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An inside into the nitrogen use efficiency and its importance in crop production

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Abstract

Agriculture is the backbone of developing countries and plays a vital role in sustaining human society. Agricultural production is highly dependent on the use of synthetic fertilizers, among all nitrogenous fertilizer rank first. With increasing use of global nitrogen (N) fertilizers tremendously; at the beginning of the decade it was around 87 million metric tons, is estimated to increase up to 236 million metric tons by 2050. Heavy use of nitrogenous fertilizer to the farmland has resulted in a dramatic increase in crop yield but with considerable negative impacts on the environment. Nitrogen use efficiency (NUE) of most of the crops particularly cereals is very low that is around 30-35%, remaining 60-65% N is lost in the environment due to runoff, leaching, volatilization, denitrification, etc. The ability of plants to effectively utilize N from the soil depends on a number of factors including N uptake, assimilation, translocation and remobilization during senescence. The innate efficiency of the plant to utilize this available N can be increased with proper phenotyping, identification of genotypes with high NUE, molecular breeding approaches, and targeting candidate genes for the improvement of NUE. The present review highlights the physiological and genetic basis of NUE and its related traits in enhancing crop productivity.

Keywords: nitrogen (N), nitrogen use efficiency (NUE), N uptake, N assimilation, N remobilization

1. Introduction

Nitrogen (N) is the integral component of proteins, nucleic acids, enzymes and is a key element required for plant growth and development (Castro Rodriguez *et al.*, 2017) [11]. N fertilization is indispensable for crop production, but the availability of N to the crops in the soil is an insufficient and external supply of N fertilizer is essential to achieve higher yield. As a result, the demand for N fertilizer is increasing. Despite increased application of N fertilizer, N use efficiency (NUE) of crops is static due to nutrient loss from the agricultural field that include; losses into groundwater, volatilization loss, denitrification loss, immobilization within the soil (Robertson, 1997; Raun and Johnson, 1999) [43, 44] and only 30% to 40% of applied N is taken up by crops, remaining is lost to the environment causing pollution (Hirel *et al.*, 2011; Robertson and Vitousek, 2009) [23, 45]. Therefore, breeding crops with improved NUE is one of the effective approaches in enhancing crop productivity (Han *et al.*, 2015; Hirel *et al.*, 2011) [22, 23]. N fertilization is one of the major input costs in crop production, especially in developing countries (Masclaux Daubresse *et al.*, 2010) [32] and it is estimated that 1% improvement in NUE could save more than US\$200 annually (Raun and Johnson, 1999) [44]. Apart from nutrient management strategies NUE involves complex networks genes that mediate uptake, assimilation, remobilization, and storage of (McAllister *et al.*, 2012; Xu *et al.*, 2012) [33, 48]. Despite the significant contribution of NUE to crop productivity, understanding the detailed mechanisms to improve NUE is relatively limited due to the complexity of mechanisms involved in it (Basra and Goyal, 2002) [6], at present the molecular basis of N metabolism is not well understood, which represents a major barrier to crop improvement.

2. Nitrogen and crop production

Nitrogen is yield-limiting nutrient in crop production. The N limitation is associated with its low efficiency (<50%) of applied fertilizer due to losses in soil-plant systems. N in crop plants has a positive association with grain yield (Fageria and Baligar, 2005) [15]. Synchronization between N supply and demand of plant is crucial in agricultural systems to increase N use efficiency (NUE), yields and to reduce environmental pollution (Richardson *et al.*, 2009) [42]. Grain N of cereals is derived largely from remobilization and translocation of N from vegetative parts after anthesis and N content in the grain is always high compared to shoot. N absorbed by crops must be partitioned between the vegetative and and reproductive or economic part of the plants like grains in order to improve the partitioning efficiency,

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which is measured as the ratio of grain N to total plant N also known as nitrogen harvest index (NHI). NHI is characteristic of genotypes, however, it can be improved by providing better growing conditions and adopting appropriate soil-plant management practices like application rate, source and timing of application, planting efficient crop species or genotypes within species and use of legumes for crop rotation.

3. Definitions of nitrogen use efficiency

Nitrogen Use Efficiency (NUE) is defined as the grain yield per unit of supplied N, also a combination of NUPE (N uptake efficiency) and NUTE (N utilization efficiency). N utilization efficiency (NUTE) includes N Assimilation Efficiency (NAE) and N Remobilization Efficiency (NRE). Another method to define NUE is the utilization index (UI), which indicates the absolute amount of biomass produced per unit of N. It can also be defined as NUEG, which indicates grain production per unit of available N. Agronomic and Physiological variations can also be used for NUE calculations (Xu *et al.*, 2012) [48].

4. Physiology of nitrogen uptake and assimilation

N uptake by plants take place through various forms such as nitrate, ammonium and other organic molecules but, nitrate in aerobic conditions and ammonium in flooded environments are the major forms of uptake (Oldroyd and Dixon, 2014) [39]. Between nitrate and ammonium, nitrate is the most predominant form of nitrogen uptake by most of the crop species with some exceptions. Uptake of nitrate is controlled by various transporters. Nitrate transporters are classified into two categories; Low affinity transporters (LATS) and High affinity transporters (HATS). Low affinity transporters are active under high N conditions and High affinity transporters are active under low N conditions. Four families of transporters are known to contribute to nitrate uptake and transport in plants; 1. Nitrate Transporter 1/Peptide Transporter (NPF) family, 2. Nitrate Transporter 2 (NRT2) family, 3. Chloride Channel (CLC) family, 4. Slow Anion Associated Channel Homolog (SLC/SLAH) family. NPF and NRT2 family of transporters are mainly involved in nitrate uptake

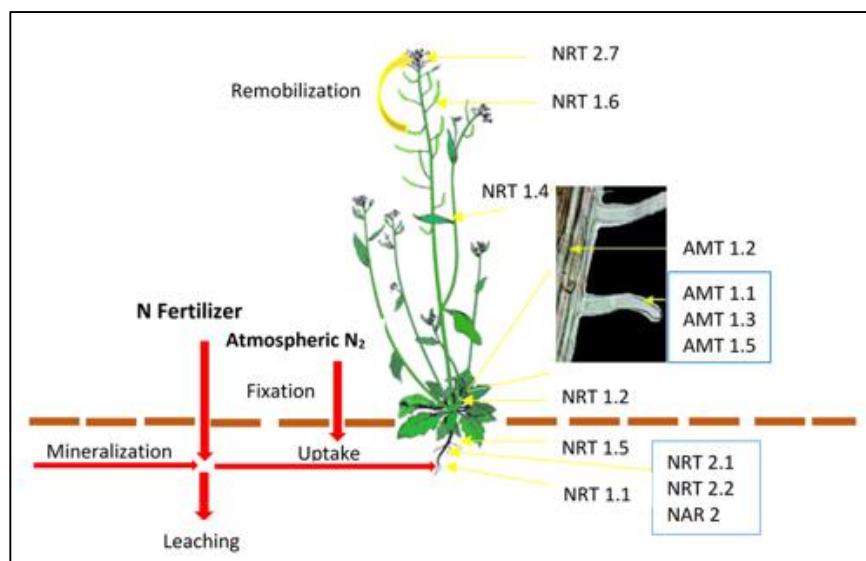


Fig1: Nitrate and Ammonium transporter genes in Arabidopsis involved in N uptake, its translocation, and remobilization.

Plants have well-developed transport systems to take up different N forms such as nitrate, ammonium, urea, amino acids. Transporters belonging to AMT1, AMT2, NRT1/NPF, and NRT2 families contribute to N uptake of plants (Crawford, 1995). Nitrate is converted into nitrite by the cytosolic enzyme Nitrate reductase (NR). Nitrite ions are reduced to ammonia by the activity of enzyme nitrite reductase (NIR). Plants avoid ammonium toxicity by converting ammonium to glutamine and glutamate by glutamine synthetase (GS) and glutamate synthase (GOGAT) respectively. There are 2 classes of glutamine synthetase, GS1 (Cytosolic) and GS2 (Plastidic). Plants contain 2 types of GOGAT, NADH-GOGAT, located in non-photosynthetic tissues like roots and vascular bundles of developing leaves and Fd-GOGAT-in chloroplasts. Glutamate dehydrogenase (GDH) catalyzes a reversible reaction that synthesizes or deaminates glutamate. An NADH-dependent form is found in mitochondria and an NADPH form is localized in chloroplasts of photosynthetic organs.

Once N has entered into the plant, it is assimilated into amino acids and other important nitrogenous compounds. The first step involved in this process is the conversion of ammonium into the organic compound which is catalyzed by the enzyme glutamine synthetase (GS). Based on the subcellular location,

GS is classified into cytosolic (GS1) and chloroplastic (GS2) isoforms. GS1 is usually encoded by a multigene family, and GS2 is often encoded by a single gene (Chardon *et al.*, 2012; Mifflin and Habash, 2002) [10, 34]. There is accumulating evidence supporting that both GS1 and GS2 play essential roles in efficient N use and high yield potential in major crops including wheat, rice and maize (Chardon *et al.*, 2012; Hirel *et al.*, 2011) [10, 23] and both of these two enzymes are being targeted through genetic engineering to improve N use efficient crops. From numerous studies on the transgenic modification of GS1, the results are positive and significant, but the experiments on GS2 are very scarce (Thomsen *et al.*, 2014) [47]. GS activity is known to be one of the best physiological markers to depict the plant N status (Kichey *et al.*, 2006) [26], and leaf GS activity was found to positively correlate with protein and N content (Habash *et al.*, 2007) [24], and grain yield, but negatively correlated with leaf senescence.

5. Nitrogen remobilization and NUE

N Remobilization Efficiency (NRE) is a major determinant of N Utilization Efficiency (NUTE) and an efficient assimilation is correlated with enhanced N remobilization to improve the seed N storage, which is essential to enhance seed yield, and

the stem may act as storage organ in case of non-synchronization between remobilization of N from source leaves and its utilization by the seeds. Photosynthesis after heading accounts for 60-90% of the total carbon accumulated in rice panicles at harvest, while around 80% of total panicle nitrogen uptake happens before heading and is continuously remobilized from leaves to grains. Proteolytic degradation of Rubisco is associated with cysteine proteases and proteasome activities and is identified as the mechanism of N remobilization in many crops. N recycling is considered to be a key factor in determining the rate of senescence and therefore, the productivity of rice plants (Yamaya *et al.*, 2002)^[50]. Senescence represents the last stage of the developmental process and plays a key role in plant life (i.e., it maintains internal cellular processes by regulating the expression of various genes with significant physiological activities). In cooperation with senescence, proteolysis plays a significant role in the recycling of nutrients, resulting in subunits like amino acids, amides, and ammonia (Diaz Mendoza *et al.*, 2014). A number of plants have various genes (*SAG13*, *SAG21*, *SAG27*) associated with senescence and they play an interactive role with other organisms (Pontier *et al.*, 1999). Changes in gene expression associated with senescence in the term, lead to enhancement in the recycling of nutrients, thereby increasing both the yield and the quality of the crop. During the decline of photosynthetic activity, there is an enhanced dependence of senescing tissues on respiratory metabolism. Thus, remobilization of reduced carbon becomes an important source for senescing leaves. Nitrogen is considered as one of the inevitable mineral nutrients in crops (Marschner, 1995), which often limits the growth, yield, and quality (Gastal and Lemaire, 2002)^[20] of crops and crop products. It is already reported that the association of nitrogen-fixing microorganisms with legumes and various other plants is costly. That is why efficient nitrogen remobilization has increased the competition with wild plants. Many plants show that during senescence, the content of organic nitrogen needed for remobilization is present in proteins; for instance, in C3 plants, 50% is soluble (Calvin cycle) and 50% insoluble (thylakoid) in chloroplast proteins, whereas 50% of plastidial nitrogen has been reported in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). During early leaf senescence, it is well known that photosynthetic capacity declines, while mitochondrial integrity and respiration are maintained to a greater extent (Gepstein, 1988). Nitrogen is the most important component for plant photosynthesis and is readily redistributed in higher plants (Martin, 1982)^[36]. It is generally mobilized in mature leaves and is not restricted to senescence (Mae and Ohira, 1981; Martin, 1982)^[31, 36]. Leaf nitrogen concentration decreases during senescence, whereas in non-senescing leaves, the export is compensated for by the nitrogen influx through the xylem. The remobilization of nitrogen from senescing leaves is an important aspect of plant physiology. According to studies on two major groups of crops, legumes, and cereals, nitrogen can be remobilized from leaves, petioles, stem, and roots.

6. The genetic basis of NUE in crops

NUE is a complex quantitative trait consisting of N uptake, its translocation, and utilization. The number of QTLs which are detected in a particular study depends on a number of different factors, including the size and type of mapping population, the trait under consideration, environmental conditions used for phenotyping, and genome coverage. The

genetic variation for NUE involves a number of loci with moderate effects, and a very large number of loci with minor effects (Kearsey *et al.*, 1998)^[28]. Researchers have mapped several QTLs related to NUE traits in a number of plants. One of the first was carried out in rice (Obara *et al.*, 2001). They mapped QTLs associated with NUE and determined whether they cosegregated with glutamine synthetase 1 (GS1) and NADH-GOGAT. They identified seven loci, cosegregated with GS1 activity and six loci that cosegregated with NADH-GOGAT activity. A number of QTLs for agronomic traits related to N use and yield have been mapped to the chromosomal regions containing GS2 in wheat and rice, which suggest that the genomic region surrounding GS2 may be important for breeding rice and wheat varieties with improved NUE and agronomic performance. However, till to date, no one has been able to introduce a GS gene into an NUE-inefficient background and show either enhanced NUE or yield. For maize, a number of experiments have been done to map QTLs associated with NUE using segregating maize populations (Gallais *et al.*, 2004)^[19]. The meta-analysis revealed 22 QTLs under low N conditions with 4 consensus QTLs located on chromosomes 1 and 4, 2 on chromosomes 3, 5, 6, and 9, and 3 on chromosome 2. Chromosomes 7, 9, and 10 harbors only 1 consensus QTL. Using 196 wheat accessions, identified 54 genomic regions through whole genome association mapping which were associated with grain yield and other traits. The study also revealed that 23 QTLs were N responsive, which can be useful for the wheat breeding programs in order to improve N responsiveness. Recently identified genomic regions using a wheat RIL population by applying a conditional analysis to detect N and P fertilization effects on QTLs. Treatment of N affected QTLs of N concentration in grain, shoots, and NUE traits, whereas P treatment affected a QTL of spikelet number per spike. It may be useful to further investigate the newly detected QTLs that include those on chromosomes 4B and 7A for yield component traits. Some QTL studies were performed with physiological traits such as N metabolic enzyme activities and N metabolites (Fontaine *et al.*, 2009)^[18]. Although these physiological traits are particularly susceptible to environmental factors and experimental settings there are potential outcomes such as metabolic QTLs (MQTLs) which provide us direct evidence to link agronomic traits with potential gene(s) underlying the QTLs (Riedelsheimer *et al.*, 2012)^[46]. In the case of Barley, most of the QTLs are mapped for yield and its component traits. Varshney *et al.*, (2012)^[49] in their study tried to find out genes which are involved in the number of traits in barley, particularly for grain yield and biomass yield, considered to be associated with NUE using association mapping approach. Berger *et al.*, (2013)^[4] in a genome-wide association study of 329 genotypes of winter barley determined genomic regions associated with a number of key agronomic traits associated with NUE. They showed two QTLs that were consistent between environments and years, 2H at 16.0 cm and 7H at 91.12 cm. Mickelson *et al.*, (2003)^[38] using genetically defined mapping populations of barley, performed a detailed analysis of QTLs associated with N storage and remobilization and identified a number of QTLs associated with NUE traits. Unfortunately owing to lack of consensus markers on this map, and several mapping inconsistencies, only a few loci associated with NUE were identified. The difference between the N acquired by the plant prior to anthesis and the N in the harvest plant product, it is found three QTLs on chromosomes 5H and 6H that explained 37.4% of the variation. In barley, (Kindu *et al.*, 2014)^[29]

found that although it was possible to detect QTLs for NUE related traits in two separate years, the QTL analysis of NUE and its components mainly indicated inconsistent results across N levels and years, in line with low correlations among environments for a given trait. However, under the different conditions and between years there were two QTLs for NUTEG (NUTE of grains), NUEG, and NHI that consistently mapped to chromosomes 2H and 3H. In wheat and barley, there is one group of QTLs that affects both NUE and GPC (Heidlebaugh, 2008) ^[25]. These loci, known as *Gpc-B1*, affect N reallocation in these plants, resulting in significant differences in NUE and N allocation within the plant. Near isolines with *Gpc-B1* in Australian varieties showed increased GPC without negative impact on grain yield. However, as noted above, GPC has a significant effect on bread quality (wheat) and malt quality (barley) and therefore must be evaluated with this in mind. To improve GPC without any penalty in grain yield is challenging, but some QTLs, such as those detected on chromosomes 1B and 2A for GPC, may be potential candidates for this purpose.

7. Strategies to improve NUE in crops

Improving the efficiency of applied fertilizer is essential for the success of meeting yield demand whilst minimizing environmental impacts. Both management practices and improved germplasm will contribute to efficient nitrogen fertilizer use and facilitate reduced inputs and reduced wastage. Some management practices that can improve N uptake and use efficiency include liming acid soils, use of adequate rate, source, and timing of application, planting N efficient crop genotypes, and use of legumes in crop rotation. Understanding the proper application of N fertilizer is, therefore, critical in reducing its environmental loading and to improve NUE in the system. The better practices of nutrient management include fertilization regime with the right rate and the right timing from the right sources at the right place (Alva *et al.*, 2011) ^[1]. Nitrogen dynamics vary with production systems such as organic and conventional or dry and humid environments. A study at a site-specific N management indicated that the key for success on-farm precision N management is the right choice and timing of N application while taking into account on farm residual N (Ahrens *et al.*, 2010) ^[2]. Selection and rate of N fertilizers, placement depth (Khalil *et al.*, 2009a) ^[27] and timing of application (Malhi *et al.*, 2010) all influence N losses from soil. These studies indicate that crops and N sources interact to cause various growth responses. Critical periods of N demand vary in crops and adjusting fertilization timing according to crop N requirements may reduce the risk of N loss and excessive N fertilizer application. Although one time application of all required N at seeding is a common and relatively effective practice for winter wheat production in drier areas. Several studies indicated that split application of N fertilizer (e.g. at seeding, tillering and stem elongation) improved NUE, the total N yield and grain yield in various growing conditions. These results clearly show that the extent of plant N uptake is associated with N demands at different plant growth stages where N is used for cell division and growth, and thus the timing of fertilizer application needs to be synchronized with the timing of crop N requirement. Besides split fertilization, application of polymer coated N fertilizers is another useful strategy to synchronize N supply and demand, and reduce N losses (Khalil *et al.*, 2009; Malhi *et al.*, 2010) ^[27]. In addition to fertilization regimes, the diversification of crops or cropping practices in crop rotation

plays a significant role in improving NUE in conventional systems. Diversified crop rotations with leguminous crops take biologically fixed N into the N cycle in situ, resulting in the reduction of N fertilizer use. Nitrogen derived from the decomposition of preceding leguminous crops compensated for a portion of the N fertilizer input. When a large volume of N is required for cereal crops, selection of N fixing crops as preceding crops could reduce N fertilizer application amount, minimizing the risk of N loss in the environment. When succeeding crops were fertilized with the recommended amount of N, increased yield of the following crops are greater than those following non-leguminous crops (Luce *et al.*, 2015) ^[30]. The amount and timing of available N in legumes depend on factors such as the C:N ratio, tillage practices, soil microbial diversity, and climate. The inclusion of post-harvest cover crops in crop rotations has been successfully practiced throughout the world. Cover crops maintain favorable soil conditions for improving NUE such as adding and conserving N, optimizing the C:N ratio of residues and preventing soil erosion (Dabney *et al.*, 2010) ^[14].

Mechanisms of each NUE component have been studied for decades, improvement of a single crop trait or cultivation technique does not necessarily result in increased crop NUE (Fraisier *et al.*, 2000) ^[17]. The integration of knowledge from breeding and agronomic strategies, therefore, may provide useful information to improve NUE although the lack of on-farm studies about optimization of N supply found under low soil N level, the primitive polyploid species had higher NUE, whereas the modern cultivated hexaploid species had better NUTE. Therefore, the selection from a wide range of germplasm under low soil N levels may be useful in breeding cultivars for different management systems. Therefore, it is useful to have more information about the behavior of important NUE related traits under different soil N concentration to breed ideal cultivars. Application of NUE knowledge is more challenging inorganic production systems due to a number of uncontrollable biotic and abiotic factors, which result in significant phenotypic variation.

Marker-assisted breeding has opened up exciting possibilities for the more effective use of variation within crop gene pools and in searching for further useful variation in the wild relatives of crops. When the variation in physiological traits and yield components were compared it was found that there was a positive correlation between nitrate content, GS activity, and yield. Loci that appeared to govern quantitative traits were determined on the map of the maize genome and the positions of the QTLs for yield components and the locations of the genes for cytosolic GS (GS1) coincided. These results suggest that it is possible that GS1 could represent a key component of nitrogen use efficiency and yield. The results of such mapping experiments have also indicated regions of the genome that are important in regulating the activity of GS, but which do not coincide with structural genes for GS. These regions could contain genes important in the control of GS activity. If these genes can be identified they might provide novel information on GS regulation and NUE.

8. Transgenics for NUE

Manipulating N assimilation genes such as NR, NIR GS, and GOGAT have been hypothesized to affect NUE in plants. First, enzymes and proteins other than those involved in primary N uptake and assimilation may be good targets for increasing plant NUE, possibly due to decreased post-transcriptional controls. Second, the selection of appropriate

promoters to control where and when the expression of transgenes occurs in NUE crops can be of significant importance. The search to identify genes that improve the NUE of crop plants will continue, with candidate NUE genes existing in pathways relating to N uptake, assimilation, amino acid biosynthesis, C:N storage and metabolism, signaling and regulation of N metabolism and translocation, remobilization and senescence. Basic insights into alterations in C:N ratio, as well as cross-talk between pathways has widened the scope of NUE targets. Furthermore, because the NUE phenotype is genetically more complex biotechnologists may need to explore stacking or pyramiding candidate genes to obtain an NUE phenotype in crop plants that remain stable in field conditions. Transgenics for NUE in crops is challenging to reduce the application of N fertilizer levels and may lead to 'Second Green Revolution' in agriculture.

9. Conclusion

Yield increments in crops with low N fertilizer consumption can be achieved by improving NUE, which includes agronomic management and breeding approaches. By understanding the basic physiological mechanisms of N uptake, assimilation and remobilization traits can be dissected for the breeding of crops with high NUE. Advanced genomics and biotechnological approaches have resulted in the identification of candidate genes for NUE. Molecular Breeding approaches such as MAS and MABC can be effectively used for identification of N efficient genotypes and introgression of candidate genes in elite varieties which otherwise can improve crop NUE. Significant research has been carried out to identify the rate-limiting steps of NUE through agronomic, physiological and molecular means. In order to improve NUE and crop productivity integration of transcriptomics, functional genomics, quantitative genetics, and physiology into explanatory models of whole plant behavior in the environment are warranting and challenging.

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