

## Root growth in response to nitrogen supply in Chinese maize hybrids released between 1973 and 2009

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Root growth has a fundamental role in nitrogen (N) use efficiency. Nevertheless, little is known about how modern breeding progress has affected root growth and its responses to N supply. The root and shoot growth of a core set of 11 representative Chinese maize (*Zea mays* L.) hybrids released between 1973 and 2009 were investigated under high N (4 mmol L<sup>-1</sup>, HN) and low N (0.04 mmol L<sup>-1</sup>, LN) levels in a solution culture system. Compared with LN, HN treatment decreased root dry weight (RDW), the root: shoot ratio (*R/S*), and the relative growth rate for root dry weight (RGR<sub>root</sub>), but increased the total root length (TRL) and the total lateral root length (LRL). The total axial root length (ARL) per plant was reduced under HN, mostly in hybrids released before the 1990s. The number of seminal roots (SRN) was largely unaffected by different N levels. More recently released hybrids showed higher relative growth rates in the shoot under both HN and LN. However, the roots only showed increased RGR under HN treatment. Correspondingly, there was a positive linear relationship with the year of hybrid release for TRL, LRL and ARL under HN treatment. Together, these results suggest that while shoot growth of maize has improved, its root growth has only improved under high N conditions over the last 36 years of selective breeding in China. Improving root growth under LN conditions may be necessary to increase the N use efficiency of maize.

### Root, *R/S* ratio, nitrogen, relative growth rate, maize breeding

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Genetic improvement has played a significant role in increasing maize grain yield over the last 50 years [1–6]. The average maize grain yield has increased linearly by about 100 kg (ha year)<sup>-1</sup> in the USA between 1939 and 2004 [2,7]. In China, the average genetic yield gain was 84–124 kg (ha year)<sup>-1</sup> for maize grown in North China since 1970 [5,8]. In maize breeding programs, plants are routinely grown with high levels of nitrogen (N) fertilizer because N is one of the most important nutrients limiting plant growth. However, the intensive use of N fertilizers in agriculture has and will continue to have major detrimental impacts on the environment

because of the inefficient use of N by crops, which typically use approximately one-third of total N applied [9].

Roots play a vital role in anchoring the plant and in water and nutrient uptake from the soil. Therefore, they directly affect yield [10]. Genotypic differences in root morphology at the seedling growth stage can markedly influence nitrate acquisition [11,12]. Genetic control of maize root traits has been shown in many studies [13]. Interestingly, some of the QTLs associated with root length density, root diameter, secondary lateral root number, and vertical root pulling resistance were closely linked with QTLs for leaf greenness and for nitrogen uptake [14–16]. For various reasons, however, the genetic variations in root traits have been ne-

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glected in modern breeding processes. Increasing crop yields through applying more fertilizer and water has reached a maximum [17]. To achieve high yielding and eco-friendly agriculture, there is significant interest in improving the capacity of plants to uptake and use nutrients, and the focus should be on improving the function of the root system [17,18].

Although some authors hypothesized that root systems of cereals could have suffered from intense selection pressure for increased above-ground biomass or grain yield [19], little is known about effects of breeding pressure on the ability of the root system to adapt to variations in environmental conditions, for example to variations in N availability [20]. In wheat, modern dwarf varieties show reduced investment in root dry matter and root length density, and thus, have a lower root to shoot ratio early in the growing season [19]. In maize, the importance of a good root system has been recognized since the beginning of modern breeding [21]. Despite the significant genetic variations in root characteristics and architecture in maize [22,23], there has been little progress made in using root traits as selection criteria to indirectly improve yield [24]. This is because of the complexity of root systems and the difficulties of investigating roots of a large number of plants, especially in field studies. Using a modeling approach, Hammer [25] provided evidence for the concept that, in comparison to shoot architecture, a change in root system architecture has the greater effect on increasing maize yield when water is available at sowing.

Evaluating root growth in the field is compounded by the high heterogeneity of soil conditions and environment× genotype interactions [26]. Root development at the seedling stage is very important for plant establishment and is closely related to grain yield [27]. In addition, root characteristics at an earlier ontogeny show higher heritability than those at later stages [28]. Feil [29] suggested that seedling root traits might be used as early selection criteria for water use efficiency in breeding studies. In this study, we aimed to evaluate whether the high N inputs used in modern maize

breeding programs affect root growth, and hence, N use efficiency. We selected 11 representative maize hybrids released from 1973 to 2009 in China to investigate root growth traits and root responses to variations in N availability using a solution culture system.

## 1 Materials and methods

### 1.1 Plant materials

Table 1 shows the 11 maize (*Zea mays* L.) hybrids released from 1973 to 2009 that were used in this study. These hybrids were the dominant hybrids used in Chinese agriculture at the time of their release. The planting area for each of the hybrids exceeded 5000000 ha in China between 1980 and 2009 [30,31]. Except for Xianyu 335, which was bred by the Pioneer Company Ltd., all the other hybrids were developed by Chinese breeders.

### 1.2 Plant culture

Seeds were sterilized in 10% (v/v) H<sub>2</sub>O<sub>2</sub> for 30 min, washed with distilled water, and then soaked in saturated CaSO<sub>4</sub> solution with continuous aeration supplied by an electric pump for approx. 12 h. Then, seeds were placed between sheets of filter paper soaked with CaSO<sub>4</sub> solution and germinated in the dark at room temperature. When the roots were approx. 1 cm long, uniform seedlings were wrapped in filter paper and carefully transferred into a plastic container filled with distilled water for continued growth. As soon as two leaves were visible the seedlings were transferred into porcelain pots containing 2 L nutrient solution. Their endosperms were removed at this time. These plants were grown in a growth chamber under the following conditions: 28/22°C, 14/10 h light/dark cycle. During the light cycle, the photosynthetic photon flux density was 250–300 μmol m<sup>-2</sup> s<sup>-1</sup> at canopy height. Plants were grown in a nutrient solution with the following constituents (mmol L<sup>-1</sup>): 0.75 K<sub>2</sub>SO<sub>4</sub>, 0.1

**Table 1** Characteristics of hybrids used in this experiment

Hybrids	Parents	Breeders	Year of release
Zhongdan 2	Mo17×Zi330	Chinese Academy of Agricultural Sciences	1973
Huang 417	Huangzao4×Mo17	Beijing Academy of Agricultural and Forestry Sciences	1978
Danyu 13	Mo17×E28	Academy of Agricultural Sciences in Dandong, Liaoning Province	1979
Shendan7	5003×E28	Academy of Agricultural Sciences in Shenyang, Liaoning Province	1982
Nongda 60	5003×Zong31	Chinese Agricultural University	1985
Yedan 13	Ye478×Dan340	Academy of Agricultural Sciences in Laizhou, Shandong Province	1989
Nongda 108	178×Huang C	Chinese Agricultural University	1991
Zhengdan 958	Zheng58×Chang7-2	Academy of Agricultural Sciences in Henan Province	1996
Jundan 20	9058×Jun92-8	Academy of Agricultural Sciences in Henan Province	1998
Xianyu 335	PH6WC×PH4CV	Pioneer Co., Ltd.	2000
Denghai 661	DH351×DH372	Shandong Denghai Seeds Co., Ltd.	2009

KCl, 0.25 KH<sub>2</sub>PO<sub>4</sub>, 0.65 MgSO<sub>4</sub>, and 0.13 EDTA-Fe, and (in  $\mu\text{mol L}^{-1}$ ): 1.0 MnSO<sub>4</sub>, 1.0 ZnSO<sub>4</sub>, 0.1 CuSO<sub>4</sub>, and 0.005 (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>. We used a completely randomized design, with four replicate pots (5 plants/pot) for each treatment. Plants were supplied with one-half strength complete nutrient solution containing 4.0 mmol L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> for 1 d and then supplied with complete nutrient solution for another 4 d. Two plants from each pot were sampled at this stage, and the remaining three plants were supplied with either 0.04 (low N, LN) or 4.0 mmol L<sup>-1</sup>NO<sub>3</sub><sup>-</sup> (high N, HN), respectively, for another 6 d. The nitrate concentration of 4 mmol L<sup>-1</sup> is approximately equal to the mean nitrate level in soil solution [32]. As determined in our previous work [12], an NO<sub>3</sub><sup>-</sup> supply at 4 mmol L<sup>-1</sup> is optimal for maximizing root growth. Nitrate was added to the substrate in the form of Ca(NO<sub>3</sub>)<sub>2</sub>. In the low NO<sub>3</sub><sup>-</sup> treatments, Ca<sup>2+</sup> was complemented by adding CaCl<sub>2</sub> to the same level as in the 4.0 mmol L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> treatments. For all growth solutions, the pH was adjusted to 6.0±0.1 with KOH. The nutrient solution was renewed every other day and was aerated continuously by an electric pump.

### 1.3 Sampling and measurements

Plants were sampled on the first day of N treatments and again on the sixth day. Shoots and roots were separated by cutting at the mesocotyl. At the second harvest, the lower two leaves of LN plants were yellow, a typical symptom of N-deficiency. Leaf length and width were measured with a ruler and leaf area was calculated by the following formula: leaf length×maximum width×*k*, where *k* is a shape factor with a value of 0.5 for partially unfolded leaves and 0.75 for completely unfolded leaves [33]. Shoots were then dried at 70°C to constant weight and weighed. The relative growth rate (RGR) was determined as described by Botella *et al.* [34], as follows:

$$\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1), \quad (1)$$

where *W*<sub>1</sub> and *W*<sub>2</sub> are the dry biomass at the first (*t*<sub>1</sub>) and second (*t*<sub>2</sub>) samplings and ln is the natural logarithm. The RGR for shoot dry weight (RGR<sub>shoot</sub>) was then calculated.

We counted the number of axial roots, including seminal roots and crown roots, and measured root length with a ruler. The roots were then scanned (Epson V700, Beijing, China). During scanning, the root was placed in a glass dish containing water to untangle the roots and to minimize root overlap. Images were analyzed for total root length per plant (TRL) using WinRHIZO (Vision Pro 5.0a, Canada). The total length of the lateral root (LRL) was calculated from the difference between TRL and ARL. The roots were then dried and weighed to calculate the RGR for root dry weight (RGR<sub>root</sub>) according to formula (1). The root to shoot ratio (*R/S*) was calculated by the ratio of root dry weight to shoot dry weight at the final harvest. The ratio of LN/HN for both

SDW and RDW was calculated, and these values were used as indicators of responses to low N supply.

### 1.4 Statistical analysis

The experimental data were analyzed by analysis of variance using the SAS statistics system (SAS, 1994). Differences were compared using LSD at the *P*<0.05 probability level. Shoot and root parameters were plotted against the year of hybrid release, and regression analysis was conducted using simple linear models (*Y*=*a*+*bX*). For the regression analysis, a few irregular data that deviated significantly from the linear relationship were omitted (as indicated in figures).

## 2 Results

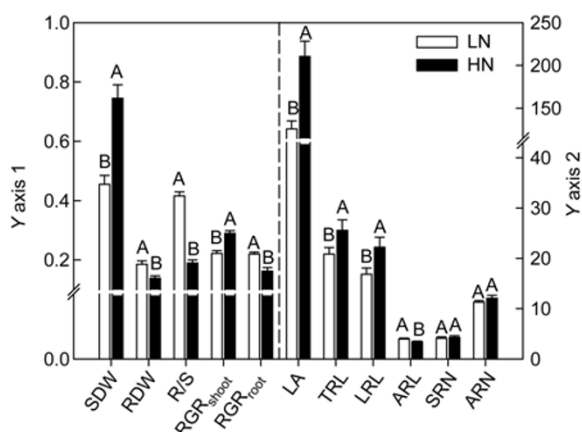
### 2.1 Effect of nitrogen×genotype (hybrid) interaction on shoot and root growth

The effect of N treatment was significant for all the root and shoot parameters tested except for the number of seminal roots (SRN) and total axial roots number (ARN) (Table 2). Compared with LN, HN increased shoot dry weight (SDW), relative shoot growth rate (RGR<sub>shoot</sub>), leaf area (LA), total root length (TRL), and total length of lateral roots (LRL), but reduced root dry weight (RDW), relative root growth rate (RGR<sub>root</sub>), root to shoot ratio (*R/S*) and total length of axial roots (ARL) (Figure 1). The genotype had a significant influence on all the root and shoot growth attributes, suggesting that both shoot and root traits have greatly changed over the past decades. The N×genotype interactions were

**Table 2** Variance analysis of effects of N, genotype (hybrid), and N×genotype interaction on shoot and root growth parameters of maize<sup>a)</sup>

Source of variation	N	Genotype (hybrid)	N×genotype
Shoot biomass	***	***	NS
Root dry weight	***	***	***
Root/shoot ratio	***	***	NS
Relative growth rate for shoot biomass	***	***	NS
Relative growth rate for root dry weight	***	***	**
Leaf area	***	***	***
Total root length	***	***	***
Total lateral root length	***	***	***
Total axial root length	***	***	***
Total axial root number	NS	***	NS
Seminal root number	NS	***	NS

a) We used 11 hybrids released in different areas in China. Plants were precultured in complete nutrient solution containing 4.0 mmol L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> for 5 d before being treated with two different NO<sub>3</sub><sup>-</sup> levels, high N (4 mmol L<sup>-1</sup>) and low N (0.04 mmol L<sup>-1</sup>) for another 6 d and then harvested. \*\* and \*\*\* denote significant at the 0.01 and 0.001 probability level, respectively. NS, Not significant.



**Figure 1** Effect of N supply on shoot and root growth traits. SDW ( $\text{g plant}^{-1}$ ), shoot dry matter; RDW ( $\text{g plant}^{-1}$ ), root dry weight; R/S, root to shoot ratio;  $\text{RGR}_{\text{shoot}}$  ( $\text{g d}^{-1} \text{plant}^{-1}$ ), relative growth rate of shoot;  $\text{RGR}_{\text{root}}$  ( $\text{g d}^{-1} \text{plant}^{-1}$ ), relative growth rate of root; LA ( $\text{cm}^2 \text{plant}^{-1}$ ), leaf area per plant; TRL ( $\text{m plant}^{-1}$ ), total root length; LRL ( $\text{m plant}^{-1}$ ), total lateral length; ARL ( $\text{m plant}^{-1}$ ), total axial root length; SRN (number  $\text{plant}^{-1}$ ), seminal root number; ARN (number  $\text{plant}^{-1}$ ), axial root number. SDW, RDW, R/S,  $\text{RGR}_{\text{shoot}}$ , and  $\text{RGR}_{\text{root}}$  were plotted against Y axis 1; LA, TRL, ARL, SRN, ARN were plotted against Y axis 2.

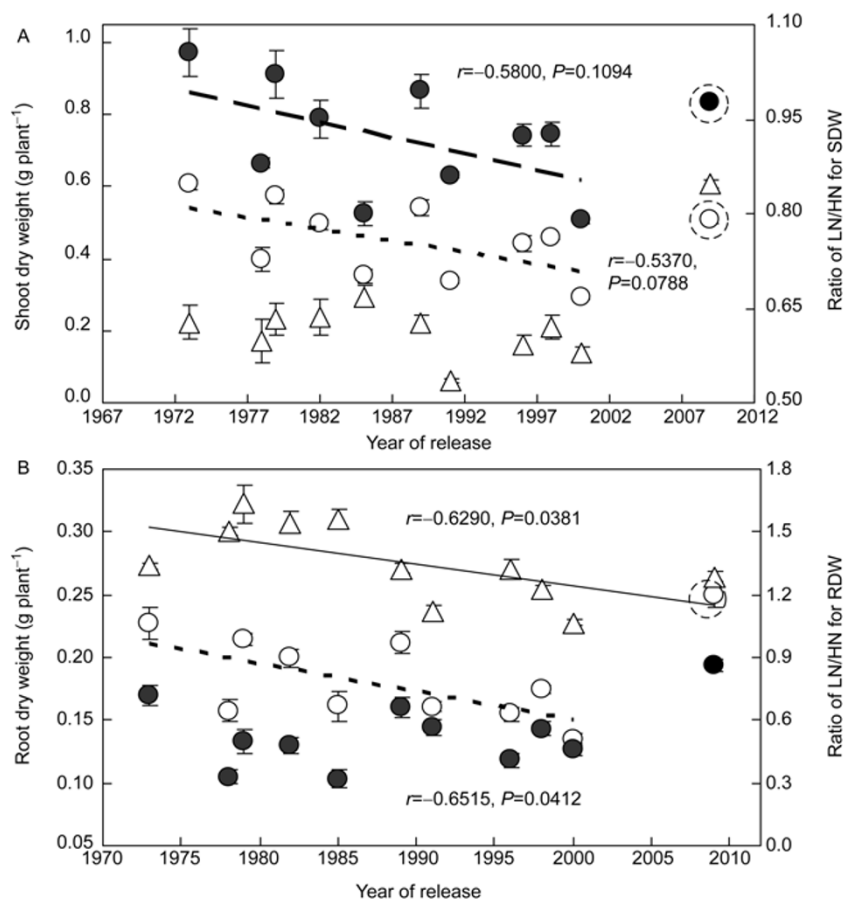
not significant for the following parameters:  $\text{RGR}_{\text{shoot}}$ , SDW, R/S ratio, SRN and ARN (Table 2, Figure 1), suggesting

that the response of the hybrids to N was similar for these parameters. However, there were significant N $\times$ genotype interactions for other traits, especially root traits. These findings imply that the response of root growth and root morphology to N levels differs among hybrids released in different eras.

## 2.2 Change in shoot and root parameters vs. year of hybrid release

### 2.2.1 Shoot and root dry weight

The total shoot dry weight (SDW) of the 11-day-old maize seedlings was lower in more recently released cultivars in both LN ( $r=-0.54$ ,  $P<0.10$ ) and HN ( $r=-0.58$ ,  $P<0.10$ ) treatments (Figure 2), except for the hybrid Denghai 661, which showed a higher value for SDW. Therefore, the response of SDW to low N stress, as indicated by the LN/HN ratio, was similar among hybrids (Figure 2A; Table 2). With increasing year of hybrid release, root dry weight (RDW) was unchanged in HN, but decreased in LN ( $r=-0.65$ ,  $P<0.05$ ) (Figure 2B). That is, the roots of the new hybrids were less responsive to LN, as indicated by the decreasing LN/HN ratio ( $r=-0.63$ ,  $P<0.05$ ).



**Figure 2** Shoot dry weight (SDW, upper panel) and root dry weight (RDW, lower panel) of maize seedlings plotted against year of hybrid release. Data represent the average of four replicates. Open circles denote low N treatment, solid circles denote high N treatments, triangles denote ratio of low N to high N treatment for either SDW or RDW. Dashed line, dotted line, and solid line are linear regressions for HN, LN and LN/HN, respectively. Data point omitted from regression analysis is circled.

### 2.2.2 Root to shoot ratio (*R/S*)

The root to shoot ratio (*R/S*) ranged from 0.38 to 0.49 under LN, and from 0.15 to 0.25 under HN. Regardless of N treatments, the root to shoot ratio (*R/S*) increased with increasing year of hybrid release (Figure 3; Table 2). Thus, the allocation of biomass to roots is greater in newly released hybrids. The only exception was hybrid ZD958, which showed the lowest *R/S* under low N.

### 2.2.3 Relative growth rate of shoot and root

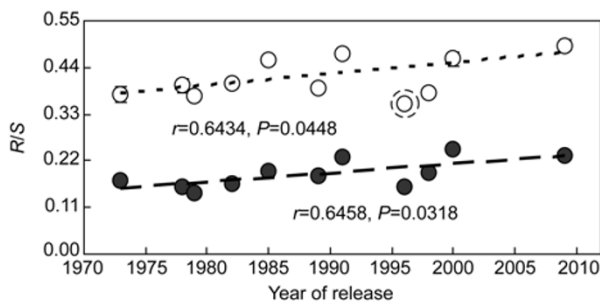
In both N treatments, the relative growth rate for shoot dry weight ( $RGR_{shoot}$ ) of maize seedlings increased with increasing year of hybrid release ( $r=0.70$  and  $0.65$  for LN and HN,  $P<0.05$ , respectively) (Figure 4A). However, the RGR for root dry weight ( $RGR_{root}$ ) was only positively correlated with the year of hybrid release under HN ( $r=0.71$ ,  $P<0.05$ ) (Figure 4B), except for hybrid ZD958, which had the least  $RGR_{root}$ . These data indicate that during the breeding process, root growth rate has generally improved under HN, but not under LN.

### 2.2.4 Leaf area per plant

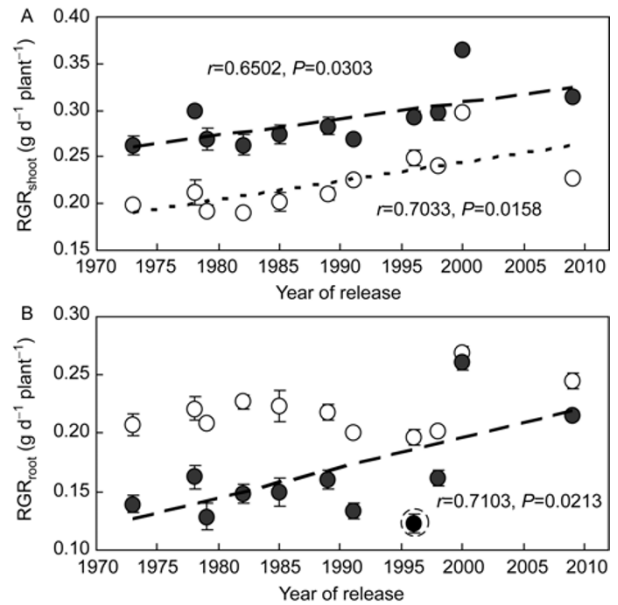
Leaf area (LA) per plant at the seedling stage decreased with increasing year of hybrid release. This trend was the same in both N treatments ( $r=-0.63$  for LN,  $P<0.10$ ; and  $r=-0.65$  for HN,  $P<0.05$ ) (Figure 5), except for hybrid Denghai 661 in which LA increased with increasing year of release. The trend in LA was consistent with that in shoot dry weight (Figure 2). In general, it appears that modern maize hybrids are smaller than older hybrids.

### 2.2.5 Root morphology

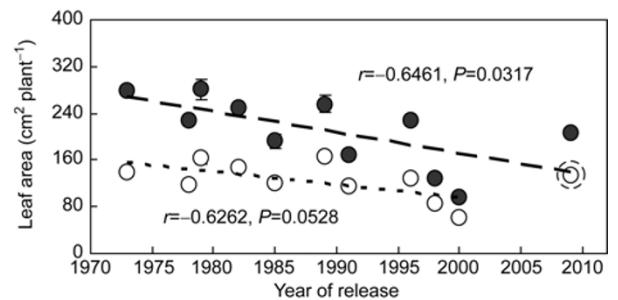
Root morphology significantly changed with increasing year of hybrid release (Figure 6). As shown in Table 2, there was a strong interaction effect of  $N \times$  genotype on root morphological traits. Total root length (TRL) was significantly positively related to increasing year of hybrid release under HN but not under LN (Figure 6A). The same trend was also observed for total lateral root length (LRL) and



**Figure 3** Ratio of root to shoot biomass (*R/S*) of maize seedlings plotted against year of hybrid release. Data represent the average of four replicates. Open circles denote low N treatment; solid circles denote high N treatment. Dashed line and dotted line are linear regressions for HN and LN, respectively. Data point omitted from the regression analysis is circled.



**Figure 4** Relative growth rate for shoot dry weight ( $RGR_{shoot}$ , above) and root dry weight ( $RGR_{root}$ , below) of maize seedlings plotted against year of hybrid release. Data represent the average of four replicates. Open circles denote low N treatment, solid circles denote high N treatment. Dashed line and dotted line are linear regressions for HN and LN, respectively. Data point omitted from the regression analysis is circled.

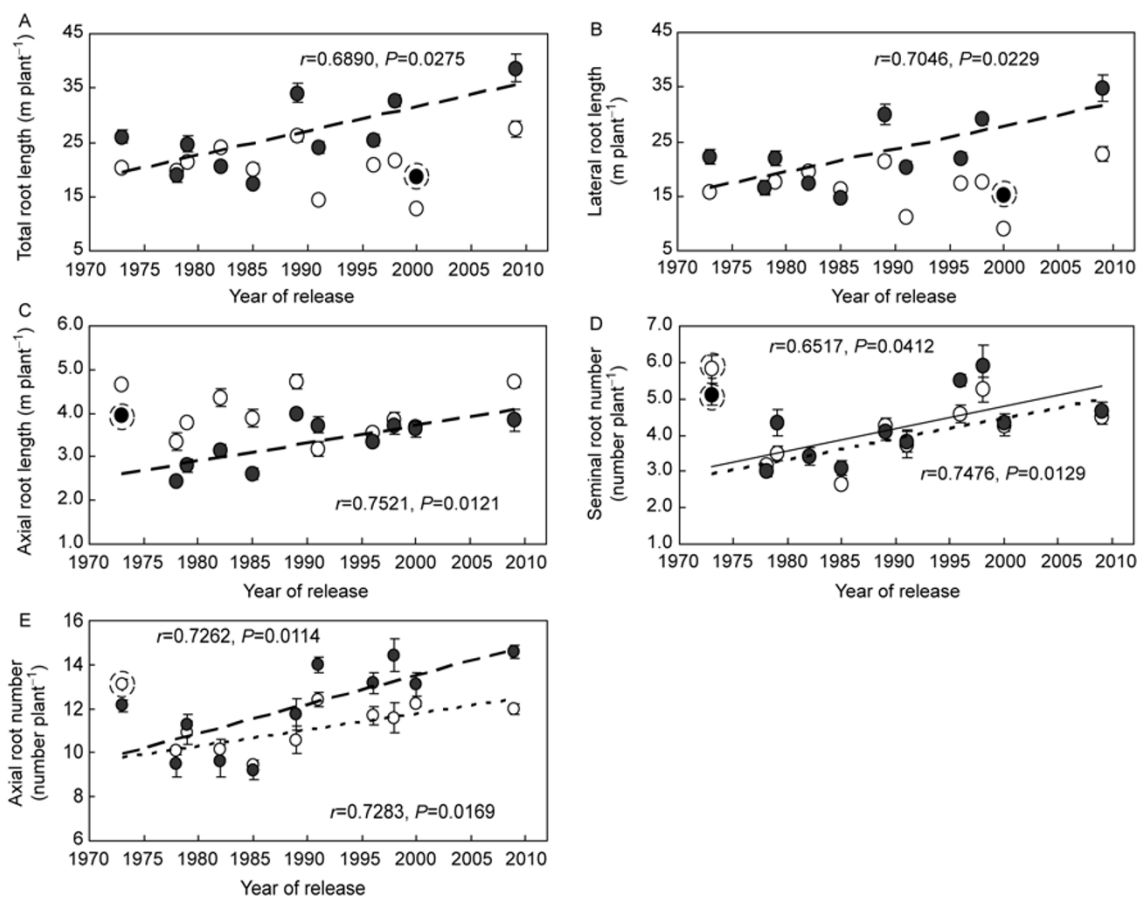


**Figure 5** Leaf area per plant (LA) of maize seedlings plotted against year of hybrid release. Data represent the average of four replicates. Open circles denote low N treatment, full circles denote high N treatment. Dashed line and dotted line are linear regressions for HN and LN, respectively. Data point omitted from the regression analysis is circled.

total axial root length (ARL) (Figure 6B and C). These results suggest that root morphology has generally improved under optimal N input conditions, but has not changed under low N conditions. In fact, under low N conditions, the response of ARL in hybrids released after the 1990s was less than that in hybrids released prior to this (Figure 6C). With the exception of hybrid ZD2, seminal root number (SRN) and total axial root number (ARN) per plant increased with increasing year of hybrid release (Figure 6D and E). Once again, the increase of ARN was greater in HN than in LN conditions (Figure 6E).

## 3 Discussion

A better understanding of the root system is required to op-



**Figure 6** Total root length (A), total lateral root length (B), axial root length (C), number of seminal roots (D) and total number of axial roots (E) of maize seedlings plotted against year of hybrid release. Data represent the average of four replicates. Open circles denote low N treatment, full circles denote high N treatment. Dashed line and dotted line are linear regressions for HN and LN, respectively. Data points omitted from regression analyses are circled.

optimize its function and to overcome the potential restrictions of soil factors (e.g., drought, waterlogging, nutrient heterogeneity, mechanical impedance, and/or pathogens) on the canopy, and to enhance yield [35]. Short-term HN treatment decreased RDW and *R/S*, compared with their respective values in LN (Figure 1), suggesting that less of the total photosynthetic carbon-assimilate was allocated to the roots [29]. However, carbon-assimilates seemed to be used more efficiently in the roots under HN, because of the greater total root length (TRL) and total lateral root length (LRL) (Figure 1). Using different maize inbred lines, Wang *et al.* [12] also observed increases in TRL and LRL under high (0.4 to 4 mmol L<sup>-1</sup>) nitrate supply, compared with low (0.04 mmol L<sup>-1</sup>) nitrate supply. Nevertheless, they did not find a correlation between TRL and N accumulation at high N supply, suggesting that a large root system may not be required for efficient N uptake in those conditions. In the present study, the number of seminal roots (SRN) was barely affected by different N levels (Table 2, Figure 1). However, the total axial root length (ARL) per plant was decreased under HN (Figure 1), indicating that root elongation may be restricted,

and that root systems tended to be shallower at high N supply. This finding is consistent with the results of Tian *et al.* [36,37] who found that plants grown under high N may decrease root elongation via increased cytokinin levels and decreased auxin levels. Taken together, these results suggest that maize plants may adapt to low N environments by allocating more carbon-assimilates to the roots and enhancing axial root elongation so that they can capture the downward-moving nitrates in the soil [38–40].

The significant genotypic effect on shoot and root growth parameters suggests that these traits have changed markedly during the breeding processes in recent decades (Table 2). However, there is little information on the changes in root traits that have occurred during maize breeding processes. Campos *et al.* [41] observed differences in water extraction between old and modern maize hybrids. During a period of water limitation, the old hybrid extracted more water from shallow soil horizons, whereas the new hybrid appeared to be more effective at extracting water at greater depths. This observation indicated that new hybrids may have greater rooting depth than old hybrids. The modeling work by

Hammer [25] suggested that changes in the architecture of the root system and water capture directly affected biomass accumulation and historical yield trends in the USA. Here, we found that values for SDW (Figure 2) and LA (Figure 5) were lower in newly developed hybrids. This may be essential for the establishment of a high density population because smaller leaves help to reduce plant-to-plant competition for light resources. In fact, high-density tolerance has been a major breeding target for maize breeding in recent years in China [42], even though there are still some cultivars with a large shoot biomass (e.g., Denghai 661 in this study). To obtain high yields (approx. 15 ton per hectare), the recommended planting density for modern hybrids (e.g., ZD958 and XY335) is up to 70000 plants per hectare [42]. Interestingly, the smaller shoot dry weight in newly released cultivars had little effect on root dry weight (Figure 2), and as a result, the  $R/S$  ratio increased (Figure 3). Also, the newly developed cultivars had higher TRL, LRL, and ARL under HN input (Figure 6). This improvement in root traits and the root-to-shoot relationship may contribute greatly to high yields and stress tolerance in new hybrids [43].

In this study, we found strong interaction effects of  $N \times$  genotype on root traits. This suggests that the breeding process has significantly affected the root response to N nutrition (Table 2). Although TRL, LRL, and ARL were higher in newly developed hybrids under HN input, they were similar to those of older hybrids under LN conditions (Figure 6), suggesting that the high N conditions used in breeding programs may not improve root morphology. In fact, RDW was significantly lower in newly released hybrids under LN conditions (Figure 2), indicating that the adaptive response of RDW to LN stress decreased with increasing year of hybrid release (Figure 2). Interestingly, the response of axial root length (ARL) to low N stress was only observed in the hybrids released prior to the 1990s, but not in hybrids released afterwards (Figure 6C). Since axial root elongation is essential for nitrate and water uptake in deep soil, these results may indicate that the adaptive response of root growth to low N has been negatively affected during the past 20 years of selective breeding.

From the 1970s to 2009, there has been a strong tendency for seminal root number and total axial root number to increase with the year of hybrid release under both low and high N inputs (Figure 6D and E). This result is consistent with those reported by Sun *et al.* [44], who found a marginal yet significant increase in nodal root number among hybrids released during the 1990s compared with those released during the 1970s. The only exception was XY335, the only hybrid developed by the Pioneer Company. It had fewer axial roots (Figure 6E) and lower TRL and LRL (Figure 6A and B) compared with other hybrids released around the same time. XY335 performs very well at high planting densities [45], but is vulnerable to root-lodging [46,47]. The results in this study provide a reasonable explanation for this phenomenon, i.e., the small root system of

XY335 reduces plant-to-plant competition for nutrient and water resources, but is detrimental for root-lodging resistance. On the other hand, the oldest hybrid (ZD2) had more axial roots, including seminal roots, and longer axial roots compared with other hybrids released in the same period (Figure 6C, D and E). This cultivar is resistant to many stresses and is still planted in large areas of Gansu province, northwestern China, where drought is a serious problem [48].

Root vigor is interpreted as the ability to develop a root system quickly. This trait is linked to the capability of plants to capture  $\text{NO}_3^-$  in soils with a high leaching potential [18]. Vigorous seedling development affects the amount of biomass at harvest in maize [49] and rice [50]. Relative growth rate (RGR) is an effective indicator of seedling vigor. This trait is heritable and is less influenced by environmental factors [51]. In this study, modern hybrids had higher  $\text{RGR}_{\text{shoot}}$  regardless of N treatments (Figure 4), suggesting that the shoot growth vigor has been increased by modern breeding. In the root, however, the genetic gain in seedling  $\text{RGR}_{\text{root}}$  was only seen under sufficient N supply, but not under LN (Figure 4). In fact, root dry weight under LN decreased with increasing year of hybrid release (Figure 2). Thus, root growth traits have been selected unconsciously to adapt and acclimate to the increasing N supply in the environment during conventional breeding, possibly because of the high level of N fertilizer in the breeding fields [52]. Under this high N environment, the early vigorous root growth may enhance early N uptake and help to reduce nitrate leaching. This differs from the situation in wheat, in which the root system of modern dwarf varieties is smaller than that of older taller varieties [19], and may be too small for optimum uptake of nutrients and further yield gains [20]. However, similar situations may occur in some modern maize hybrids, e.g., ZD958 in the present study, which had a lower  $\text{RGR}_{\text{root}}$  and a low  $R/S$  ratio compared with those of hybrids released in the same decade (Figures 3 and 4).

#### 4 Conclusion

Root growth and its response to N availability in Chinese maize hybrids have changed greatly between 1973 and 2009, as a result of high-yield breeding processes. While shoot growth vigor has improved at sufficient and limited N supply, the root growth rate, total root length, total lateral root length, total axial root length, seminal root number and axial root number have only improved under N-sufficient conditions. Furthermore, an adaptive response of axial root length to low N stress was only observed in hybrids released before the 1990s, not in those released afterwards. Therefore, root growth traits have been selected unconsciously to adapt and acclimate to the increasing N supply in the environment over the past few decades. To increase N acquisi-

tion efficiency under limited N supply, root growth rate and root morphology should be taken into consideration in maize breeding programs in the future.

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