
Primary root length	−0.19	0.34**	−0.35**	−0.08
Average seminal root length	−0.04	0.29*	0.06	−0.13
Average nodal root length	−0.33**	0.17	−0.24*	−0.38**
Total axial root length	−0.34**	0.33**	−0.33**	−0.41**
Total root length	−0.30**	0.53**	−0.36**	−0.13
Total lateral root length	−0.25*	0.52**	−0.32**	−0.04
Seminal root number	−0.23*	−0.06	−0.17	−0.12
Nodal root number	−0.11	0.16	−0.18	−0.17
Lateral density on primary roots	−0.15	−0.03	−0.15	−0.02
Lateral density on seminal roots	−0.12	0.18	0.12	0.36**
Lateral density on nodal roots	−0.23*	0.11	−0.09	−0.06
Apical unbranched zone of primary roots	−0.13	0.07	−0.27*	−0.20
Specific root length	0.01	−0.11	0.12	0.32**
Root diameter	−0.18	0.10	−0.19	−0.36**
Root surface area	−0.32**	0.52**	−0.38**	−0.21
Root volume	−0.32**	0.49**	−0.38**	−0.27*
Root length at 0–20 cm depth	−0.26*	0.42**	−0.39**	−0.09
Root length at 20–40 cm depth	−0.27*	0.45**	−0.23*	−0.15
Root length at 40–60 cm depth	−0.23*	0.51**	−0.22*	−0.06
Root length below 60 cm depth	−0.11	0.45**	−0.34**	−0.07

Note: N efficiency was evaluated as the ratio of shoot dry weight under LN and the control treatments. * and ** significant at $P \le 0.05$ and $P \le 0.01$, respectively.

effect was that the average length of primary, seminal and nodal roots was generally increased in N-efficient and medium genotypes but was reduced in N-inefficient genotypes by LN stress (Tables 2 and S4). The seedling N efficiency under LN stress showed a positive correlation to primary root length ($r = 0.34$) and average seminal root length ($r = 0.29$), rather than average nodal root length (Table 4), which implies that in terms of axial roots, primary and seminal roots play a vital role in the N foraging and utilization in maize during early growth under suboptimal N conditions. It was previously reported that LR growth in maize responded inconsistently to N deficiency.[5,7-9,13] In our study, total LR length increased in N-efficient and medium genotypes, but decreased in N-inefficient genotypes (except Zhengdan958) after LN treatment (Tables 3 and S4). The result was that in response to LN stress the N-efficient and medium genotypes mostly exhibited longer total root length, whereas the opposite was found in N-inefficient genotypes (Tables 3 and S4). The similar pattern was observed in the root elongation of N-efficient or inefficient genotypes in different growth layers, especially in the deeper layer (Fig. 3). In addition, generally limiting N supply was also accompanied by the decreased lateral density on seminal and nodal roots (though the lateral density on the primary roots were differently affected) (Tables 3 and S5). Therefore, we concluded that N-efficient maize genotypes have a plasticity response that would lead to not only fewer laterals,[36] but also enhanced root elongation both as a whole and at different growth strata under LN conditions.

Enhancement in root elongation is commonly regarded as an important strategy for acquisition of mobile resources like water and nitrate in leaching environments.[19,20] Therefore, N-efficient genotypes having longer axial or LRs are better able to capture mobile nutrient N and improve N uptake per unit root length. Since LRs demand more metabolically per gram of tissue than axial roots, an optimal degree of LR development could balance the need for resource exploration and exploitation with their metabolic demands and the consequent effects on the development of other root classes.[20] A model analysis using *SimRoot* also indicates that sparse (with an optimum at 2–7 branches cm^{-1}) LR branching should allow internal reallocation of carbon and other resources for axial and/or LR elongation.[36] This can improve N capture efficiency per unit of root length in maize plants by reducing strong competition between neighboring LRs for limited N, and increasing carbon budgets of plants for greater growth of other root system classes including axial roots or the shoot. Under LN stress, the seedling N efficiency was more correlated with total LR length ($r = 0.52$) than with total axial root length ($r = 0.33$)

(Table 4), reflecting the greater contribution of total LR length to the N acquisition/utilization and thus plant growth under N deficient conditions. This is supported by the results of Chun *et al*. and Gaudin *et al*.,[7,8] but contradicts with other studies on maize plants in which total LR length was declined due to LN stress.[5,9] These inconsistencies may be a consequence of the different cultivation methods, intensity and duration of N stress used in the experiments. Further, it was found that under LN stress, root length in each foraging layer positively correlated with the seedling N efficiency (Table 4), implying that the root elongation at different rooting depths could contribute to the N capture and utilization in maize under LN supply.

The apical unbranched zone in primary roots was normally elongated in all the genotypes grown under LN stress (Tables 3 and S5), which suggests that N deficiency could induce an increase in the elongation zone of primary roots in maize plants.[5,22] The elongation zone, however, is reduced in response to other abiotic stressors including phosphorus deficiency, drought and excess sodium.[37] Similarly, the specific root length was increased in all tested maize genotypes under LN treatment (Tables 3 and S5), hence, it can be concluded that the increment of specific root length is another critical acclimation response to LN stress[8] likewise. This decreased the investment for biomass per unit root length, and may indirectly influence NUE through the regulation of root volume, since the negative correlation ($r = -0.30$) existed between specific root length and root volume only under LN stress (Table S6).

Under LN supply, the shoot N content was decreased in maize genotypes (Fig. 2). However, no significant correlation was observed between the N accumulation in shoots and **s**eedling N efficiency (Table 4). This probably indicates that shoot N accumulation was not a direct limiting factor to enhancement in N-efficiency of maize plants grown under N deficiency. It is hypothesized that shoot N content functions as a signal controlling root morphogenesis, such as root-to-shoot ratio in tobacco[38] and axial root elongation in maize.[5] In the current study, we found a positive relationship between shoot N content and specific root length under LN stress (Table 4), suggesting that specific root length of maize plants can be positively regulated by low N availability.

CONCLUSIONS

This study evaluated genotypic variability in 20 maize genotypes contrasting in root morphology subjected to LN stress during the early growth. Among them, eight N-efficient, four medium, and eight N-inefficient genotypes were identified based on their differences in N efficiency (relative shoot dry weight at deficient and sufficient N supply). The stem width, root width, total root length, root surface area, root volume, and root length in different rooting depths contributed differently to N efficiency in maize. The improved N foraging and utilization in maize, depending on axial root elongation though, was mainly attributed to the increase in total LR length under LN conditions. The selected N-efficient genotypes, such as Shengrui999, Xundan29 and Zhongke11, have potentials for breeding high-yielding N-efficient maize hybrids in future.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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