

MAIZE ROOT MORPHOLOGY AND NITROGEN USE EFFICIENCY - A REVIEW

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ABSTRACT

The increased population pressure has led to the maximum use of chemical fertilizers especially in the major crops such as rice, wheat and maize production. In the United States, excess application of nitrate-nitrogen in maize cultivation is becoming a major issue because of increased ground water contamination. The nitrogen used by the maize plant is very low; often most of the applied nitrogen is lost through leaching. Root traits, amount of nitrogen present in the soil, soil pH, competition for available nitrogen between the weed and crop plant are challenges in improving nitrogen use efficiency (NUE) in maize breeding program. Effective root system is important in breeding maize for NUE as it is the root system that takes up nutrients from the soil and can help prevent leaching of nitrogen. The trait – NUE often becomes a limiting factor due to low frequency of NUE alleles in maize. Identifying genotypes with efficient root system and improved NUE for developing suitable hybrids would reduce yield loss in maize. It will also have positive effect on the environment by lowering down the excessive nitrogenous fertilizer application.

Key words: Maize, Nitrogen, Yield losses, Root, Nitrogen use efficiency (NUE).

Nutrient stress especially nitrogen deficiency is a common phenomenon all over the world. Low nitrogen stress hinders leaf expansion and photosynthetic rate (Muchow, 1989). Yield is also severely affected by lower grain number and less grain weight which is caused by fewer fertilized ovum, kernel abortion and other changes at physiological and biochemical level (Uhart and Andrade, 1995a). Generally, maize yield is high with high fertilizer dose as maize grain yields are highly responsive to supplemental nitrogen (Moose and Below, 2008); because of this reason the amount of applied nitrogen is increasing every year (average applied nitrogen amount is 10 million metric tons annually) worldwide (FAO, 2004). High nitrogen fertilizer application not only causes increase in input cost, but also has negative impact on the environment. Soil, air and water quality is degraded due to excess fertilization and residual effect (Tilman et al., 2002). Maize fertilization recommendation is generally made based on previous yields, expected yield based on the environmental conditions and by testing the available soil-nitrate. This leads to over fertilization in some areas and under fertilization in some areas (Mamo et al., 2003). Using N\textsubscript{15} isotope it was found that unfavorable environmental conditions decreased crop yield, but it was not due to less nitrogen content in the soil; the percentage of N recovered by plants were low though significant proportion of N was found in the soil (MacDonald et al., 1997). This emphasizes the fact that plant breeders need to develop varieties and hybrids with high nitrogen use efficiency (NUE).

Due to challenges of lack of knowledge regarding physiological and genetic mechanism of NUE in plants, environmental interactions and difficulty in evaluating NUE the progress for breeding plants with higher NUE has been impeded (Moose and Below, 2008). Increasing demand of maize for biofuel production and poultry feed also put pressure on increasing the yield in terms of grain production as well as total biomass production. Efficient root system provides anchorage preventing the yield loss
from lodging and enhances nutrient uptake. Thus the in-depth study of maize root architecture for improving NUE is necessary to secure staple food source for increasing population of the world, especially when there are challenges from global climate change, and higher cost of energy, fertilizers and water (Hochholdinger and Tuberosa, 2009). A better understanding of maize yield response to nitrogen deficiency, genetic basis of NUE and knowledge of root structure and its relation with NUE is necessary to improve the selection efficiency for low nitrogen condition.

In this review, an overview is presented on the maize root morphology, nitrogen assimilation, how maize root structure is directly related with nitrogen use efficiency and indirect impact on the ultimate grain production. The concept of NUE, current maize breeding programs for developing hybrids with high NUE have also been explored.

1. Maize root structure

Although information on root distribution and root spread is fundamental for proper understanding of a wide range of crop processes, such as water and nutrient uptake and root competition, the methodological difficulties associated with data sampling are responsible for the lack of knowledge of root distribution (Liedgens and Richner, 2001) especially under field conditions. In general, a root system can be divided into axial roots and lateral roots. Root architecture of any plant is mainly determined by the distribution of axial roots. Increased length of axial roots help help avoid low N levels under water deficiency provided there is relatively more nitrogen in the deeper soil layers (Jordan et al., 1983). The effect of N stress on increasing the root/shoot ratio has been documented (Eghball and Maranville, 1993; Feil et al., 1990; Vamerali et al., 2003). But it is not clear which kind of roots or what combination of root traits would be preferable in a low N environment. For instance, there are opposite results concerning whether or not a low N level will lead to an increase (Eghball and Maranville, 1993) or decrease (Maizlish et al., 1980; Sattelmacher et al., 1990; Wang et al., 2004) in lateral root growth. The maize root system is composed of embryonic primary and seminal roots and postembryonic shoot-borne and lateral roots (Hochholdinger et al., 2004). Early vigor of the maize seedlings is mainly controlled by embryonic roots and later during maturity period the maize root stock is dominated by shoot-borne nodal roots, which can have a significant influence on grain yield in water-limited conditions (Hochholdinger and Tuberosa, 2009). Liedgens and Richner (2001) observed that root density increased to a maximum level at 25cm depth and decreased at further depth. Hirel et al. (2007) suggested that sorghum’s better performance under low nitrogen condition over maize may be due to more developed and branched root system of sorghum than maize. Various models and softwares have been developed for understanding plant root system in a better way. The most widely employed methods for root analysis are based on the line intersect principle, which was first devised by Newman (1966) and later modified (Marsh, 1971; Tennant, 1975). Since this method relies on visual counting of grid line–root intercepts, it can be time consuming and prone to inaccuracy, especially when measuring samples with a large number of fine roots (Smit et al., 1994). SimRoot is an architectural root model. It simulates the growth of a root system over time in a 3d virtual space. Growth is predefined in SimRoot. An infinite number of root classes can be defined, each with its own growth and branching pattern. Collection of the growth and branching patterns takes time, but is possible using several methods outlined on this site such as pouch studies, crown evaluation and root coring. Currently input files for maize and bean are available (Lynch et al., 1997).

2. Molecular basis of maize root system

Although there are limited informations about the genes associated with root development in cereals, the genes discovered have shown to be examples of conserved and divergent aspects of root form, development and function (Hochholdinger and Zimmermann, 2008). The introduction of modern DNA-based molecular markers, allows for unprecedented opportunities to identify the genetic factors (quantitative trait loci, QTLs) uncovering the variation of quantitative traits (Frova et al., 1999; Sari-Gorla et al., 1999) and to understand to what extent linkage and/or pleiotropy may influence various traits’ association (Sanguineti et al., 1999). Maize root architecture is controlled by many genes; it is a quantitative trait which is inherited
discontinuously in the maize genotypes. Mutant studies in maize revealed the development of roots and how the genes are involved in this process. Genes regulating shoot-borne root initiation (RTCS) and root hair elongation (RTH1 and RTH3) have also been identified through mutant analysis (Hochholdinger and Tuberosa, 2009). The mutant rtcs (rootless concerning crown and seminal roots) is affected in the initiation of the embryonic seminal and the postembryonic shoot-borne roots. Map-based cloning revealed that RTCS encodes an auxin-inducible LOB domain transcription factor which is involved in the early events leading to the initiation and maintenance of seminal and shoot-borne root primordia (Taramino et al., 2007). Compared to the biparental linkage mapping used so far to detect root QTLs in maize, association mapping (Ersoz et al., 2007), and nested association mapping (NAM) (Yu et al., 2008) have proved to be new tools for QTL discovery, with the latter allowing for an improved level of genetic resolution in dissecting quantitative traits. Most of the QTLs regulating root traits are undetected due to the small magnitude of their effect on phenotypic variation and the low heritability of many root features (Hochholdinger and Zimmermann, 2008).

3. Nitrogen assimilation processes in plants

Nitrogen, an abundant (most abundant component in the atmosphere in terms of volume, approximately 78%) and yet limiting nutrient for crop and food production, enters the plant as nitrate or ammonium (rice), or as dinitrogen through biological fixation by prokaryotes associated with the plant. Managing N supply to achieve a balance between sustainable crop productions and environmentally sound levels of NO₃⁻ in water supplies should be every crop producer’s main aim. N is lost mainly through leaching, denitrification, volatilization, crop removal, soil erosion and runoff. During decomposition of organic matter in most agricultural soils, excess NH₄⁺ not utilized by microbes is released, and usually oxidized mostly by autotrophic bacteria to NO₂⁻, and then NO₃⁻. Nitrite does not usually accumulate except in some situations where pH is above 7 and excess NH₃·H₂O (or urea hydrolyzing to NH₃·H₂O) together inhibit the NO₂⁻ oxidizers. When atmospheric N₂ is fixed, the first form of nitrogen to appear is NH₄⁺, and most of it is immediately assimilated into organic forms so that very little is exuded to soil. The direction of N-transformation processes is dictated by carbon: nitrogen (C: N) ratio in the soil. In dry soils, nitrogen mineralization proceeds so slowly that it is difficult for crops to grow (Franco and Munns, 1982). In some situations nitrate accumulates before reduction. Nitrate uptake rates are initially exponential and then linear (Chantarotwong et al., 1976; Hallmark and Huffaker, 1978). Hallmark and Huffaker (1978) found that nitrate reduction was more affected by increased temperature than was uptake. Nitrate reduction occurs in two steps: 1. First mediated by nitrate reductase (NRase) 2. Second by nitrite reductase (Ni-reductase), both nitrate inducible (Breteler et al., 1979). After nitrate uptake by means of specific transporters present in the root cell membrane (Orsel et al., 2002), the assimilation of nitrate is a two-step process. First, nitrate reductase catalyzes the reduction of nitrate to nitrite. Then nitrite reductase mediates the reduction of nitrite to ammonium. Root-specific transporters (Glass et al., 2002) can allow direct absorption of ammonium (Loqué and von Wirén, 2004) when available in the soil or under certain conditions. Ammonia, which is the ultimate form of inorganic nitrogen available to the plant, is then incorporated into an organic molecule by glutamine synthetase (GS) to synthesize glutamine (Hirel and Lemaire, 2005). The reaction catalyzed by the enzyme GS is considered to be the major factor for the incorporation of inorganic nitrogen into organic molecules in conjunction with the Ferredoxin-dependent glutamate synthase, which recycles glutamate and incorporates carbon skeletons into the cycle (Franco and Munns, 1982). Both glutamine and glutamate are further used as amino group donors to other amino acids used for transport and protein synthesis and to nucleotides used as basic biomolecules for RNA and DNA synthesis. (Hirel and Lea, 2001). Enzymes involved in nitrogen assimilation in plant roots (Oaks, 1992) are Nitrate reductase (NR), Nitrite reductase (NiR), Glutamine synthetase (GS), Glutamate synthase (GOGAT), Glutamate dehydrogenase (GDH), Phosphoenolpyruvate carboxylase (PEPcase), Ferredoxin pyridine nucleotide reductase, Asparagine synthetase, Glutamate-oxaloacetate aminotransferase.
3.1. Nitrogen use efficiency (NUE)

Moll et al. (1982) defined NUE as the yield of grain per unit of available N in the soil (including the residual N present in the soil and the fertilizer). NUE can further be divided into two processes: 1. nitrogen uptake efficiency and 2. nitrogen utilization efficiency. Nitrogen uptake efficiency is defined as a quotient of plant nitrogen uptake (Nupt) and total crop nitrogen supply (Nsup) (fertilizer plus soil mineral nitrogen) (Samborski et al., 2008). Nitrogen uptake efficiency is also called nitrogen recovery efficiency (REN), which defines the capacity of the plant to acquire nitrogen from the soil. According to Baligar et al. (2001), nitrogen uptake efficiency is attributed to morphological, physiological and biochemical processes in plants and their interaction with climate, soil, fertilizer, biological, and management practices. The ratio of yield to nitrogen uptake is nitrogen utilization efficiency (Moll et al., 1982). Moll et al. (1982) recommended selecting cultivars with both nitrogen uptake efficiency and nitrogen utilization efficiency whereas Liu (1991) recommended selecting appropriate cultivars to cope up with the current environmental and cultural conditions to improve efficient use of nitrogen. For cereal crops, the ratio of plant nitrogen content to the supplied nitrogen does not exceed 50% whatever the level of nitrogen fertilization (Malagoli et al., 2005), which suggests that there is possibility and need for improvement of cereal crop species with regard to NUE (Hirel et al., 2007). In maize, 45-65% of the grain nitrogen is provided from the pre-existing nitrogen in the stover before silking and 35-55% is from post-silking nitrogen uptake (Gallais and Coque, 2005). N rates affect three essential components of root morphology: 1) length; 2) number of apices and 3) frequency of branching (Maizlish et al., 1980). Drew et al., (1973) and Tennant (1976) found that root length increased with N applied to either part or all of the root system of temperate cereals. Costa et al., (2002) reported that the mean diameter of maize roots was greater in the absence of N application than at a rate of 127.5 or 255 kg N ha⁻¹. This finding contradicts the general observation that finer-diameter roots are formed under low N (Fitter, 1996). Costa et al., (2002) also found that greater root length and root surface area were obtained at an N fertilization rate of 127.5 kgNha⁻¹ compared with either the absence of fertilizer N or at an N rate of 255 kg N ha⁻¹.

3.2. Correlation of water stress and nitrogen deficiency

Maize production is affected by water and nitrogen (N) deficit either separately or joined, but this fact is not completely defined (Rimski-Korsakov et al., 2009). The most sensitive period of maize to drought is between 2 weeks before and 2–3 weeks after silking (Otegui et al., 1995). Sharp and Davies (1979) found that mild water stress may initially increase root growth, but severe and long-lasting drought decreases root growth (Eghball and Maranville, 1993). Pandey et al. (2000) and Moser et al. (2006) found that when maize was subjected to drought, a lower yield with high N doses. However, Eck (1984) and Al-Kaisi and Yin (2003) observed that high N doses do not affect yield in water-stressed plants. Eghball and Maranville (1993) found that root biomass was not affected by the interactive effect of N rate and water deficit. These results show the fact that drought treatments are difficult to standardize due to each local environmental characteristics, soil properties and crop attributes (Rimski-Korsakov et al., 2009). To quantify the proportion of N from fertilizer taken by the crops or following other destination 15N isotope can be used (Herzog and Götz, 2004). This is a direct method to determine the fate of N from fertilizer through the soil and plant compartments and through the air and water (Carter and Rennie, 1987). Fertilization increased the uptake of soil N in the roots, independently from water treatment (Rimski-Korsakov et al., 2009). Campos et al. (2004) observed that during a period of water limitation, the old maize hybrid extracted more water from shallow soil depth, whereas the new hybrid plant appeared to be more effective at deep layer.

3.3. Molecular genetics of Nitrogen use Efficiency

Recent plant molecular biotechnology, modern physiological and biochemical studies, have helped in better understanding of the regulatory mechanisms controlling the steps of inorganic N assimilation and the biochemical pathways in N supply for secondary metabolism (Hirel and Lemaire, 2005). Bartin and Gallais (2001) found most of the chromosomal regions related to grain composition (starch, protein and amino acids), yield
and traits related to NUE corresponded to the quantitative trait loci (QTLs) detected at high input. Studies on local nitrate-induced lateral root formation in maize have shown strong interactions among hormonal pathways and local nitrate signaling pathways in microarray experiments (Liu et al., 2008). Genes related to nitrate uptake and assimilation, sugar transport and utilization, and cell division and expansion are induced by local nitrate application implying a role of these transcripts in the root system response to nitrate. Agronomic studies on maize have demonstrated that there is genetic variability for NUE, though understanding about corresponding physiological traits is not yet explored fully. Studies have been conducted to find the roles of different proteins and enzymes involved in mineral N-uptake, and assimilation (Lea and Ireland, 1999). Most of the approaches involving either whole plant physiology or the use of transgenic plants or mutants have not contributed to an understanding of the physiological and genetic basis of NUE in a more integrated manner (Gallais and Hirel, 2004).

Quantitative genetic studies associated with the use of molecular markers may be an effective way of identifying Quantitative Trait Loci (QTL) involved in the genetic variation of a complex character like NUE. If there is co-mapping with genes encoding enzymes involved in N-assimilation, it will allow the identification of ‘candidate’ genes, i.e. genes for which allelic variation could be responsible for a part of the observed variation. After identifying a good candidate gene, to validate it, the favorable allele can be transferred to a genotype with an unfavorable allele to test whether there is the expected effect. Although QTLs for adaptation to environmental stresses such as drought resistance (Agrama and Moussa, 1996; Ribaut et al., 1997; Tuberosa et al., 1998), and tolerance to phosphorus stress (Reiter et al., 1991) have been detected in maize, few studies are published on QTL identification for adaptation to low N condition (Gallais and Hirel, 2004). Agrama et al. (1999) found common and specific QTLs for high and low N-input whereas Bertin and Gallais (2001) showed that QTLs detected at high N-input were different from those detected at low N-input. The present studies are the initial steps in the development of crop genotypes tolerant to low nitrogen stress. In near future molecular tools will prove to be the essential part of crop improvement and further research regarding the inheritance of nitrogen transport related genes are needed to get clear picture about nitrogen assimilation in the crop plants.

4. Characteristics associated with nitrogen stress tolerance

Physiological mechanisms related to abiotic stress tolerance have been reported in various studies in tropical maize (Duque Vargas et al., 1994; Lafitte and Edmeades 1994a,b,c; Chapman and Edmeades, 1999; Edmeades et al., 1999) though it is less understood how these mechanisms might improve the yield of the crop under abiotic stress conditions (Bänziger et al., 2002). Tollenaar and Wu (1999) indicated that increased leaf longevity, increased water and nutrient uptake, and greater assimilate supply during grain filling were related to increased low N tolerance in Canadian maize hybrids. More number of ears and kernels were observed in stress tolerant hybrids than stress susceptible hybrids under both N and weed stress (Tollenaar et al., 1997). Nitrogen stress cause reduction in leaf area, enhances leaf senescence, and decreases radiation use efficiency (Uhart and Andrade, 1995b). Carbohydrate reserves typically accumulate in the stem (Mumera and Below, 1993) rather than ears which may be one of the reasons for poor development of harvestable plant parts. Below et al., (2000) found that N plays an important and direct role in kernel development by regulating the enzymatic activities involved in the translocation of sucrose from stem to the developing ovaries. Bänziger et al., (2002) found among the traits that changed with selection for drought tolerance, ASI (Anther silking interval) showed the largest increase under severe N stress whereas increases in grain yield, grain N content, grain N concentration, ears per plant and kernel weight at maturity due to selection were all alike from high to low.

5. Breeding for efficient root system and NUE

Overuse of nitrogen fertilizers has already deteriorated the soil quality and in future also it will have detrimental impacts on the biodiversity especially of soil microflora, animals and plant ecosystem. The challenge of twenty-first century is to feed over 6 billion people along with shrinking
agricultural land. Therefore there is a strong need in developing the crop genotypes that can yield more with less nutrients especially inorganic nitrogen supply (Delmer, 2005). High yield of rice, wheat and maize largely contributed to increased global food supply since 1967 (Cassman, 1999). It is therefore of major importance to identify the critical steps that controls plant NUE (Hirel et al., 2007) so that development of NUE efficient cultivars will be both economic and environment friendly. In conditions where excess of N cannot be totally avoided, it is important to improve genotypes with high absorbing capacity. This will help in accumulating excess N in order to reduce the quantity of N remaining in soils after harvest and thus decrease the risk of leaching (Herel and Lemaire, 2005).

Presterl et al. (2003) showed that when genotypes are selected for low nitrogen conditions yield less significantly. Muruli and Paulsen (1981) reported that genotypes selected for low nitrogen conditions are not truly adapted to nitrogen rich soils. So, it is a difficult and challenging task for the maize breeders to select and breed genotypes for low nitrogen soils without reducing the yield. Local landraces might serve important source for this type of breeding program and further research should be to know why some landraces perform better under low input conditions. Especially research related to improved root system would be a better choice for developing varieties and hybrids with low nitrogen stress tolerance. Combining the breeding objectives of yield improvement for high input of N fertilizers and yield improvement conditions with low N input conditions would be desirable. Two breeding strategies have been suggested (Atlin et al., 2000; Falconer, 1952): (i) Indirect improvement through selection at only one N level, whereby performance at the other N level improved by correlated response; (ii) Combined improvement through selection based on an index of the weighted performance means at high and low input of N. If combination strategy proves to be ineffective, it would be desirable to breed for the high nitrogen and low nitrogen conditions separately (Presterl et al., 2003).

Knowledge of genotypic variance components, heritabilities, coefficients of genotypic correlation, as well as economic weights for yield under high and low N conditions is necessary to decide which of the above strategies would be the most appropriate (Presterl et al., 2003).

Root branching and architecture are strongly related to plant survival under various abiotic (drought, flood, nutrient deficiencies) and biotic (competition among plants, diseases, pests) stress conditions (Bohn et al., 2006). Breeding with the objective of improved root system is always a big question for the breeders. Root complexity and root development depend on genetic and environmental factors and their interactions (O'Toole and Bland, 1987) A better understanding of biochemical and physiological mechanisms controlling different metabolic processes (nitrate uptake, nitrate partitioning between roots and shoots, nitrate reduction and its subsequent assimilation and transfer into organic molecules) and their relationships will be useful for selecting target plant criteria and physiological traits which can be used as markers for breeding NUE (Good et al., 2004). The efficiency of selection for yield under low-N conditions may be improved through selection for correlated secondary traits (Blum, 1988). These traits are related to efficient N uptake and metabolism such as improved N uptake characteristics by seedlings (Teyker et al., 1989) and high plant nitrate content (Mollaretti et al., 1987). Genetic variation for mobilization of N from leaves and stems to grain have also been described (Eghball and Maranville, 1991). Developing cultivars that accumulate large amounts of N during the vegetative stage and redistribute it efficiently during grain production (Beauchamp et al., 1976) would become important objective of maize breeders in near future. Difficulty in measuring such traits precisely and quickly has limited maize breeding programme for improved NUE. There is a strong urgency in developing cultivars with better root system as the ability of plants to grow and produce seeds is governed by a functional and efficient root system. There are limited studies in terms of genetic studies of root architecture and development of abiotic stress tolerant genotypes under field conditions The reason might be labor-intensive procedure, destructive digging required for collecting root samples, complex and different responses even in the same field. Complex root systems are often characterized by a high number of branching points, having a higher probability of
finding adequate resources by exploring a large portion of the soil than root systems with less complex root systems (Bohn et al., 2006).

**CONCLUSION**

Improved NUE can help to enhance yield under low nitrogen and thus improve crop nutritional quality and it will also reduce ground water contamination by excess nitrates (Hirel and Lemaire, 2005). Maize root architecture is very much influenced by its interaction with the soil environment and its adaptability to changing environmental conditions. Recently, proteomic studies have provided new insights into the regulation of maize root development (Hochholdinger et al., 2006). Such experiments either generated reference maps of the mostly available proteins of a particular stage of root development or compared differential protein accumulation levels among different genotypes or treatments of a particular root type. Gene expression and protein accumulation studies related to maize root formation can be used for in silico QTL studies to identify novel genomic regions that are important in the process of maize root development. Non-destructive measurement of roots in soil through nuclear magnetic resonance (NMR) imaging coupled with modeling tools will facilitate the research of root characteristics in soil and the prediction of their effects on field performance (Menzel et al., 2007). The combination of these novel tools will increase the knowledge about the association of root architecture and yield under different environmental conditions and will ultimately help breeders in developing superior maize hybrids via marker-assisted selection of key root features (Hochholdinger and Tuberosa, 2009). Effective nitrogen uptake and utilization in maize production is of utmost importance because of high cost associated with high-input practices and because of its effect on groundwater quality. Increased yield potential in high-input cropping systems have not been much effective for resource-poor farmers in marginal areas (Edmeades et al., 1990). Low soil nitrogen can also be a serious problem where N is applied at below-optimal levels because of the potential for low economic return resulting from drought or excessive leaching of nitrate (Lafitte and Edmeades, 1994a,b). Development of cultivars that are superior in the utilization of available N may prove to be one approach to reduce the impact of N-deficiency in maize production. Integration of genetic, physiological, biochemical and agronomic studies of plant nitrogen response to understand the regulation of NUE is necessary. This suggests that in order to develop an integrated research program for developing cultivars with improved capacity to utilize available soil nitrogen and discovering genes for NUE, a complete and extensive phenotyping, comprising agronomical, physiological, molecular and biochemical studies on crops grown under low and high N fertilization applications will be key issue that needs to be addressed.

**REFERENCES**


