A genetic relationship between nitrogen use efficiency and seedling root traits in maize as revealed by QTL analysis

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Abstract

That root system architecture (RSA) has an essential role in nitrogen acquisition is expected in maize, but the genetic relationship between RSA and nitrogen use efficiency (NUE) traits remains to be elucidated. Here, the genetic basis of RSA and NUE traits was investigated in maize using a recombination inbred line population that was derived from two lines contrasted for both traits. Under high-nitrogen and low-nitrogen conditions, 10 NUE- and 9 RSA-related traits were evaluated in four field environments and three hydroponic experiments, respectively. In contrast to nitrogen utilization efficiency (NutE), nitrogen uptake efficiency (NupE) had significant phenotypic correlations with RSA, particularly the traits of seminal roots (r = 0.15–0.31) and crown roots (r = 0.15–0.18). A total of 331 quantitative trait loci (QTLs) were detected, including 184 and 147 QTLs for NUE- and RSA-related traits, respectively. These QTLs were assigned into 64 distinct QTL clusters, and ~70% of QTLs for nitrogen-efficiency (NUE, NupE, and NutE) coincided in clusters with those for RSA. Five important QTLs clusters at the chromosomal regions bin1.04, 2.04, 3.04, 3.05/3.06, and 6.07/6.08 were found in which QTLs for both traits had favourable effects from alleles coming from the large-rooted and high-NupE parent. Introgression of these QTL clusters in the advanced backcross-derived lines conferred mean increases in grain yield of ~14.8% for the line per se and ~15.9% in the testcross. These results reveal a significant genetic relationship between RSA and NUE traits, and uncover the most promising genomic regions for marker-assisted selection of RSA to improve NUE in maize.

Key words: Genetic relationship, nitrogen uptake efficiency, nitrogen use efficiency, QTL analysis, QTL clusters, roots, root system architecture, Zea mays L.

Introduction

Nitrogen (N) is quantitatively the most important mineral nutrient for plant growth and development. In the past decades application of synthetic N fertilizer has increased crop yield significantly. However, N fertilizer production consumes ~1% of the world’s total annual energy supply and results in significant amounts of greenhouse gas emissions (Smith, 2002;
Zhang et al., 2013). Moreover, the overuse of N fertilizer in many regions of the world causes serious damage to the environment, including soil acidification, and water and air pollution (Galloway et al., 2008; Guo et al., 2010; Liu et al., 2013). To simultaneously ensure food security and environmental quality, it is important to cultivate crops that are able to uptake and utilize N efficiently (Good and Beatty, 2011).

Maize (Zea mays L.) is one of the world’s major crops and ~967 million metric tons were produced in 2013 for food, feed, and industrial uses (Ort and Long, 2014). Meanwhile, global maize production consumes almost one-fifth of total N fertilizer (FAO, 2000). In terms of N use efficiency (NUE), genetic variation in the maize germplasm implies that the selection of better NUE varieties can be achieved by breeding processes (Paponov et al., 2005; Uribelarraea et al., 2007). However, developing maize cultivars for NUE traits is challenging because of the genetic complexity and strong interaction with the environment. The general definition of NUE is plant yield in grain per unit of available N in soils (Moll et al., 1982). NUE consists of two main components: N uptake efficiency (NupE), the ability of plants to remove N from the soil; and N utilization efficiency (NutE), the ability of plants to use N to produce grain yield (GY). Correlation studies in maize between these components of NUE have revealed that variation in NupE likely contributes more to variation in NUE under both high-N and low-N conditions, while NutE contributes more at the low-N input (Mi et al., 1998; Bertin and Gallais, 2000; Presterl et al., 2002). In addition, in regions where N fertilizer is overused, maize cultivars with high NupE can help accumulate excess N and subsequently reduce N leaching into the environment.

Roots are essential for the acquisition of mineral nutrients, including N. Effective root system architecture (RSA) is important in breeding maize genotypes for high NupE and helping prevent N leaching (Mackay and Barber, 1986). In maize, the hypothetical ideotype RSA for efficient N acquisition has been proposed in several physiological studies on different genotypes (Mi et al., 2010; Lynch, 2013). In general, increases in root size (root dry weight, root length, and root density) improve N uptake ability and yield formation in maize (Chen et al., 2013; Mu et al., 2015). Given the considerable carbon costs for root growth, the optimal number of crown roots (CRs) and lateral roots (LRs) is essential for N acquisition from low-N soil (Trachsel et al., 2013; Postma et al., 2014; Saengwilai et al., 2014). Besides the morphology, the architecture of roots also plays an important role in N acquisition; for example, a steeper and deeper root more efficiently absorbs N in deep soil layers (Wiesler and Horst 1994; Lynch, 2013; Trachsel et al., 2013). In addition, maize RSA can be strongly influenced by the N availability in the soil to efficiently capture N resources. Under low-N conditions, maize plants reduce the number of CRs but increase the total root length (Feil et al., 1990; Chun et al., 2005; Liu et al., 2008). In N-rich soil, the LRs are stimulated to branch and elongate (Granato and Raper, 1989; Liu et al., 2010). Thus, the components of RSA themselves and their plasticity to soil N availability deserve important consideration as potential traits for genetic improvement of NupE in maize.

Both NUE and RSA are complex traits, depending on both genetic and environmental factors and their interactions. In maize, poor understanding of the genetic basis of NUE as well as limited knowledge about RSA and its relationship with NUE have hindered selection efficiency of RSA-based NUE traits. Recently, mapping quantitative trait loci (QTL) has become a powerful tool to identify genomic regions and even putative candidate genes involved in the genetic variation of complex traits. Many maize QTLs for NUE traits at agronomic and physiological levels have been identified, including traits for NupE and NutE (Bertin and Gallais, 2000; Coque and Gallais 2006; Coque et al., 2008), N grain uptake (Coque et al., 2006), post-silking N-uptake (Gallais and Hirel, 2004; Coque et al., 2008), N remobilization (Gallais and Hirel, 2004; Coque et al., 2006; Coque et al., 2008), and N metabolism (Zhang et al., 2010; Cañas et al., 2012). Likewise, many maize QTLs that regulate RSA have been identified in several linkage populations, and meta-analysis has further determined the putative consensus root-QTL clusters (Lebreton et al., 1995; Landi et al., 2002; Tuberosa et al., 2002, 2003; Zhu et al., 2006; Liu et al., 2008; Hund et al., 2011). Despite these extensive advancements in the genetic knowledge of RSA and NUE, it is not clear if genetic relationships between, and common QTLs for both traits exist. Guingo et al. (1998) evaluated RSA traits for root lodging at flowering stage in the field using a maize recombinant inbred line (RIL) population. Although some of the identified QTLs for root density, diameter, and dry weight were later found to co-localize with QTLs for NUE, the correlations between RSA and NUE at the phenotypic level have not been reported (Coque et al., 2008). Additionally, because it easy to select for root traits at the seedling stage in breeding programmes, establishing the relationship between RSA and NUE at the seedling stage is more promising than at other stages. Therefore, a deep investigation is required to directly uncover the genetic relationship between seedling root traits and NUE traits in a population specific to RSA, and also in response to different levels of N supply.

In the present study, a maize RIL population derived from a cross of two parental lines Ye478 and Wu312 with contrasting NUE and RSA traits was evaluated (Liu et al., 2009). Phenotyping of NUE and RSA in response to two N levels was performed for adult plants in field trials and seedling programmes, establishing the relationship between RSA and NUE at the seedling stage is more promising than at other stages. Therefore, a deep investigation is required to directly uncover the genetic relationship between seedling root traits and NUE traits in a population specific to RSA, and also in response to different levels of N supply.

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**Materials and methods**

**Plant materials**

The RIL population, consisting of 218 F8 lines, was derived from a cross between two inbred lines, Ye478 (female parent) and Wu312 (male parent), as described by Liu et al. (2011). An advanced-backcross line (ABL) population, consisting of 187 BC2F3 lines, was
generated from advanced backcross processes using Ye478 as the donor parent and Wu312 as the recurrent parent (Liu et al., 2011; Cai et al., 2012). Each BC2F2 line was further crossed with a tester line 178 to produce the F1 testcross population. Ye478 was developed in China during the 1990s and was the female parent of more than 50 high-yielding hybrids (Yu and Zhu 1996; Li et al., 2005). Wu312 was derived from an unknown hybrid. In previous studies, Ye478 was characterized as a nitrogen use efficient inbred, and Wu312 as a nitrogen use inefficient inbred (Tian et al., 2006; Liu et al., 2009; Peng et al., 2010). Ye478 had higher GYs and shoot biomass, and took up more N in both HN and LN conditions than Wu312. The root system of Ye478, as indicated by root length, root number, root biomass, and root to shoot ratio, was larger than Wu312 both in hydroponics and field conditions.

Field experiments
Field experiments for 218 RILs and the two parent lines were conducted at six environments, including the China Agricultural University (CAU, Beijing, China) experimental station in Dongbeiwang (DBW) (40°03′ N, 116°18′ E, 60 m above sea level) in 2006 and 2007, at the CAU experimental station in Changping (CP) (40°06′ N, 116°19′ E, 45 m above sea level) in 2007 and 2008, and at the CAU experimental station in Shangzhuang (SZ) (40°06′ N, 116°11′ E, 46 m above sea level) in 2009 and 2010 (Supplementary Table S1). In SZ, field experiments for an advanced 187 BC2F2 line population and the two parents, together with the testcross F1 hybrids, were also performed for two years (2009 and 2010). The nutritional composition of the soils before sowing in each field and the information of fertilizer treatments are summarized in Supplementary Table S1. Before sowing, the fields were supplied with 135 kg/ha KCl and with 750 kg/ha CaH2PO4; in DBW and SZ, and 422 kg/ha in CP. The N supply in soils varied from 37–84 kg/ha across the six environments, representing a mild LN condition. For the HN treatment, an additional urea-based N fertilizer (391 kg/ha for DBW and SZ, and 290 kg/ha for CP) was applied (40% of N applied before sowing and 60% at the V9 stage). In addition to soil residual and fertilizer N, other N supplies from soil mineralization and atmosphere deposition could have been available under both HN and LN conditions, but were not taken into account here.

At each location the population was evaluated in a completely randomized block design of one-row plots with three replicates. In DBW and SZ, each row was 4 m long, 0.5 m wide, and contained 13 plants. The planting density was 60,000 plants per hectare. In CP, each row was 2 m long, 0.5 m wide, and contained 11 plants. The planting density was 100,000 plants per hectare. Standard cultivation management practices were performed. The anthesis date (AD) of the two parental lines was similar, whereas those within the RIL population varied by 3 weeks. At maturation stage, the healthy plants within each row (11–13 individuals) were harvested and pooled for the measurements as described in Table 1. Ears were air-dried and then threshed to determine GY. Stover tissues were over-dried for determining stover yield (SY). Nitrogen concentration in grain (GNC) and stover (SNC) was measured using a standard Kjeldahl procedure. NUE, NupE, NutE, harvest index (HI), and nitrogen harvest index (NHI) were calculated as described in Table 1. GY and NUE were evaluated across six environments (E1–E6), whereas other traits were evaluated across four environments (E1–E4). Considering the N supply was constant, the complete correlation between GY and NUE, and between nitrogen uptake (Nup) and NupE, was expected. Therefore, GY and NUE were integrated into a single trait (GY/NUE), and Nup and NupE into a single trait (Nup/NupE), in the phenotypic correlation and principal component analysis (PCA). Because the same QTLs for GY and NUE, and for Nup and NupE, were also expected, GY and NUE traits were omitted from the subsequent QTL analysis.

Hydroponics experiments
Maize seeds were sterilized for 20 min in a 10% solution of H2O2, washed with distilled water, soaked in saturated CaSO4 for 6 h, and then germinated in the dark on moist filter paper at room temperature. After 2 days the germinated seeds were wrapped in a moist filter paper roll and grown until the stage of two visible leaves. The uniform seedlings were then selected and transferred into a plastic tube (60 × 40 × 15 cm, length × width × height) containing 40 L nutrient solution. The distance between two neighbouring plants was 3.3 cm in the row and 2.8 cm in the column. For HN treatment, the nutrient solution consisted of (mM): 2.0 mM Ca(NO3)2, 0.75 K2SO4, 0.65 MgSO4, 0.1 KCl, 0.25 KH2PO4, 1 × 10−3 H2BO3, 1 × 10−4 MnSO4, 1 × 10−3 CuSO4, 1 × 10−3 ZnSO4, 5 × 10−6 (NH4)6Mo7O24, 0.1 Fe-EDTA, with

### Table 1. Summary of the investigated traits in this study and the measurements

| Classification          | Trait                          | Abbreviations | Units       | Trait measurements                                                                 |
|-------------------------|--------------------------------|---------------|-------------|-----------------------------------------------------------------------------------|
| **NUE-related traits**  | Grain yield                    | GY            | g m⁻²       | —                                                                                  |
|                         | Nitrogen use efficiency        | NUE           | g/g         | Grain yield/total amount of nitrogen supply                                        |
|                         | Nitrogen uptake                | Nup           | g m⁻³       | GY × GNC + SY × SNC                                                                 |
|                         | Nitrogen uptake efficiency     | NupE          | g/g         | Total N uptake/total amount of nitrogen supply                                      |
|                         | Nitrogen utilization efficiency| NutE          | g/g         | Grain yield/total nitrogen content                                                  |
|                         | Stover yield                   | SY            | g m⁻³       | —                                                                                  |
|                         | Harvest index                  | HI            | g/g         | GY/(GY + SY)                                                                       |
|                         | Grain nitrogen concentration   | GNC           | %           | Kjeldahl method                                                                    |
|                         | Stover nitrogen concentration  | SNC           | %           | Kjeldahl method                                                                    |
| **RSA-related traits**  | Nitrogen harvest index         | NHI           | g m⁻³/g m⁻² | GY × GNC / (GY × GNC + SY × SNC)                                                   |
|                         | The number of seminal roots    | SRN           | number      | Average number of the two plants                                                   |
|                         | The number of crown roots      | CRN           | number      | Average number of the two plants                                                   |
|                         | The number of lateral roots    | LRN           | number      | Count number of the lateral root within 5 cm from the first emerged lateral root in the primary root. |
|                         | The length of primary roots    | PRL           | cm          | Measured with a ruler                                                              |
|                         | The length of seminal roots    | SRL           | cm          | Measured with a ruler                                                              |
|                         | The length of crown roots      | CRL           | cm          | Measured with a ruler                                                              |
|                         | Root dry weight                | RDW           | mg plant⁻¹ | Dried and weighted using a balance (1/1000g)                                      |
|                         | Shoot dry weight               | SDW           | mg plant⁻¹ | Dried and weighted using a balance (1/1000g)                                      |
|                         | Root to shoot ratio            | R/S           | mg/mg       | Root dry weight/shoot dry weight                                                   |
pH 6.0. For LN treatment, the 2.0 mM Ca(NO$_3$)$_2$ was replaced by 0.02 mM Ca(NO$_3$)$_2$ plus 1.98 mM CaCl$_2$ in the first week, and was then substituted by 2.0 mM CaCl$_2$ until the harvest. The nutrient solution was renewed every 2 days and aerated by a pump. The maize seedlings were grown in a growth chamber with controlled conditions: 28/22°C during a 14/10h light/dark cycle, with a light density of 250–300 µmol m$^{-2}$·s$^{-1}$ that was measured at canopy height.

The hydroponically grown two parent lines and 218 RILs were evaluated by three independent experiments (E7–E9). Each experiment was conducted in a completely randomized design with three replicates for two N treatments. The value of each replicate was represented by the means of two seedlings for each RIL or six seedlings for each parental line. The seedlings were harvested at the developmental stage with five to six visible leaves (~20 days after seed germination), and the plants grown under LN condition showed typical N-deficiency symptoms. Roots were separated from the shoot and stored at ~20°C before the measurements as described in Table 1. The seminal root numbers (SRN) and crown root numbers (CRN) were counted. The number of lateral roots (LRN) was evaluated within a 5 cm region of the primary root starting from the first emerged LR. The primary root length (PRL), seminal root length (SRL), and crown root length (CRL) was measured with a ruler. The roots and shoots were oven-dried at 70°C until a constant weight, and root dry weight (RDW), shoot dry weight (SDW), and root to shoot ratio (R/S) were evaluated.

**Data analysis**

Phenotypic data was analysed with software SAS 9.0 (SAS Institute Inc., NC, USA) using the GLM procedure. Combinations of year-location were treated as environments (E). Genotype (G) was treated as fixed, and E and interaction of genotype-by-environment (G × E) as random. The procedure LSMEANS was then performed to estimate phenotype values for the genotypes that were used for the subsequent phenotypic analysis and correlation analysis. The procedure VARCOMP was conducted to estimate genotypic variance ($\sigma^2_G$), G–E interaction variance ($\sigma^2_{G × E}$) and error variance ($\sigma^2_e$). The broad-sense heritability ($h^2$) of each measured trait was calculated as previously described by Hallauer and Miranda (1981). Pearson correlation coefficients and PCA were calculated using SPSS Statistics 17.0 (SPSS, Inc., Chicago, IL, USA) and further visualized using the R package (R Development Core Team, 2013).

Detection of QTL was performed by the composite interval mapping method (Zeng, 1994) using the software Windows QTL Cartographer version 2.5 (Model 6, Wang et al., 2005). The genotypic data for the RIL population was obtained from Liu et al. (2011), showing that a molecular map comprising 184 simple sequence repeat markers covered 2084.1 cM on 10 chromosomes with an average interval of 11.3 cM. Forward regression was analysed using a window size of 10 cM, a walk speed of 2 cM and five control markers. The threshold limit of detection (LOD) values were determined with 1000× permutations at $P < 0.05$ level (Churchill and Doerge, 1994), and LOD thresholds were set at 2.5 for all investigated traits. QTL positions were assigned at the point of maximum LOD score. A QTL meta-analysis was used to investigate coincidences of several QTLs for different traits in a given genetic population, and subsequently, to identify the QTL clusters. Within a cluster, the coincidence of QTLs for two traits was considered to be positive if the allele effect of both QTLs from the same parent showed the same sign, whereas it was considered negative if the two signs were different (Coque et al., 2008). The software MetaQTL Version 1.0 was applied to detect QTL clusters, according to the procedure initiated by Goffinet and Gerber (2000) and further improved by Veyrieras et al. (2007).

**Results**

**Evaluation of NUE-related traits in the field**

NUE-related traits were evaluated in field experiments across four environments, including plant biomass (GY, SY, and HI), plant N concentration (GNC, SNC, and NHI), and N efficiency (NUE, Nup, NupE, and NutE). Under both HN and LN conditions, the parental line Ye478 had higher GY and SY than those of parental line Wu312 (Fig. 1, Table 2, and Supplementary Table S2). Under LN conditions, GY of both lines decreased by 9.5% and SY by 13.6%, but the differences were not significant. Total N accumulation as indicated by Nup significantly decreased by up to 29.4% because N concentration in grain and stover of plants were decreased by up to 16.1% and 28.7%, respectively. Nup in Ye478 reduced 16.8% owing to LN stress, but by almost double that (29.4%) in Wu312, suggesting a lower sensitivity of Ye478 to N deficiency (Supplementary Table S2). Importantly, Ye478 had ~32–37% higher NUE than that of Wu312 under both N conditions. Of the two main components of NUE, NupE was 25–33% higher in Ye478, but NutE did not significantly differ between the two genotypes (Fig. 1, Table 2, and Supplementary Table S2).

Within the RIL population generated by a cross of Ye478 and Wu312, the NUE-related traits were extensively segregated and normally distributed, and considerable phenotypic variation existed as revealed by the coefficient of variation (CV) ranging from 8.6% to 32.7% (Table 2). At LN level for RILs, average GY did not significantly decrease and Nup decreased by up to 18%. Accordingly, GNC and SNC were further decreased by up to 6.3% and 25%, respectively. For each NUE-related trait a significant correlation between HN and LN conditions, the parental line Ye478 had higher GY and SY than those of parental line Wu312 (Fig. 1, Table 2, and Supplementary Table S2). Under LN conditions, GY of both lines decreased by 9.5% and SY by 13.6%, but the differences were not significant. Total N accumulation as indicated by Nup significantly decreased by up to 29.4% because N concentration in grain and stover of plants were decreased by up to 16.1% and 28.7%, respectively. Nup in Ye478 reduced 16.8% owing to LN stress, but by almost double that (29.4%) in Wu312, suggesting a lower sensitivity of Ye478 to N deficiency (Supplementary Table S2). Importantly, Ye478 had ~32–37% higher NUE than that of Wu312 under both N conditions. Of the two main components of NUE, NupE was 25–33% higher in Ye478, but NutE did not significantly differ between the two genotypes (Fig. 1, Table 2, and Supplementary Table S2).

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![Fig. 1](image.png)
and LN values was observed (Table S5). Besides genotype, significant effects were observed for N levels, environments, and their corresponding interactions, indicating strong G × E interaction (Supplementary Table S4). Nevertheless, the heritability ($h^2$) of NUE-related traits were rather high, varying from 48.0% to 75.8% under HN and from 52.9% to 73.1% under LN (Table 2).

As shown in Supplementary Figure S1A and Table S5, the phenotypic correlation between NupE and NUE was significant. The coefficients were higher under the LN condition ($r = 0.52$) than under the HN condition ($r = 0.36$). NutE had the higher correlation with NUE under both N levels ($r = 0.57–0.62$). As expected, NupE was highly related to plant biomass (GY and SY, $r = 0.36–0.69$), while NutE was highly positively correlated with HI for carbon related to plant biomass (GY and SY, $r = 0.36–0.69$), while NutE had the higher correlation with NUE under both N conditions ($r = 0.52$) than under the HN condition ($r = 0.36$). The presence of strong environmental effects was revealed by the significant variance of G × E interaction (Table S4). Moreover, N deficiency for RILs resulted in increases in average RL (6.6–26.1%), RDW (23.3%), and R/S (14.5%) (Supplementary Table S3).}

### Evaluation of RSA-related traits in hydroponics

Three independent hydroponic experiments were used to evaluate different root traits under both HN and LN growth conditions (Fig. 1, Table 2, and Supplementary Table S3). The investigated RSA-related traits consisted of root biomass (RDW), root number (RN: SRN, CRN, LRN), and root length (RL: PRL, SRL, and CRL). Irrespective of N levels, Ye478 had higher RN and RL than Wu312, as revealed by 42–45% more LRs, 62–77% more seminal roots, and 59–73% more RDW. Under LN stress, RL of Ye478 (PRL, SRL, and CRL) increased ~20%, twice as much as those of Wu312. RDW and R/S also increased more in Ye478. These results show that, compared with Wu312, Ye478 had a larger root system and showed a stronger root growth response to N deficiency (Fig. 1, Table 2, and Supplementary Table S3).

Within the RIL population, as for NUE-related traits, considerable phenotypic variation also existed for RSA-related traits (CV values ranged from 13.0% to 26.2%; Table 2). Each RSA-related trait significantly correlated under both HN and LN levels. Among the different root types, CR had the highest correlation with RDW (52.9%), but little change in RN (0–3.5%). The heritability ($h^2$) of root-related traits were moderate, ranging from 38.9% to 68.8% under HN and from 36.4% to 62.5% under LN condition (Table 2).

As shown in Supplementary Figure S1B and Table S5, RDW was significantly correlated with RN and RL irrespective of N levels. Among the different root types, CR had the higher value of coefficients with RDW ($r = 0.40–0.54$). The RN and RL were correlated in the same root type with high coefficients (SR, $r = 0.59–0.65$; CR, $r = 0.47–0.59$). PRL also significantly associated with SRL and CRL ($r = 0.24–0.55$). Among all root traits, LRN showed no correlation with others, except a very small correlation with RDW ($r = 0.11–0.24$). Because it was counted within a 5 cm region of the primary roots, LRN investigated here was more representative of the density of LRs. In spite of the stimulating effects on root
growth, it is unlikely that LN stress affected the overall correlation among different root traits (Supplementary Figure S1B).

**Phenotypic relationship between RSA- and NUE-related traits**

To investigate the contribution of roots to plant N efficiency, the Pearson correlation between RSA- and NUE-related traits was determined, and a PCA performed to visualize the correlation (Table 3, Supplementary Table S5, Fig. 2). Overall, correlations between RSA- and NUE-related traits were much lower than those within RSA-related traits or NUE-related traits. Nevertheless, significant correlations were observed between some root traits and GY/NUE (r = 0.14–0.27), Nup/NupE (r = 0.15–0.31), and SY (r = 0.10–0.26). By contrast, no significant correlation was determined between most root traits and NutE, GNC, SNC, HI, or NHI (Table 3 and Supplementary Table S5). PCA demonstrated that root traits except LRN were most closely related with NupE under both HN and LN conditions (Fig. 2), indicating that NupE is more likely to be genetically associated with root traits. Irrespective of N levels, SRN and CRN appeared more related to NupE and NUE than other root traits (Table 3). The association between SRL and NupE was also relevant, and significantly greater under LN conditions with the highest coefficient value (r = 0.31). These results suggest that both SR and CR traits have a closer relationship with NUE and NupE than they do with NutE, and the relationship between SR and NupE was even strengthened under N-deficient conditions.

**Detection of QTLs for NUE- and RSA-related traits**

In the RIL population, a total of 331 putative QTLs for all investigated traits through all independent experiments were identified across all 10 maize chromosomes, including 184 QTLs for NUE-related traits detected in the field and 147 QTLs for RSA-related traits detected in hydroponics (Fig. 3, Supplementary Table S6). Similar numbers of QTLs were detected under HN and LN conditions for both NUE-related traits (94 under HN, 90 under LN) and root-related traits (72 under HN, 75 under LN) (Supplementary Table S6). Within the identified QTLs for NUE, a similar proportion of QTLs carried the favourable allele that originated from either the parental line Ye478 or Wu312. By contrast, ~70% of identified QTLs for roots had the favourable alleles from the larger-rooted parent Ye478. Total phenotypic variation explained by the QTLs for each NUE-related trait ranged from 4.2% to 53.6%, and those for each root-related trait ranged from 4.7% to 29.7% (Supplementary Table S6).

Those QTLs repeatedly detected across the different environments were considered to be stable QTLs (sQTLs). sQTLs also had more than 1-LOD confidence interval overlapping and a positive additive effect from alleles of the same parental line (Supplementary Table S7). Constitutive sQTLs were defined for NUE-related traits if they were detected in at least three of four environments under both HN and LN conditions, and for RSA-related traits if they were detected in at least two of three independent hydroponics experiments. The QTLs that were exclusively detected in at least two environments under either HN or LN conditions were represented as HN-specific or LN-specific sQTLs. Thus, a total of 39 sQTLs were generated that contained 101 QTLs, about 30% of the total number of individual QTLs (Fig. 3 and Supplementary Table S7). NUE-related traits had 18 sQTLs, including 10 constitutive sQTLs, 3 HN-specific sQTLs and 5 LN-specific sQTLs (Fig. 3A and Supplementary Table S7). RSA-related traits had 21 sQTLs and most of them were constitutively expressed except for two LN-specific QTLs (Fig. 3B and Supplementary Table S7). Therefore, these sQTLs most likely contributed significantly to the genetic basis of NUE and RSA traits, and were relatively less influenced by the environments.

For NUE traits, a total of 29 significant QTLs were detected with LOD scores ranged from 2.6 to 6.8, and

**Table 3. Pearson’s correlation coefficients between RSA-related traits and NUE-related trait evaluated in the field and in hydroponics, respectively, under HN and LN levels**

| Treatment | Traits | SRN (217) | CRN (217) | LRN (218) | PRL (218) | SRL (216) | CRL (217) | RDW (217) | SRN (214) | CRN (218) | LRN (218) | PRL (217) | SRL (217) | CRL (218) | RDW (218) |
|-----------|--------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| HN        | NUE/GY (213) | 0.18** | 0.16* | 0.00 | 0.16* | 0.19** | 0.09 | 0.14* | 0.18** | 0.17* | -0.02 | 0.12 | 0.27** | 0.18** | 0.16* |
|           | NupE/NupE (208) | 0.13 | 0.15* | -0.08 | 0.05 | 0.06 | 0.09 | 0.06 | 0.15* | 0.12 | -0.07 | 0.07 | 0.21** | 0.12 | 0.05 |
|           | NutE (199) | 0.00 | 0.04 | -0.03 | 0.12 | 0.03 | 0.03 | -0.01 | -0.06 | 0.01 | -0.03 | 0.06 | 0.04 | 0.03 | 0.01 |
| LN        | NUE/GY (216) | 0.17* | 0.05 | 0.05 | 0.07 | 0.11 | -0.05 | 0.11 | 0.15* | 0.10 | 0.04 | 0.09 | 0.20** | 0.05 | 0.13 |
|           | NupE/NupE (207) | 0.23** | 0.13 | -0.07 | 0.03 | 0.11 | -0.04 | 0.03 | 0.31** | 0.15* | -0.06 | 0.04 | 0.31** | 0.02 | 0.05 |
|           | NutE (200) | -0.01 | -0.09 | 0.02 | 0.07 | 0.01 | -0.06 | 0.00 | -0.04 | -0.09 | -0.01 | 0.03 | 0.09 | -0.08 | 0.04 |

* The number of points used to calculate the Pearson’s correlation coefficients. * Significant at P < 0.05, ** Significant at P < 0.01.
explained 5.8–19.0% of phenotypic variation (Fig. 3A and Supplementary Table S6). Of these QTLs, four sQTLs were found and only one constitutive sQTL at chromosomal region bin2.04 had an allelic effect from Ye478 (Supplementary Table S7). Twenty-six QTLs for NupE were identified that explained 7.1–35.4% of phenotypic variation. Twenty-five QTLs for NutE were identified that explained 8.0–40.5% phenotypic variation. An HN-specific and two LN-specific sQTLs were found from the NupE and NutE QTLs, respectively. By contrast, for the traits of tissue N concentration (GNC and SNC), relatively fewer QTLs (14 and 17) and only a few sQTLs were detected (Supplementary Tables S6 and S7).

For RSA traits, a total of 54 significant QTLs were associated with RN, included 18 for SRN, 19 for CRN, and 17 for LRN (Fig. 3B and Supplementary Table S6). Relatively fewer QTLs (44) were associated with RL traits, including 14 for PRL, 15 for SRL, and 15 for CRL. Most root QTLs had minor genetic effects because they all explained less than 20% of the phenotypic variation (Supplementary Table S6). More sQTLs for RN (12) were found than for RL (3), indicating that RN traits were relatively less influenced by the environments. Favourable alleles from Ye478 were most influential in QTLs for PR- and SR-related traits, but not for CR-related traits (Supplementary Table S6). A total of 16 and 13 QTLs were detected for RDW and SDW, respectively, which were largely driven by the alleles from Ye478. Other than those for NUE, most of the identified sQTLs for roots were constitutively expressed.

Identification of QTL clusters for NUE- and RSA-related traits

By a method of meta-analysis, the identified 331 QTLs for all investigated traits across all the environments were assigned into 64 distinct QTL clusters (Cl) (Fig. 4, Supplementary Figure S2 and Supplementary Table S8). Each QTL cluster consisted of 5.2 QTLs on average with a range of 1–17. The QTLs for NUE-related traits were grouped into 52 QTL clusters. Among them, 39 QTL clusters contained QTLs for at least one trait of NUE, NupE, or NutE were defined as NE-QTL clusters. Nine NE-QTL clusters (Cl1.4, 1.5, 3.2, 3.5, 4.4, 7.2, 7.4, 8.2, and 8.6) had QTLs for both traits of NUE and NupE, and five NE-QTL clusters (Cl1.6, 3.2, 3.5, 4.3, and 5.2) for both traits of NUE and NutE (Supplementary Figure S3A and Supplementary Table S8). Two NE-QTL clusters (Cl3.2 and 3.5) at chromosome 3 simultaneously harboured QTLs associated with NUE, NutE, and NupE (Fig. 4 and Supplementary Figure S3A), and the QTLs for NupE were contributed to by alleles coming from Ye478 (Fig. 4 and Supplementary Table S7). All 134 RSA-related QTLs except those for SDW were assigned into 48 QTL clusters that were defined as RSA-QTL clusters (Fig. 4 and Supplementary Table S8). More than half of QTLs for RN and RL presented as co-localized clusters (Supplementary Figure S3C). Two QTL cluster (Cl3.5 and 2.1) contained QTLs for RN, RL, and root biomass (Fig. 4 and Supplementary Figure S3C). By contrast, less than one-third of QTL clusters overlapped between each different root type (CR, SR, PR, and LR), and no common clusters for all root types was observed (Fig. 4 and Supplementary Figure S3D). Two of the largest QTL clusters for roots, Cl3.5 and Cl7.3, harboured up to nine and seven root-related QTLs at the chromosomal regions bin3.04 and bin7.03, respectively. Cl3.5 had QTLs for most root traits (RN, RL, and root biomass), while Cl7.3 had QTLs more specific for RN. Both QTL clusters had the favourable alleles coming from Ye478. In addition, Cl1.3 and Cl2.2 were the QTL clusters for the LR traits, and had favourable effects from alleles coming from Ye478. Cl1.4 had QTL clusters for CR traits with favourable alleles coming from Wu312 (Fig. 4).
Determination of QTL clusters in which NE- and RSA-QTLs were co-localized

Of 39 NE-QTL cluster, 28 (~70%) were associated with RSA-QTL clusters and were defined as RSA-NE-QTL clusters (Fig. 4 and Supplementary Figure S3A). Among them, 15 QTL clusters overlapped between RSA and NUE traits, 13 between RSA and NupE, and 12 between RSA and NutE. Most common QTL clusters between NUE and NupE were associated with RSA-QTL clusters (Supplementary Figure S3A). More than 50% of RSA-QTLs expressed under both HN and LN condition co-localized with NE-QTL clusters (Supplementary...
Genetic relation of roots to NUE for maize

Figure S3B. Additionally, 75% of HN- and 62.5% of LN-specific RSA-QTLs co-localized with NE-QTL clusters. Irrespective of RN, RL, or root biomass, more than 50% of QTL cluster co-localized with NE-QTL clusters (Supplementary Figure S3C). In terms of root types, ~50% of CR- and SR-related QTL clusters, and up to 60% of PR- and LR-QTL clusters, co-localized with those for NE traits (Supplementary Figure S3D).

The largest QTL cluster, Cl3.5, harboured QTLs for all traits of most root types (except LRN), and was also associated with QTLs for NE traits (NUE, NupE, and NutE) under both HN and LN conditions (Fig. 4). Both Cl3.2 and
Cl3.6 contained QTLs for the trait of PRL, and was associated with NupE under HN conditions. Cl1.4 for CR and Cl8.2 for SR traits co-localized with QTL clusters for both NUE and NupE detected under LN conditions. Cl1.3 and Cl2.2 contained QTLs mainly for LRN and co-localized with NUE QTL clusters. Cl6.7 harboured QTLs for NupE at HN conditions, and was associated with QTLs for PRL and SRL at LN levels. By contrast, a relatively smaller number of QTL clusters were found to be shared between the traits of RSA and NutE. Cl6.2 and Cl8.5 contained QTL clusters for SR and CR, respectively, which co-localized with QTL clusters for NutE (Fig. 4). Therefore, the presence of a considerable number of QTL clusters in which both NE- and RSA-QTLs were co-localized indicated the significant genetic contribution of RSA to NUE traits in maize.

Performance of backcross lines containing RSA-NE-QTL clusters both per se and in hybrid combination

From a breeding point of view, five RSA-NE-QTL clusters (Cl1.3, 2.2, 3.5, 3.6, and 6.7) were considered because they had positive additive effect from the alleles coming from the large-rooted and high-NUE parent Ye478 (Fig. 5). Of these QTL regions, Cl3.6 at the chromosomal region bin3.05/3.06 and Cl6.7 at bin6.07/6.08 contributed positively to PRL and SRL traits, while both Cl1.3 at bin1.04 and Cl2.2 at bin2.04 most likely affected LRN. Cl3.5 at bin3.04 had a favourable effect on root systems in general. To evaluate the breeding value of RSA-NE-QTL clusters on the genetic improvement of GY/NUE, an ABL population (BC4F3) was generated by introgression of Ye478 genomic regions into the Wu312 background. The performance of the BC4F3 line per se and their testcrosses with line 178 were tested over two years under HN and LN levels (Fig. 5; Supplementary Table S9). Based on the flanked simple sequence repeat markers for Cl1.3, 2.2, 3.5, 3.6, and 6.7, the corresponding 17, 6, 12, 9, and 9 ABLs were selected (Fig. 5). These ABLs showed more than 90% genetic similarity to recurrent parent Wu312 (Supplementary Table S9). Compared to recurrent background Wu312, the GY/NUE of these ABLs showed apparent increases of 0.6–34.8% (mean, 13.8%) under HN and of 5.9–29.8% (mean, 15.9%) under LN conditions (Fig. 5A and Supplementary Table S9). At the hybrid level, the ABL testcross containing the targeted QTLs showed GY/NUE improvements of 7.8–16.2% (mean, 11.0%) under HN and of 13.4–30.2% (mean, 20.8%) under LN conditions (Fig. 5B and Supplementary Table S9). These results implicate the application of these QTL clusters as the targets for marker-assisted selection to improve GY/NUE in maize breeding programmes.

Discussion

Given the important function of roots, plant breeders are turning their attention to roots to increase crop yield with the sustainable use of natural resources. Previous attempts...
to enhance phosphorus and water use efficiency have been achieved by genetic improvements of RSA in crops (Steele et al., 2006; Landi et al., 2010; Chin et al., 2011). From a physiological perspective, selecting optimal RSA in breeding programmes has the potential to enhance N acquisition (Mackay and Barber 1986; Lynch et al., 2013). However, this type of breeding practice is limited because of a lack of knowledge on genetic relationship between RSA and NUE and the corresponding genomic regions for targeted genetic manipulation. In this study, a maize RIL population was generated that possessed traits for both RSA and NUE to uncover the significant genetic associations between RSA and NUE, and to identify and validate several important underlying QTL clusters. These findings allow the establishment of an RSA-based approach for genetically improving NUE in maize.

A RIL population for the genetic dissection of RSA- and NUE-related traits

The existence of valuable phenotypical variation for the targeted traits between the parental lines and within the corresponding genetic population allows the effective dissection of their genetic basis and identification of genomic regions for genetic improvements. In this study, the two parental lines, Y478 and Wu312, had contrasting phenotypes for both RSA and NUE traits (Fig. 1 and Supplementary Table S2 and S3). Compared to Wu312, Ye478 had the larger root system in hydroponics as well as the higher NupE in the field irrespective of HN and LN conditions. Previous studies on these two lines observed similar phenotypes at the seedling stage, and also suggested that the higher N uptake capacity in Ye478 relies on an advanced RSA performance and its response to N availability, rather than a higher N transport activity (Tian et al., 2006; Liu et al., 2009). At the lateral developmental stages (the silking and maturation stages) as revealed in the field, Ye478 also has the longer CR and LR length, and greater root biomass (Cai et al., 2012; Peng et al., 2010). For both RSA and NUE traits, the phenotypic variation within this RIL population is considerable larger, with modest to high heritability values (Table 2). As expected, QTL analysis revealed the additive effect of 70% of RSA-QTLs was derived from the donor allele of Ye478, given the genetic contribution of Ye478 to RSA traits (Supplementary Table S6). Consequently, this established RIL population from two contrasted lines in NUE and RSA was suitable for determining the relationship between RSA and NUE traits, and identifying favourable alleles for the RSA coming from Ye478 that can directly contribute to improving NupE.

Genetic relationship between RSA and NUE traits in maize

The existence of a genetic relationship between RSA and NUE traits makes it essential for breeders to consider RSA as a selection criterion to improve NUE in maize breeding programmes. In this study, two lines of genetic evidence revealed the significant associations between RSA and NUE. First, the phenotypical correlation analysis and PCA showed that RSA significantly associated with NUE (r = 0.14–0.27) (Fig. 2 and Table 3). Importantly, RSA had a positive correlation with NupE (r = 0.15–0.31) but no correlation with NutE, suggesting that N acquisition, rather than N utilization, is most likely related to the function of roots. Among RSA traits, the contribution of SR and CR, in particular for length and number of SR under LN conditions, seems to be more relevant to NupE (r = 0.31) (Table 3). Besides bi-parental population, a similar correlation study using a diverse set of 74 inbred maize plants also showed that SR length of maize seedling plants was most correlated with GY under HN (r = 0.36) and LN (r = 0.24) levels (Abdel-Ghani et al., 2013). Therefore, the selection of optimal root traits at the seedling stage is a promising approach to optimize maize NupE. Under field conditions, a significant correlation (r = 0.30–0.43) was also found between GY and RSA at an early development stage using the same advanced backcross population in this study (Cai et al., 2012). Given that evaluation of maize RSA directly in the field is technically difficult, time- and labour-consuming, and strongly affected by the soil environments, plant breeders are likely to find high throughput, low-cost, and relatively stable analysis of RSA using hydroponic systems more acceptable for selection of root traits. Additionally, the significant relationships found between maize RSA and GY under water deficiency (r = 0.2–0.3; Tuberosa et al., 2002) and phosphorus deficiency (r = 0.2–0.25, Zhu et al., 2006) further implicate the essential function of RSA on adaptation of maize plants to abiotic stress in general. It is worth noting that under severe stress conditions the trade-off between root growth and above-ground growth leads to a negative correlation between roots and GY (Chen et al., 2013).

Second, the coincidence of QTL clusters as revealed in this study further supports the genetic relationship between RSA and NUE traits (Fig. 4, Supplementary Figure S3A, and Supplementary Table S8). A large proportion of NE-related QTLs (70%) is associated with QTL clusters for RSA (Supplementary Figure S3A). Importantly, several QTL clusters contained QTLs for both RSA and NUE traits (Fig. 4). Most of these QTL clusters had favourable effects from alleles coming from the parent Ye478, which has a better root system than Wu312. Likewise, QTL analysis of another maize RIL population also revealed three QTL clusters for both N-uptake and root traits, and they also had favourable effect s from alleles coming from a parent with a superficial root system (Coque et al., 2008). It is worth noting that the relationship between RSA and NupE traits at QTL clusters may correspond to control of pleiotropic genes or to different closely linked genes. Nevertheless, the presence of significant phenotypic correlation and co-localized common QTLs provide the solid genetic basis for establishing the association between RSA and NUE.

The AD is considered an important trait in maize, and often affects other physiological and agronomic traits directly or indirectly. In the present study, however, no phenotypic correlation was obtained between AD and NupE or RSA-related traits (Table S10). Furthermore, no QTLs for AD were found to co-localize with those of NupE or RSA-related QTLs, except one AD-QTL region for NutE and NHI traits.
(Table S11). Thus, these results indicate that the genetic variation of AD in this RIL population did not affect the phenotypic behaviour in terms of NupE or RSA traits.

**Five important QTL clusters for a root-based approach to improve NUE in maize**

The coincidence of QTL clusters for RSA and NUE provides clues on their genetic association. More importantly, identification of the best alleles among these QTL clusters can help maize breeders implement high-NUE cultivars via a marker-assisted selection approach. In the present study, five important QTL clusters were identified in which QTLs for RSA and NUE coincided (Fig. 4). These QTL clusters contained root QTLs mainly for traits of RDW, LRN, PRL, and SRL, implying that these root traits could be the targets for selection of high-NUE maize. In comparison to LR traits, the RDW, PRL, and SRL traits seem to be more promising as selection criteria because they are easily measured and less affected by the environment. Similarly, Abdel-Ghani et al. (2013) highlighted that the selection of RDW and SRL in maize seedling could lead to an increase in selection efficiency for GY of adult plants.

Of these important QTL clusters, the CI2.2 cluster was localized on chromosome region bin2.04, and comprised NUE- and RSA-related traits (PRL and LRN). Tuberosa et al. (2003) discovered QTLs for root traits, i.e. brace root number and root pulling resistance, in a similar genomic region. A putative QTL, root-ABA1, that regulates both root size and GY also localized in this genomic region (Landi et al., 2006). The largest QTL clusters located at chromosomal region bin3.04 were identified for RSA and NUE, in particular for NupE (Fig. 4). QTLs have previously been determined in this region for a large number of root traits (Qiu et al., 2007; Liu et al., 2008), as well as for yield-related traits under different environmental conditions (Agrama et al., 1999; Messmer et al., 2009; Qiu et al., 2007). Coque et al. (2008) found a QTL for whole-plant N uptake at maturity (WpNup) in the genomic region bin6.07, which most likely co-localized with Cl6.7 in this study (Fig. 4). Collectively, these QTL clusters seem to be common for regulating maize RSA traits across different genetic backgrounds, and further involved in maize plants for improving water, nitrogen, and phosphorus use efficiency.

The QTLs with a significant impact on variation of NUE at hybrid level would have a greater breeding value, because hybrid varieties are often used by farmers. However, QTLs detected for NUE-related traits for per se value are different from the QTLs detected for testcross preformation (Coque and Gallais, 2008). In this study, the identified five important QTL clusters revealed a greater contribution to GY at both line per se and in testcross evaluation (Fig. 5 and Supplementary Table S9). Therefore, these alleles can confer a >20% gain of GY at hybrid level under LN stress conditions. Therefore, discovery of these QTLs opens a new and exciting opportunity for the manipulation of RSA via marker-assisted selection QTLs for improving NUE in maize.

**Conclusions**

Despite the highly complex nature of NUE and RSA traits, this study shows the presence of genetic relationships and of major QTL clusters that coincide for both traits, implicating the potential genetic improvement for efficient N acquisition in maize. Where previous studies have investigated at the physiological level, the findings presented here provide a better knowledge of the genetic factors regulating RSA to produce N-efficient maize genotypes. From a breeding point of view, five identified QTL clusters, Cl1.3 (bin1.04), CI2.2 (bin2.04), CI3.5 (bin3.04), CI3.6 (bin3.05/3.06), and Cl6.7 (bin6.07/6.08) have a positive effect on maize NUE at per se and hybrid levels, representing valuable targets for marker-assisted selection. Because it is impossible to distinguish a pleiotropy or linkage between close loci at the current level of QTL clustering, fine mapping and positional cloning are required to identify the underlying gene or genes to further promote root-based approaches to genetically improving maize NUE.

**Supplementary data**

Supplementary data are available at *JXB* online

**Supplementary File 1**
- Table S1. Summary of soil environment and fertilizer supply at different environments (E1-E6).
- Table S2. Statistics for NUE-related traits of the parent lines across six environments (E1-E6).
- Table S3. Statistics for RSA-related traits of the parent lines across three independent experiments (E7-E9).
- Table S4. ANOVA analysis for the traits across different environments and N levels.
- Table S6. Main features of the QTLs detected for all investigated traits across all the environments.
- Table S7. Summary of sQTLs for all investigated traits across all the environments.
- Table S10. Pearson’s correlation coefficients between AD and NUE- or RSA-related traits under HN and LN levels.
- Table S11. Detected QTLs for AD under HN and LN levels.

**Figure S1.** Network diagrams representing the phenotypic correlations between two traits within NUE-related traits and RSA-related traits based on their Pearson coefficients.

**Figure S2.** QTL clustering determined by MetaQTL software.

**Figure S3.** Numbers of QTL clusters for NUE- and RSA-related traits.

**Supplementary File 2**
- Table S5. Pearson’s correlation coefficients (r) between all investigated traits of RILs grown under HN and LN levels.
- Table S8. Summary of detected QTLs for all investigated traits in different environments and the identification of QTL clusters.
- Table S9. The performance of inbred lines and testcrossed lines under HN and LN levels in 2009 and 2010.
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References
Abdel-Ghani AH, Kumar B, Reyes-Matamoros J, Gonzalez-Portilla PJ, Jansen C, San Martin JP, Lee M, Lübbertstedt T. 2013. Genotypic variation and relationships between seedling and adult plant traits in maize (Zea mays L.) inbred lines grown under contrasting nitrogen levels. *Euphytica* 189, 123–133.

Agrama HAS, Zakaria AG, Said FB, Tuinstra M. 1999. Identification of quantitative trait loci for nitrogen use efficiency in maize. *Molecular Breeding* 5, 187–195.

Bertin P, Gallais A. 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. *Maydica* 45, 53–66.

Cai HG, Chen FJ, Mi GH, Zhang FS, Maurer HP, Liu WX, Reif JC, Yuan LX. 2012. Mapping QTLs for root system architecture of maize (Zea mays L.) in the field at different developmental stages. *Theoretical and Applied Genetics* 125, 1319–1324.

Cañas RA, Quillere I, Gallais A, Hirl P. 2012. Can genetic variability for nitrogen metabolism in the developing ear of maize be exploited to improve yield? *New Phytologist* 194, 440–452.

Chen XC, Zhang J, Chen YL, Li Q, Chen FJ, Yuan LX, Mi GH. 2013. Changes in root size and distribution in relation to nitrogen accumulation during maize breeding in China. *Plant and Soil* 374, 121–130.

Chin JH, Gamuyao R, Dalid C, et al. 2011. Developing rice with high yield under phosphorus deficiency: Pup1 sequence to application. *Plant Physiology* 156, 1202–1216.

Chun L, Mi GH, Li JS, Chen FJ, Zhang FS. 2005. Genetic analysis of maize root characteristics in response to low nitrogen stress. *Plant and Soil* 276, 369–382.

Churchill GA, Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138, 963–971.

Coque M, Bertin P, Hirl P, Gallais A. 2006. Genetic variation and QTLs for N-15 natural abundance in a set of maize recombinant inbred lines. *Field Crops Research* 97, 310–321.

Coque M, Gallais A. 2006. Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. *Theoretical and Applied Genetics* 112, 1205–1220.

Coque M, Gallais A. 2008. Genetic variation for N-remobilization and poststicking N-uptake in a set of maize recombinant inbred lines. 2. Line performance and comparison with testcross performance. *Maydica* 53, 29–38.

Coque M, Martin A, Veyrieres JB, Hirl P, Gallais A. 2008. Genetic variation for N-remobilization and poststicking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences. *Theoretical and Applied Genetics* 117, 729–747.

FAO. 2000. Fertilizer requirements in 2015 and 2030, ISBN–9789251044506.

Feil B, Thiraporn R, Geisler G, Stamp P. 1990. Root traits of maize seedlings—indicators of nitrogen efficiency? *Plant and Soil* 123, 155–159.

Gallais A, Hirl B. 2004. An approach to the genetics of nitrogen use efficiency in maize. *Journal of Experimental Botany* 55, 295–306.

Galloway JN, Townsend AR, Erismann JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889–892.

Goffinet B, Gerber S. 2000. Quantitative trait loci: a meta-analysis. *Genetics* 155, 463–473.

Good AG, Beatty PH. 2011. Fertilizing nature: a tragedy of excess in the commons. *PLoS Biology* 9, e1001124.

Granato TC, Raper CDJ. 1989. Proliferation of maize zea-mays I. Roots in response to localized supply of nitrate. *Journal of Experimental Botany* 20, 263–276.

Guing E, Hebert Y, Charcosset A. 1998. Genetic analysis of root traits in maize. *Agronomie* 18, 225–235.

Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KW, Vitousek PM, Zhang FS. 2010. Significant acidification in major chinese croplands. *Science* 327, 1098–1101.

Hallauer AR, Miranda JB. 1981. *Quantitative Genetic Maize Breeding*. Iowa State University Press, Ames, IA.

Hund A, Reimer R, Messmer R. 2011. A consensus map of QTLs controlling the root length of maize. *Plant and Soil* 344, 143–158.

Landi P, Giuliani S, Salvi S, Ferri M, Tuberosa R, Sanguineti MC. 2010. Characterization of root-1.0.6, a major constitutive QTL for root and agronomic traits in maize across water regimes. *Journal of Experimental Botany* 61, 3553–3562.

Landi P, Sanguineti MC, Darrah LL, Giuliani MM, Salvi S, Conti S, Tuberosa R. 2002. Detection of QTLs for vertical root pulling resistance in maize and overlap with QTLs for root traits in hydroponics and for grain yield under different water regimes. *Maydica* 47, 233–243.

Landi P, Sanguineti M, Liu C, Li Y, Wang TY, Giuliani S, Bellotti M, Salvi S, Tuberosa R. 2006. Root-ABA1 QTL affects root lodging, grain yield, and other agronomic traits in maize grown under well-watered and water-stressed conditions. *Journal of Experimental Botany* 58, 319–326.

Lebretón C, Lázicójancic V, Steed A, Pekic S, Quarrie SA. 1995. Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. *Journal of Experimental Botany* 46, 853–865.

Li DH, Mao LH, Yang JS, Liu JG, Zhang YH. 2005. Breeding process and utilization of excellent maize inbred line 478. *Journal of Laiyang Agricultural College* 22, 159–164.

Liu JC, Cai HG, Chu Q, Chen XH, Chen FJ, Yuan LX, Mi GH, Zhang FS. 2011. Genetic analysis of vertical root pulling resistance (VRPR) in maize using two genetic populations. *Molecular Breeding* 28, 463–474.

Liu JC, Li JS, Chen FJ, Zhang FS, Ren TH, Zhuang ZJ, Mi GH. 2008. Mapping QTLs for root traits under different nitrogen levels at the seedling stage in maize (Zea mays L.). *Plant and Soil* 305, 253–265.

Liu JX, An X, Cheng L, Chen FJ, Bao J, Yuan LX, Zhang FS, Mi GH. 2010. Auxin transport in maize roots in response to localized nitrate supply. *Annals of Botany* 106, 1019–1026.

Liu JX, Chen FJ, Olokhunu C, Glass ADM, Tong YP, Zhang FS, Mi GH. 2009. Root size and nitrogen-uptake activity in two maize (Zea mays L.) inbred lines differing in nitrogen-use efficiency. *Journal of Plant Nutrition and Soil Science* 172, 233–236.

Liu XJ, Zhang Y, Han WX, Tang A, Shen J, Cui Z, Vitousek P, Erismann JW, Goulding K, Christie P, Fangmeier A, Zhang F. 2013. Enhanced nitrogen deposition over China. *Nature* 494, 459–462.

Lynch JP. 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Annals of Botany* 112, 347–357.

Mackay AD, Barber SA. 1986. Effect of nitrogen on root growth of corn genotypes in the field. *Agronomy Journal* 78, 699–703.

Messmer R, Fracheboud Y, Baen zigzer M, Vargas M, Stamp P, Ribaut J M. 2009. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. *Theoretical and Applied Genetics* 119, 913–930.

Mi GH, Chen FJ, Wu QP, Lai NW, Yuan LX, Zhang FS. 2010. Ideotype root architecture for efficient nitrogen acquisition by maize in intensive cropping systems. *Science China-Life Sciences* 53, 1369–1573.
Mi GH, Liu JN, Zhang FS. 1998. Analysis on agronomic nitrogen efficiency and its components of maize hybrids. Journal of China Agricultural University 3, 97–104.

Moll RH, Kamprath EJ, Jackson WA. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. Agronomy Journal 74, 562–564.

Mu XH, Chen FJ, Wu QP, Chen QW, Wang JF, Yuan LX, Mi GH. 2015. Genetic improvement of root growth increases maize yield via enhanced post-silking nitrogen uptake. European Journal of Agronomy 63, 55–61.

Ort DR, Long SP. 2014. Limits on yields in the corn belt. Science 344, 483–489.

Paponov IA, Sambo P, Erley GSm, Presterl T, Geiger HH, Engels C. 2005. Grain yield and kernel weight of two maize genotypes differing in nitrogen use efficiency at various levels of nitrogen and carbohydrate availability during flowering and grain filling. Plant and Soil 272, 111–123.

Peng YF, Niu JF, Peng ZP, Zhang FS, Li CJ. 2010. Shoot growth potential drives N uptake in maize plants and correlates with root growth in the soil. Field Crops Research 115, 85–93.

Postma JA, Dathe A, Lynch J. 2014. The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiology 166, 590–602.

Presterl T, Groh S, Landbeck M, Seitz G, Schmidt W, Geiger HH. 2002. Nitrogen uptake and utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. Plant Breeding 121, 480–486.

Qiu FZ, Zheng YL, Zhang ZL, Xu SZ. 2007. Mapping of QTL associated with waterlogging tolerance during the seedling stage in maize. Annals of Botany 99, 1067–1081.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Saengwilai P, Tian XL, Lynch J. 2014. Low crown root number enhances nitrogen acquisition from low nitrogen soils in maize (Zea mays L.). Plant Physiology 166, 581–589.

Smith BE. 2002. Nitrogenase reveals its inner secrets. Science 297, 1654–1655.

Steele KA, Price AH, Shashidhar HE, Witcombe JR. 2006. Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theoretical and Applied Genetics 112, 208–221.

Tian QY, Chen FJ, Zhang FS, Mi GH. 2006. Genotypic difference in nitrogen acquisition ability in maize plants is related to the coordination of leaf and root growth. Journal of Plant Nutrition 29, 317–330.

Trachsel S, Kaeppler SM, Brown KM, Lynch JP. 2013. Maize root growth angles become steeper under low N conditions. Field Crops Research 140, 18–31.

Tuberosa R, Salvi S, Sanguineti MC, Maccaterri M, Giuliani S, Landi P. 2003. Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. Plant and Soil 255, 35–54.

Tuberosa R, Sanguineti MC, Landi P, Michelena Giuliani M, Salvi S, Conti S. 2002. Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Molecular Biology 48, 697–712.

Uribelarraea M, Moose SP, Below FE. 2007. Divergent selection for grain protein affects nitrogen use in maize hybrids. Field Crops Research 100, 82–90.

Veyrieras J-B, Goffinet B, Charcosset A. 2007. MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. BMC Bioinformatics 8, 1–16.

Wang S, Basten CJ, Zeng ZB. 2005. Windows QTL cartographer 2.5. Department of Statistics , North Carolina State University, Raleigh, NC.

Wiesler F, Horst WJ. 1994. Root-growth and nitrate utilization of maize cultivars under field conditions. Plant and Soil 163, 267–277.

Yu X, Zhu X. 1996. Excellent germplasm resource in maize (Zea mays L.). China Agriculture Press, Beijing (in Chinese).

Zeng ZB. 1994. Precision mapping of quantitative trait loci. Genetics 136, 1457–1468.

Zhang NY, Gibon Y, Gur A, Chen C, Lepak N, Höhne M, Zhang Z, Kroon D, Tschoep H, Stitt M, Buckler E. 2010. Fine quantitative trait loci mapping of carbon and nitrogen metabolism enzyme activities and seedling biomass in the intermated maize IBM mapping population. Plant Physiology 154, 1753–1765.

Zhang WF, Dou ZF, He P, Ju XT, Powison D, Chadwick D, Norse D, Lu YL, Zhang Y, Wu L, Chen XP, Cassman KG, Zhang FS. 2013. New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. Proceedings of the National Academy of Sciences of the United States of America 110, 8375–8380.

Zhu JM, Mickelson SM, Kaeppler SM, Lynch JP. 2006. Detection of quantitative trait loci for seminal root traits in maize (Zea mays L.) seedlings grown under differential phosphorus levels. Theoretical and Applied Genetics 113, 1–10.