

Physiological and Genetic Mechanisms for Nitrogen-Use Efficiency in Maize

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Abstract

Due to the strong influence of nitrogen (N) on plant productivity, a vast amount of N fertilizers is used to maximize crop yield. Over-use of N fertilizers leads to severe pollution of the environment, especially the aquatic ecosystem, as well as reducing farmer's income. Growing of N-efficient cultivars is an important prerequisite for integrated nutrient management strategies in both low- and high-input agriculture. Taking maize as a sample crop, this paper reviews the response of plants to low N stress, the physiological processes which may control N-use efficiency in low-N input conditions, and the genetic and molecular biological aspects of N-use efficiency. Since the harvest index (HI) of modern cultivars is quite high, further improvement of these cultivars to adapt to low N soils should aim to increase their capacity to accumulate N at low N levels. To achieve this goal, establishment and maintenance of a large root system during the growth period may be essential. To reduce the cost of N and carbon for root growth, a strong response of lateral root growth to nitrate-rich patches may be desired. Furthermore, a large proportion of N accumulated in roots at early growth stages should be remobilized for grain growth in the late filling stage to increase N-utilization efficiency. Some QTLs and genes related to maize yield as well as root traits have been identified. However, their significance in improving maize NUE at low N inputs in the field need to be elucidated.

Key words: nitrogen use efficiency, root response, maize (*Zea mays* L.)

Maize is an important multi-purpose crop used for food, fodder, chemicals and biofuels. According to the FAO, total world maize production reached 6.37×10^{12} tons (http://nue.okstate.edu/Crop_Information/) in 2003, higher than wheat or rice. In developing countries such as China, a huge demand for maize is expected due to the increase in animal production and biofuel requirements. Yield increase in maize is largely due to larger nitrogen (N) fertilizer inputs (Dai, 1998). While N fertilizer is a limiting factor for maize production in marginal areas and in developing countries, excessive input of N fertilizers in intensive agricultural areas is causing serious environmental problems such as nitrate leaching and nitrous oxide emissions, especially where there is heavy rainfall during the maize growing season. In the latter situation, the N fertilizer recovery rate is typically around 20-30% (Ju et al. 2004). Improving N fertilizer application techniques can greatly increase N-use efficiency. Developing N-efficient cultivars which take up N and/or utilize plant N efficiently provides an alternative strategy. Knowledge

on the physiology and genetics of N uptake and utilization is crucial to the development of an N-efficient cultivar. Taking maize as a model crop in this review, we discuss the response of maize plants to low N stress and the possible physiological and genetic mechanisms determining N-use efficiency.

Response of maize plants to N supply

Spatial nutrient availability is largely determined by root distribution in the soil. A typical response of maize plants to low N supply is an increase in root-to-shoot ratio resulting from relatively more assimilate allocated from shoot to root. The change in root system architecture (RSA) on response to N supply is much more complicated. In general, elongation of the axial and the lateral roots is enhanced at relatively low N supply (Chun et al. 2005a; Tian et al. 2005; Wang et al. 2003) (Fig. 1). Nevertheless, lateral root elongation is inhibited if the N supply is extremely low (Chun et al. 2005b; Guo et al. 2005a). Under this situation, a local supply of nitrate can increase lateral root elongation significantly (Guo et al. 2005b). In arable soils in which N distribution is extremely heterogeneous, changes in maize root morphology may represent a combination of the above-mentioned responses, and it is difficult to distinguish one

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response from another.

Low N stress induces N translocation from older to younger leaves and from vegetative to generative organs. This retranslocation of N results in chlorophyll degradation in older leaves which makes them appear yellow, a typical N deficiency symptom (Fig. 1). Nevertheless, under field conditions maize plants tend to keep a relatively homeostatic N concentration in the leaves by reducing leaf expansion at the seedling stage. It is only at anthesis and kernel-filling stages that a dramatic N retranslocation from vegetative organs to ears occurs and older leaves therefore show typical symptoms of chlorosis.

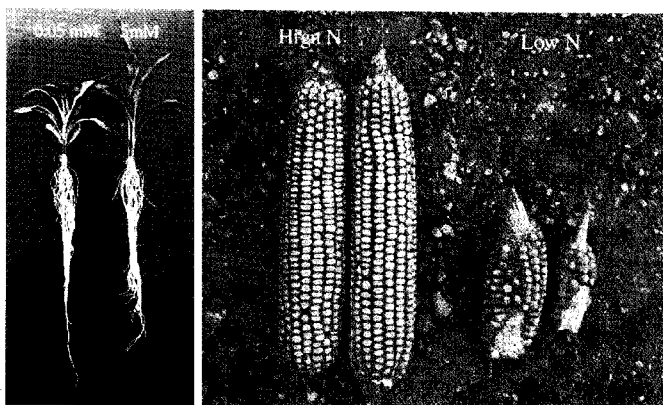


Fig. 1. Response of maize plants to low N stress. Left: roots, right: ears.

Ear and grain development is severely inhibited by N deficiency (Below 1996). It was found that yield reduction at low N stress is largely due to increased kernel abortion and fewer kernels per ear (Below 2002) (Fig. 1). It appears that N metabolism in kernels has a direct effect on kernel development and productivity since the provision of N to developing maize kernels can increase their capacity to synthesize protein and to utilize sugars for the biosynthesis of starch (Singletary and Below 1990).

Genotypic differences among maize cultivars in N-use efficiency

From an agronomic perspective, N-use efficiency (NUE) of a genotype refers to its grain yield at available N supply from both the soil and fertilizers (Moll et al. 1982). NUE therefore tends to increase with decreasing N fertilizer input. An N-efficient cultivar may produce a higher yield at low N and/or at high N applications compared to the inefficient cultivars (Fig. 2). In general, however, a cultivar which attains higher yields at relatively low N inputs is referred to as an N-efficient genotype.

Genotypic differences in NUE were observed as early as the 1930s (Smith 1934; Stringfield and Salter 1934). Interest in identifying genotypic differences in NUE has been intensifying since the 1980s as crop scientists have recognized this variation as one method to reduce N inputs and N losses to the environment. Large variation among maize genotypes in NUE indicates that this trait is genetically determined and may be improved by breeding. Modern maize breeding programs have been mostly conducted with sufficient N inputs. This raises the question of whether modern cultivars are less N efficient than traditional

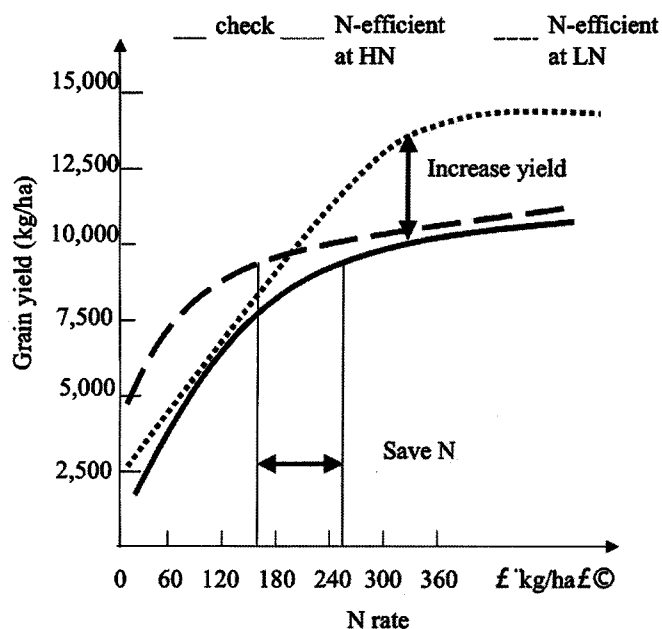


Fig. 2. Conceptual fertilizer N - grain yield curves of genotypes with different N-use efficiencies.

cultivars when grown in low N soils. In fact, modern cultivars usually achieve higher yields than the old cultivars not only at high N but also under low N supply. In general, a genotype \times N rate interaction cannot be observed when a large number of hybrids are compared (Below, 2002; Mi et al., 2004). Modern cultivars are therefore more N efficient because the physiological traits related to yield formation and resistance are comprehensively improved in modern cultivars. As a result, the traits related to N-use efficiency have been simultaneously modified. Nevertheless, this does not mean that the physiological and morphological traits in a modern high-yielding cultivar have been optimized for adaptation to low N conditions. These cultivars still show large variation in yield formation at low N stress conditions, indicating the possibility that NUE can be further improved by breeding.

Physiological basis for nitrogen-use efficiency

Nitrogen-use efficiency of a cultivar is roughly determined by two factors. One is the efficiency of a plant in recovery of N from the soil, namely N-uptake efficiency. The other is the efficiency of a plant in the utilization of N to produce grain yield, namely N-utilization efficiency, or physiological N-use efficiency (Moll et al. 1982). Grain yield is ultimately limited by N uptake (Sinclari and Vadez 2002). In most cases, a significant positive correlation has been established between N accumulation and NUE (Fig. 3), suggesting that total N accumulation might be more important than N utilization in plants for NUE (Mi et al. 1998; Horst et al. 2003). This opinion is supported by the fact that no significant relationship was found between grain yield and N-harvest index among maize hybrids (Wang et al. 2005). Nevertheless, other reports suggest that N utilization can be more important in determining NUE at low N inputs (Moll et al. 1982; Bertin and Gallais 2000). In fact, a strong interaction has been shown between N acquisition and N utilization, that is,

the pattern of plant utilization of N has a profound influence on N acquisition. For example, a plant that has a fast growth rate will tend to take up N more quickly. It may therefore not be possible to separate the relative contributions of N uptake and N utilization to NUE.

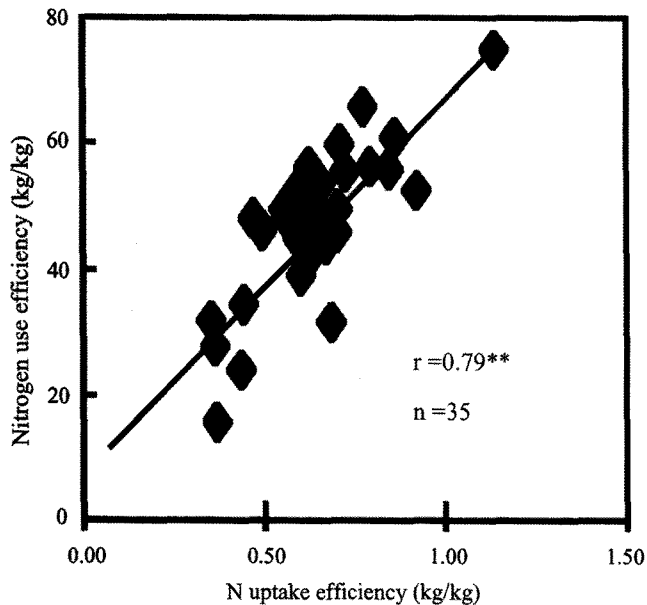


Fig. 3. Correlation between N-uptake efficiency and N-use efficiency in 35 maize hybrids. N application rate was 75 kg N/ha. Calculation of NUE and N-uptake efficiency is based on fertilizer N. (Mi et al. unpublished data).

N acquisition

In the plant as a whole, N accumulation is regulated by plant demand (Ismande and Touraine 1994). This N demand can be achieved by two factors, namely root size and the N-uptake system in the roots. Plant nitrate uptake systems are composed of two types of transporting proteins. At higher nitrate supply a non-saturated, low-affinity, nitrate transport system operates. This transport system is performed by nitrate transporters of the NRT1 family. When the external nitrate supply is lower than 0.1mmol/L, a saturated, high-affinity nitrate transport system is responsible for nitrate uptake. This system is mainly conducted by nitrate proteins of the NRT2 family, although a NRT1 family protein, AtNRT1.1, also functions as a high-affinity transporter in *Arabidopsis* (Crawford and Glass 1998). Two full-length, high-affinity nitrate transporter genes ZmNRT2.1 (AY129953) and ZmNRT2.2 (AY559405) and one low-affinity nitrate transporter gene (AY187878) from maize have been submitted to Genebank. However, their function and regulation are still largely unknown. Quaggiotti et al. (2002) compared the expression of ZmNRT2.1 between two maize hybrids with different N-uptake efficiencies but they did not find any difference. Using two inbred lines with different N-use efficiency, namely 478 (N efficient) and Wu312 (N inefficient), we compared ZmNRT2.2 expression in roots after nitrate induction. We also found no difference (unpublished data). In fact, the N-uptake rate of 478 is lower than that of Wu312 (Tian et al. 2006), suggesting that effi-

cient N acquisition may not depend on the N-uptake system. In arable soils, the average N concentration is usually around 4.5 ± 9.8 mmol/L (Wolt 1994). Under this nitrate regime, N uptake should mainly depend on the low affinity nitrate transport system. Since this N-uptake system has non-saturating characteristics, it is unlikely to be a limiting factor in efficient N uptake.

It is usually taken for granted that root size and spatial distribution may not be limiting factors for N acquisition under sufficient N supplies because N is mobile in soil and usually moves rapidly by mass flow (Sinclair and Vadez 2002). However, when N supply is not sufficient or N movement in soils is limited by water shortage, N in mass flow may not be adequate to meet the N demand for plant growth. In these cases, root size and morphology will be very important for the utilization of the spatially distributed N, especially newly mineralized N (Marschner 1998). It was shown that, under N limitation, there is a close relationship between total root length and N accumulation in maize seedlings (Wang et al. 2004). In field conditions, N-efficient maize hybrids had large root systems compared to inefficient hybrids, especially at late growth stages. (Fig. 4). Furthermore, root senescence of N-efficient hybrids was very slow (Chun et al. 2005a). To build and maintain such a large root system, N-efficient maize genotypes tend to allocate relatively more N to the roots at early growth stages (Li et al. 2001; Niu et al. 2007) and take up more N after anthesis (Chun et al. 2005a). Sustainable post-anthesis N uptake, and therefore staying green at maturity, are essential features for modern high-yielding hybrids (Dai et al. 1998; Below 2002; Mi et al. 2003). These two traits also appear to be important under low N supply conditions for a plant to maintain root longevity and activity for N uptake (Horst et al. 2003; Chun et al. 2005a).

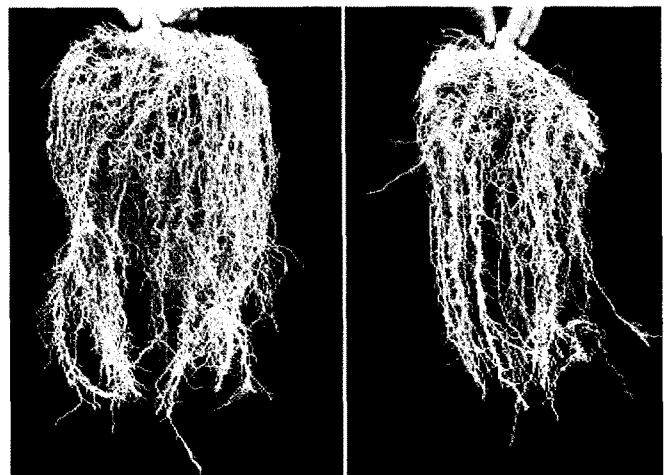


Fig. 4. Roots of a N-efficient hybrid (NE1, left) and a N-inefficient hybrid (SD19, right) at kernel filling stage.

The response of lateral root growth to local nitrate stimulation may also have a role in efficient N uptake under conditions of low N supply in the field. Because of the characteristic heterogeneity of soil N, a plant can save much N and energy if it proliferates lateral roots only in nitrate-rich patches. In a comparison of N-efficient inbred line 478 with N-inefficient Wu312,

it was found that 478 had a stronger capacity to proliferate lateral roots towards nitrate patches (Guo et al. 2005).

N utilization in plants

The way in which plants utilize accumulated N may also have a fundamental influence on grain yield. As root size is much more important for N uptake over the whole growth period, early establishment of a large root system may be essential. It was found that N-efficient maize inbred lines allocate more N to root development (Li et al. 2001; Niu et al. 2007). At the same amount of accumulated N, the N-efficient genotype can develop more roots compared to the inefficient genotype (Tian et al. 2006).

It is well established that leaf photosynthesis rate is largely determined by foliar N content. One strategy for efficient use of N in the plant is to utilize limited N to produce as much photosynthate as possible. In comparison with N-inefficient hybrids, N-efficient maize hybrids had a higher net photosynthetic rate at the kernel filling stage, although their N concentrations in the vegetative organs was similar (Chen et al. 2006). One possible explanation is that the photosynthetic N-use efficiency is higher in N-efficient cultivars. Indeed, as a C₄ plant, maize has much higher photosynthetic N-use efficiency than other cereals (Sinclair and Vadez 2002). An alternative explanation might be that there is weak negative feedback on photosynthetic rate from sink requirement in N-efficient cultivars because they have more fertile kernels compared with the inefficient cultivars.

It seems that the capacity of the maize plant to supply N to the ears was more limiting than the capacity to provide photosynthate. One of the important roles of N in ensuring high productivity of crop plants is establishment of reproductive sink capacity (Below 2002). In N-efficient maize hybrids, the number of kernels per ear is much higher than in N-inefficient hybrids (Chun et al. 2005a), even when their above-ground vegetative biomass is similar (Chen et al. 2006).

Taken together, an N-efficient maize hybrid may have the following characteristics: (1) a large root system for efficient N accumulation; (2) efficient N utilization in the plant for root construction, leaf and ear development, and kernel set; and (3) slow leaf senescence through maintenance of post-anthesis N uptake (Fig. 5).

Genetic and molecular biological aspects of NUE in maize

As NUE is defined as the production of grain yield per unit of N from soil and fertilizers, NUE must be a multi-gene controlled trait. Numerous studies suggest that NUE and its related physiological traits such as N accumulation and re-translocation are mainly controlled by additive gene effects (1988; Below 1996; Chen et al. 2003; Pollmer 1979). Therefore, identifying quantitative traits loci (QTLs) linked to NUE is a promising way for genetic improvement of NUE. Agrama et al. (1999) found significant differences in QTLs controlling grain yield and its components under high and low N supply, and the contribution of these QTLs to the phenotypes is between 11.8 (grain weight)

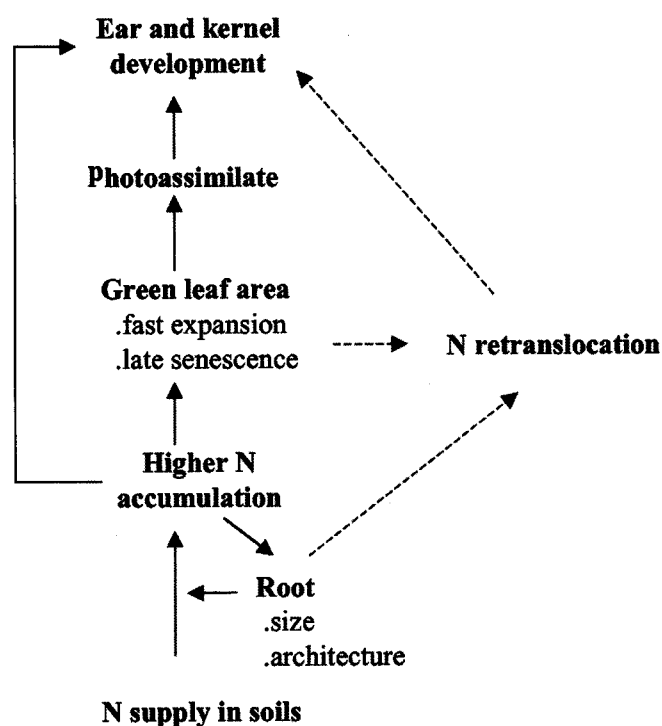


Fig. 5. key physiological processes determining NUE in maize

and 42.1% (yield). In a later report, Bertin and Gallais (2001) found QTLs related to NUE at high rather than low N supply, possibly because plant response to N is higher at high N inputs. They also identified more QTLs controlling N accumulation at high N supply, but more QTLs controlling N utilization at low N inputs. The same group further confirmed that on chromosome 1 the region defined by locus *bnlg1643* may be involved in grain yield determinism in optimal N-fertilization, probably through efficient plant N uptake. Moreover, at the end of chromosome 6, locus *umc1653* plays a role in the adaptation to N stress conditions through efficient grain filling (Coque and Gallais 2006). They also concluded that QTLs for root architecture and glutamine synthetase activity may be important for grain yield whatever the rate of N fertilization.

Analyzing coincidences between QTLs for agronomic and physiological traits and key genes of N uptake and metabolism provides a new approach to identify genes/QTLs involved in the variation NUE. By this means, one QTL for leaf GS1 enzyme activity has been shown to be coincident with a QTL for yield, one QTL for thousand kernel weight was coincident with the Gln3 (Gln1-4) locus and two QTLs for thousand kernel weight and yield were coincident with the Gln4 (Gln1-3) locus (Hirel et al. 2001). Based on this information, mutant *gln1-3*, *gln1-4* as well as the *gln1-3/gln1-4* double mutant were isolated and analyzed. The *gln1-4* phenotype displayed reduced kernel size and *gln1-3* reduced kernel number, with both phenotypes displayed in *gln1-3/gln1-4*. Moreover, when Gln1-3 was overexpressed constitutively in the leaves, kernel number increased by 30%, indicating that GS1-3 plays a major role in kernel yield (Martin et al. 2006). These results highlight the possibility of increasing maize yield by optimizing N metabolic traits. It would be inter-

esting to see if the GS overexpressed maize plant will also achieve higher yield under low N supply.

As discussed above, root size and architecture have substantial roles in N uptake capacity under low N conditions. In recent years, genetic information and QTLs controlling root traits of maize have been largely investigated (Chun et al. 2005b; Tuberosa et al. 2003). The most important QTL effects were detected on chromosome bins 1.03, 1.06, 1.08, 2.03, 2.04, 7.02, 8.06 and 10.04 (Tuberosa et al. 2003). More studies are required to establish which QTLs are closely related to N acquisition and grain yield formation at low N stress.

Breeding for nitrogen efficient maize hybrids

Since the 1990s, breeding for low N tolerant maize hybrids for farmers in developing countries has been a goal of CIMMYT (Edmeades et al. 1996). Some secondary physiological traits for selection of N-efficient genotypes have been established. These traits include, *inter alia*, more ears per plant, delayed leaf senescence and short anthesis-to-silking interval, etc (Bänziger and Lafitte 1997). Selection under low N field conditions will greatly increase the breeding efficiency (Bänziger et al. 1997). Despite numerous reports on the existence of genetic variability for many of the physiological characters and their relation to yield, direct selection may not increase grain yield because the physiological processes are easily affected by environmental factors. Besides, monitoring of the physiological and morphological traits involved in N-use efficiency is costly and laborious. Direct selection for yield under low N supply is still the main method in breeding programs for N-efficient hybrids. Presterl et al. (2002) developed hybrids under low and high N conditions. They showed that the average yields of the hybrids developed at low N conditions were 11.5% higher at low N supply than those selected under high N conditions. There was no significant difference in yield between the two hybrid types at high N supply. In addition, the N-efficient hybrids showed significantly higher N uptake at low N levels than the hybrids selected under high N. No differences in N-utilization efficiency were observed. Similar results were obtained in our research (Chen et al. 2005). It may therefore be possible to increase maize yields at reduced N supply while maintaining the yield potential under high N inputs. This should be achieved by increasing total N accumulation under conditions of low N supply.

Concluding remarks

It has been a major challenge to increase crop yields while reducing N fertilizer inputs. Although modern breeding programs have aimed to increase maize yields at high N inputs, the ability to take up more N by modern hybrids under low N supply has been improved simultaneously. Nevertheless, there is still scope to increase the NUE of maize at low N supply by exploring the genetic resources. Since the harvest index of maize plants is already quite high (Sinclair and Vadez 2002), increasing total N uptake, therefore the total plant biomass and grain yield is likely the way to increase NUE at low N supply. As in other crops, the potential of increasing N acquisition through the over expression of nitrogen transport systems may be unlikely to

succeed (Britto et al. 2004). By establishing a large root system at an early stage of growth, a maize plant can acquire more N resources from the soil for plant growth, and this is especially true when soil water content is too low for mass flow of N in the soil. To reduce competition for N and C from the shoots by the roots, an efficient root architecture is desirable that costs less N and C. In this context, a strong response of lateral roots to local nitrate stimulation would be helpful because lateral roots will only elongate at nitrate-rich regions. Furthermore, N used for root construction should be remobilized and translocated into the grain for reutilization at maturity. By means of cooperative work by scientists in agronomy, plant physiology and molecular biology, it should be possible to identify key genes controlling NUE-related traits in the future and to improve maize NUE by integrating them into one genotype.

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