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## 4.16 Improving Plant Nitrogen-Use Efficiency

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### Glossary

**crop** The cultivated produce of the ground while growing or when gathered.

**fertilizer** Any substance used to fertilize the soil, especially a commercial or chemical manure.

**genotype** The genetic makeup of an organism or a group of organisms with reference to a single trait, set of traits, or an entire complex of traits.

**patent** The exclusive right granted by a government to an inventor to manufacture, use, or sell an invention for a certain number of years.

**phenotype** The observable constitution of an organism.

**quantitative trait loci (QTL)** Quantitative trait loci refers to the inheritance of quantitative traits or polygenic inheritance of a phenotypic characteristic that varies in degree and can be attributed to the interactions between two or more genes and their environment.

**transgenics** The branch of biology concerned with the transfer of genes to other species.

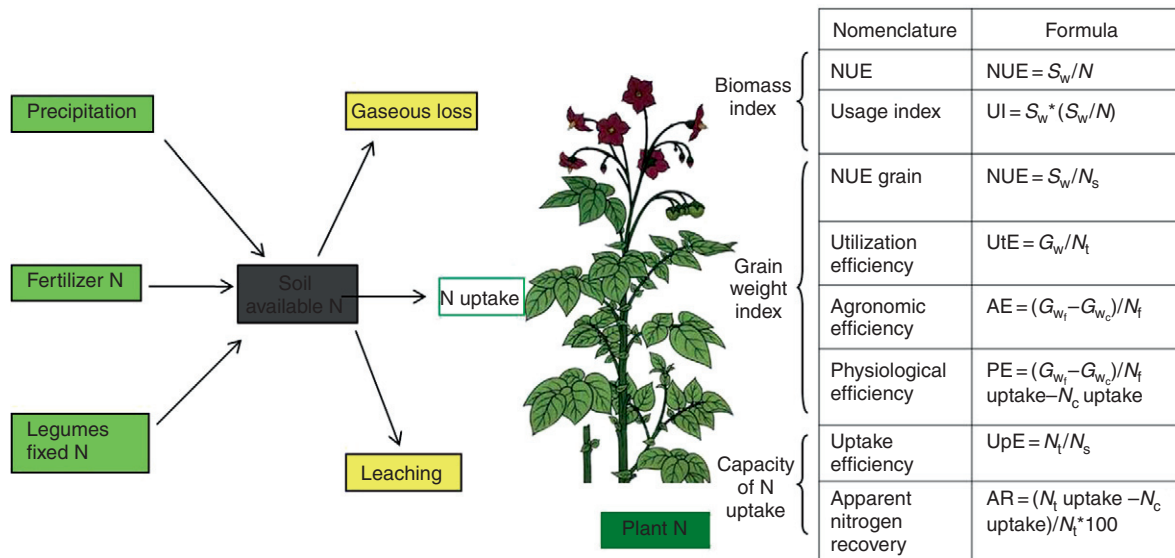
### 4.16.1 Introduction

Agriculture is the mainstay of most developing economies across the globe and continues to play a vital role in the sustenance of human society. Most agricultural practices depend heavily on the use of inorganic nitrogenous fertilizers, with global use of nitrogen (N) at the beginning of this decade amounting to 87 million metric tonnes and projected to increase to 236 million metric tonnes by the year 2050. However, the ability of plants to effectively utilize N from the soil depends on a number of variables, which is further compounded by the fact that close to 50–75% of N applied to agricultural land is used by microorganisms or lost through leaching. Naturally, there is growing interest in reducing fertilizer-N inputs by improving plant N-use efficiency (NUE).

Although the amount of N available to the plant can be improved through fertilizer–soil–water–air interactions, the innate efficiency of the plant to utilize this available N necessitates biological interventions. They may include biological processes, such as N uptake, distribution and assimilation, and their optimal contribution toward agricultural outputs, such as biomass growth and/or increased grain/leaf/flower/fruit/seed output. The identification of appropriate phenotypes, genotypes, molecular markers, and target candidates for the improvement of NUE pose a formidable challenge. The present article discusses NUE as a concept and its relevance with respect to environment and society, the different approaches for enhancing NUE and perspectives on its scope and avenues for future development.

### 4.16.2 What Is NUE?

As a concept, NUE includes N uptake, utilization, or acquisition efficiency, expressed as a ratio of output (total plant N, grain N; biomass yield, grain yield) and input (total N, soil N, or N-fertilizer applied). From one of the earliest definitions of NUE that considered the amount of plant yield in terms of either grain per unit of applied N (NUE grain) or biomass per unit of applied N



**Figure 1** The key events involved in uptake and utilization of nitrogen in plants.  $S_w$ , shoot weight;  $N$ , total nitrogen content of shoots;  $G_w$ , grain weight;  $N_s$ , nitrogen supplied in gram per plant;  $N_t$ , total nitrogen in plant;  $G_{w_f}$ , grain weight with fertilizer;  $G_{w_c}$ , grain weight without fertilizer (control);  $N_f$ , nitrogen fertilizer applied;  $N_f$  uptake, plant nitrogen with fertilizer;  $N_c$  uptake, plant nitrogen unfertilized control; PE, physiological N-use efficiency.

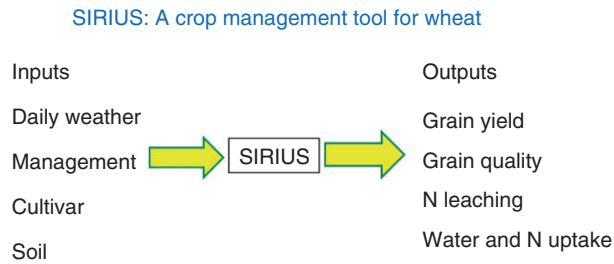
[10] to a recent report on the N characteristics of two rice cultivars who defined it as plant-N content expressed over the total N supplied to the plant; there are a range of interpretations of NUE (Figure 1).

Over the past few decades, a number of parameters have been put forth for evaluating NUE. One of the most widely used approach measures either total biomass or grain weight [10]. Apart from this, efficiency of extracting N from soil is another important measure of NUE for crop plants. In agronomic terms, the product physiological efficiency (PE) and apparent recovery is used to arrive at the net agronomic efficiency (AE), which in combination with NUEg (product of uptake efficiency (UpE) and utilization efficiency) provide a reflection of the overall efficiency of the applied N in producing grain yield. Craswell and Godwin [7] offered a different perspective, in which an unfertilized control was used as an initial starting point for calculating NUE that also included physiological factors. Their fertilizer efficiency parameters include AE, apparent N recovery (AR), and PE. AR reflects the efficiency of the crop in obtaining N-based fertilizer from the soil, whereas PE can be viewed as the efficiency with which crops use N in the plant for the synthesis of grain.

However, the appropriate way to estimate NUE eventually depends on the crop, its harvest product, and specific physiological processes involved in NUE. For example, most internal estimates of NUE for monocot plants are represented as UtE or NUEgrain to include grain yield and express yield in relation to N supplied. In most cases, UtE is considered to be a better reflection of the ability of plants to use N because NUEgrain is influenced by N uptake as well as internal utilization or partitioning. This has been revealed in a number of breeding programs that showed considerable variation in UpE and UtE in several important crop species including rice, maize, and barley by considering N content, grain number, and yield as well as environment or genetic effects [4]. Although breeding research has typically focused on trends across cultivars/lines without addressing the mechanistic reasons for high NUE germplasm, molecular approaches have concentrated on studying the mechanisms and analyzing various factors such as amino acid pools and photosynthesis measurements without investigating physiological factors contributing directly to yield. However, recent biotechnological interventions that focus on creating transgenic NUE lines have taken a more inclusive approach by combining multiple factors to assess the net improvement in NUE.

### 4.16.3 Agronomic Approaches for Improving NUE

Crop production in most countries is highly dependent on the supply of exogenous N fertilizers. Regardless of whether the source of N is chemical fertilizers, biofertilizers, animal dung, residue burning, or farm-waste manure, the adverse consequences of their accumulation in the environment are the same, barring the additional dependence of synthetic fertilizers on fossil fuels. This necessitates consideration of the overall NUE in food grain production expressed as partial factor productivity of N (PFPN). PFPN is an aggregate efficiency index that includes contributions to crop yield derived from uptake of indigenous soil N, fertilizer N UpE, and the efficiency with which N acquired by the plant is converted to grain yield. Fertilizer NUE can be controlled by crop demand for N, supply of N from the soil, fertilizer and manure, and loss of N from the soil-plant system. Recent literature emphasizes on achieving greater synchrony between crop N demand and N supply throughout the growing season by using a combination of



**Figure 2** Various input and output parameters calculated by SIRIUS.

anticipatory (before planting) and responsive (during the growth season) decisions. Improved synchrony can be achieved by more accurate N prescriptions based on the projected crop N demand and the levels of mineral and organic soil N, and also through improved rules for splitting of N applications according to phenological stages, using decision aids to diagnose soil and plant N status during the growing season (models and sensors), or using controlled-release fertilizers or inhibitors [18].

As described earlier, NUE is interplay of several complex interdependent parameters that need close monitoring in order to arrive at a deterministic approach for its improvement. Some effects of changes in a single trait on crop performance can theoretically be determined in a field experiment. However, crop responses also depend on weather and environmental conditions due to large and variable effects. Naturally, experimental determination of how new plant characteristics, either individually or in combination, will affect crop performance under a wide range of growing conditions becomes an unfeasible task. This analysis could be performed *in silico* by replacing a real plant with a crop model based on physiological and environmental parameters. SIRIUS is one such simulation model for wheat that calculates biomass from intercepted photosynthetically active radiation and grain growth from simple partitioning rules. It is based on mechanistic descriptions of wheat phenology and N uptake and redistribution, which makes possible to link model cultivar parameters with simple physiological traits. Also considered are several simple crop traits for sensitivity analysis of NUE that include traits controlling wheat development, some determining sizes of N storage pools in the plant and others responsible for UpE of roots for water and N. Using this tool, researchers have demonstrated that a crop-simulation model could be a powerful tool for deconvoluting complex traits in wheat with the potential to facilitate genetic, and subsequent genomics, research by focusing experiments on those wheat traits that are identified by modeling as the most promising (Figure 2).

Apart from the above, there are other cropping practice-based or crop management-based methods to improve NUE, such as crop rotation with N-fixing crops and optimization of chemical, biofertilizers, manures, and other N sources, as well as selection of natural plant varieties with better NUE. Biological interventions are aimed at further improvement of the natural or genetically inherent NUE using plant breeding and molecular marker-assisted methods, and recently transgenic interventions for improving NUE.

#### 4.16.4 Transgenic Efforts to Improve NUE

NUE is a multigenic trait spread across hundreds of genes that extends beyond primary nitrate assimilation and metabolism. Naturally, transgenic efforts have concentrated on diverse targets that include genes belonging to uptake, translocation, remobilization, and carbon metabolism. In addition, signaling targets and regulatory elements have recently emerged as prospective candidates for biotechnological interventions. The following sections summarize various transgenic attempts to improve NUE based on the processes targeted.

##### 4.16.4.1 NUE – Transgenic Approach

Biotechnological interventions to improve NUE have largely revolved around manipulation and overexpression of many crucial candidate genes apart from using knockout mutations to assess its effects on biomass and plant N status and overall yield [10, 14, 15]. N assimilatory pathway is one of the most widely chosen targets for improvement of NUE. The primary nitrate assimilation pathway that involves nitrate transporters, nitrate reductase (NR), nitrite reductase (NiR), plastidic glutamine synthetase (GS2), and ferredoxin glutamate synthase (Fd-GOGAT) along with secondary N assimilation and remobilization that includes cytosolic glutamine synthetase (GS1), reduced nicotinamide adenine dinucleotide glutamate synthase (NADH-GOGAT), glutamate dehydrogenase (GDH), and various aminotransferases; offers myriad opportunities of intervention at the uptake, assimilation, translocation, and remobilization stages.

Emerging evidences from these efforts reveal that attempts to improve NUE in plants need to target different organs for individual plants. For example, in cereals, the total grain biomass or grain N content would be most suitable indicators of NUE, rather than any other organ. Thus, areas where NUE can be targeted is to improve the distribution of N between canopy (leaves and stem) and roots, better photosynthetic rate/unit leaf N, reduced leaf senescence, transgenics developing C<sub>4</sub> options for rice and

wheat, or otherwise increasing the efficiency of net photosynthesis in warmer environments by modifying Rubisco, Rubisco activase, and the enzymes that modulate photorespiration in C<sub>3</sub> plants.

#### 4.16.4.2 Manipulating Genes of N Uptake

N uptake is the first step in nitrate assimilation, which can be manipulated to enhance NUE. Two types of nitrate uptake systems: low-affinity transport system encoded by NRT1 gene family and high-affinity transport system encoded by NRT2 gene family. In addition, a number of ammonium transporters and putative amino acid transporters have also been identified in *Arabidopsis*. In the last decade, several attempts of overexpression of high-affinity nitrate/nitrite transporters in tobacco and *Arabidopsis* [15] reported enhanced nitrate uptake, but concomitant increase in assimilation and NUE was not observed. Though the various nitrate and ammonium transporters in plants are very well characterized and their differential regulation mechanisms are well known, overexpression studies involving their genes have not been conclusive (Table 1).

#### 4.16.4.3 Manipulating Genes of N Assimilation

Several attempts at transgenic manipulation of the enzymes of primary and secondary assimilation have been made as described in Table 1. Primary nitrate assimilation involves NR, NiR, plastidic GS (GS2), and Fd-GOGAT, while cytosolic GS (GS1) and NADH-GOGAT are a part of secondary ammonia assimilation and remobilization. NR is considered as a rate-limiting step in nitrate metabolism. There have been reports of overexpression of two genes *nia1* and *nia2* for NR in *Arabidopsis*, tobacco, potato, and lettuce, without any specific improvement in NUE. Similarly, little improvement in phenotype and NiR activity levels were observed when *nii* gene was overexpressed in tobacco and *Arabidopsis* under CaMV 35S promoter, though there was an increase in the NiR transcript level. Overexpression of GS2 has also been reported in rice and tobacco, but no significant improvement in terms of NUE was observed. Though, transgenic tobacco plants overexpressing GS showing improved capacity for photorespiration and an increased tolerance to drought while transgenic rice plants showed only better growth rate. The potential of transgenic plants with overexpressed Fd-GOGAT gene has not been tested yet, although barley mutants with reduced Fd-GOGAT revealed changes in various nitrogenous metabolites, decreased leaf protein, rubisco activity, and nitrate contents.

The genes of secondary ammonia assimilation have also been overexpressed in a variety of crops for developing transgenic with enhanced NUE. GS1 has emerged as a potential candidate from among all the genes that have been tested so far. Overexpression of GS1 gene has been tried in several plants such as wheat, tobacco, and maize have resulted in higher grain yield and biomass with improved N content. Transgenic overexpression and antisense technology has been employed to modulate the expression of NADH-GOGAT gene in rice and alfalfa plants. Though the genes of secondary ammonia assimilation appear to be good candidates for improving NUE in short run, the results may vary with crop to crop variation.

#### 4.16.4.4 Manipulating Genes of N Translocation and Remobilization

N remobilization is proposed as one of the key steps in improving NUE in plants [14]. In cereals, the main source of N for the grains is N remobilized from the vegetative parts. This source accounts for 60–92% of the N accumulated in the grains at maturity. The amount of N remobilized depends on N remobilization efficiency and the amount of N available. It is also well known that genotype and many environmental factors are known to affect N translocation, which makes the genes involved in remobilization and translocation attractive targets for improvement of NUE.

There are several reports of transgenic overexpression of AS genes that are actively involved in remobilization and translocation of amino acids resulting in enhanced seed protein content and total protein content. Molecular manipulation of asparagine synthetase (encoded by *Aln1* gene) has been attempted recently along with reports of genetically engineered plants overexpressing alanine aminotransferase [11]. Taking a cue from some of these studies, genetically engineered rice (*Oryza sativa* L.) was developed by introducing barley alanine aminotransferase complementary DNA (cDNA) driven by tissue-specific *OsAnt1* promoter [17]. These plants showed improved biomass and grain yield along with significant change in key metabolites and nitrate content confirming increased NUE. Further, Beatty *et al.* [2] reported the involvement of some candidate genes through root and shoot transcriptome analysis in engineered rice overexpressing alanine aminotransferase (AlaAT) under the control of a tissue-specific promoter showing a strong NUE phenotype. Though the importance of GDH in higher plant N remobilization is still controversial, transgenic plants overexpressing *gdhA* gene were shown to have improved amino acid content, higher yields in maize and wheat [13]. More recently, *Arabidopsis* NRT1 transporter NRT1.7 has been implicated in nitrate remobilization from source to sink tissues by mutant analysis [9].

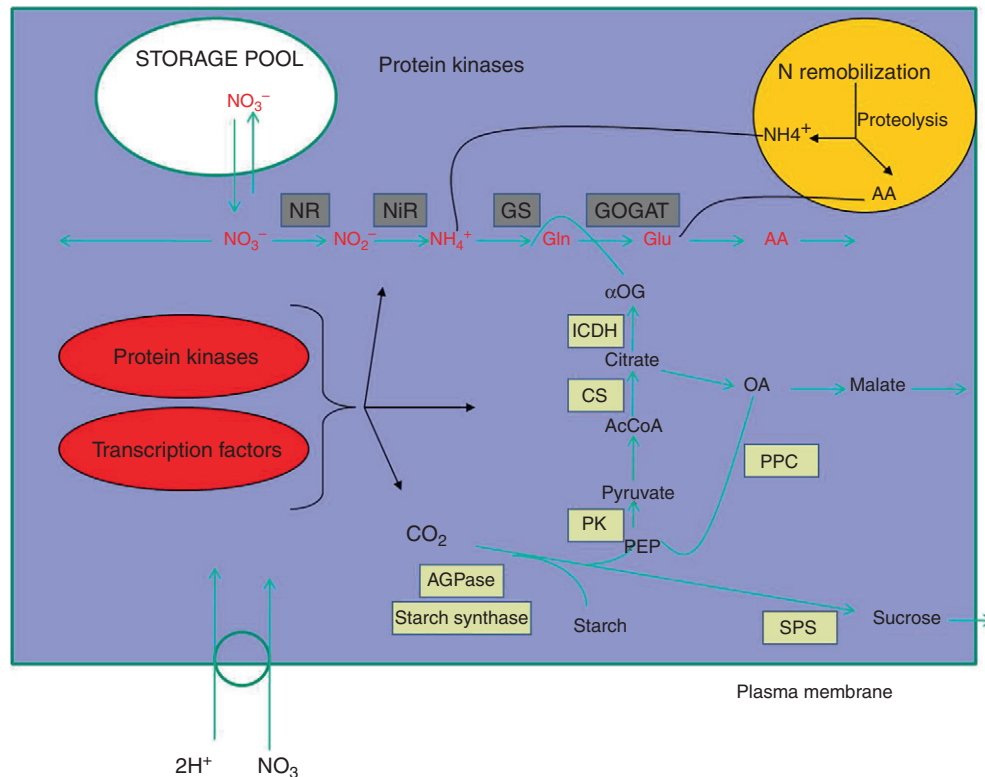
#### 4.16.4.5 Manipulating Genes of C Metabolism

Sugars play an important role in plant growth and metabolism by providing carbon skeletons and energy for cellular metabolism. However, sugar metabolism and signaling influences a number of processes involved in plant growth and development, such as seed germination, embryogenesis, flowering, and senescence, and have also been implicated in hormone signaling. The genes involved in N metabolism and nitrate signaling are also tightly regulated by sugar signaling mechanisms. A coordination between N and C metabolism is required at the amino acid synthesis level due to the requirement of carbon skeletons for their synthesis. SnRK1, a principal regulator in carbon signaling, is known to be linked to N and amino acid metabolism. Similarly to CDPK, GCN2



**Table 1** List of plant transgenics and observed phenotypes

Gene product and gene source	Promoter	Target plant	Phenotype observed
Nrt1.1 – high affinity nitrate transporter ( <i>Arabidopsis</i> )	CaMV 35S	<i>Arabidopsis</i>	Increase in constitutive nitrate uptake but not in induced
Nrt2.1 – high affinity nitrate transporter ( <i>N. plumbaginifolia</i> )	CaMV 35S, rol D	<i>N. tabacum</i>	Increased nitrate influx under low N conditions
NR – nitrate reductase	<i>N. plumbaginifolia</i> CaMV 35S	<i>N. tabacum</i>	Three to four fold drop in NR protein and activity, no change in NR transcript
	<i>N. plumbaginifolia</i>	<i>N. tabacum</i>	Increased NR activity, biomass, drought stress
Nia – nitrate reductase	<i>N. tabacum</i> CaMV 35S	<i>L. sativa</i>	Reduced nitrate content, chlorate sensitivity
	<i>N. tabacum</i>	<i>N.plumbaginifolia</i>	Nitrite accumulation in high nitrate supply
Nia2 – nitrate reductase ( <i>N. tabacum</i> )	CaMV 35S	<i>S. tuberosum</i>	Reduced nitrate levels
NiR – Nitrite reductase	<i>N. tabacum</i> CaMV 35S	<i>N. plumbaginifolia</i> , <i>Arabidopsis</i>	NiR activity, no phenotypic difference
	<i>S. oleracea</i>	<i>Arabidopsis</i>	Higher NiR activity, higher nitrite accumulation
GS2 – chloroplastic glutamine synthetase	<i>O. sativa</i> CaMV 35S	<i>N. tabacum</i>	Improved photorespiration capacity, and increased resistance to photo-oxidation
	<i>O. sativa</i>	<i>O. sativa</i>	Enhanced photorespiration, salt tolerance
	<i>N. tabacum</i> Rubisco small subunit	<i>N. tabacum</i>	Enhanced growth rate
Fd- GOGAT – Fd dependent glutamate synthase ( <i>N. tabacum</i> )	CaMV 35S	<i>N. tabacum</i>	Diurnal changes in NH <sub>3</sub> assimilation
GS1 – cytosolic glutamine synthetase	<i>G. max</i> CaMV 35S <i>G. max</i> rol D <i>P. vulgaris</i> Rubisco small unit	<i>L. corniculatus</i> <i>L. japonicus</i> <i>T. aestivum</i>	Accelerated senescence Decrease in biomass Enhanced capacity to accumulate nitrogen
	<i>M. sativa</i> CaMV 35S	<i>N. tabacum</i>	Enhanced growth under N starvation
	<i>G. max</i> CaMV 35S	<i>M. sativa</i>	No increase in GS activity
	Pea CaMV 35S	<i>N. tabacum</i>	Enhanced growth, leaf soluble protein, ammonia levels
	<i>P. sylvestris</i> CaMV 35S	Hybrid poplar	Enhanced growth rate, leaf chlorophyll, total soluble protein
	<i>G. max</i> CaMV 35S	<i>P. sativum</i>	No change in whole plant N
	<i>Alfalfa</i> CaMV 35S	<i>L. japonicus</i>	Higher biomass and leaf proreins
NADH-GOGAT – NADH-dependent glutamate synthase	<i>O. sativa</i> <i>O. sativa</i> CaMV 35S	<i>O. sativa</i> <i>N. tabacum</i>	Enhanced grain filling, increased grain weight Higher total C and N content, increased dry wt
GDH – glutamate dehydrogenase	<i>E. coli</i> CaMV 35S	<i>N. tabacum</i>	Increased biomass and dry weight
	<i>E. coli</i> CaMV 35S	<i>N. tabacum</i>	Increased ammonium assimilation and sugar content
	<i>L. esculentum</i> CaMV 35S	<i>L. esculentum</i>	Twice GDH activity, higher mRNA levels, and twice glutamate concentration
	<i>E. coli</i> OsUB1	<i>Z. mays</i>	Increased N assimilation , herbicide tolerance, biomass, grain aa content
	<i>C. sorokiniana</i> CaMV 35S	<i>T. aestivum</i>	Schmidt and Miller, 2009 (patent no. 627,886
	<i>C. sorokiniana</i> CaMV 35S	<i>Z. mays</i>	Schmidt and Miller, 2009 (patent no. 627,886
ASN1 – glutamine-dependent asparagine synthetase ( <i>A. thaliana</i> )	CaMV 35S	<i>A. thaliana</i>	Enhanced seed protein
ASNI – asparagine synthetase ( <i>P. sativum</i> )	CaMV 35S	<i>N. tabacum</i>	Reduced biomass and increased level of free asparagine
AspAT – mitochondrial aspartate aminotransferase (prosomillet)	CaMV 35S	<i>N. tabacum</i>	Increased AspAT, PEPCase activity
AlaAT – alanine aminotransferase(barley)	btg26	<i>Brassica napus</i>	good yields even with 50% less N fertilizer
ANR1 – MADS transcription factor ( <i>Arabidopsis</i> )	CaMV 35S	<i>Arabidopsis</i>	Lateral root induction and elongation
GLB1 – PII regulatory protein ( <i>Arabidopsis</i> )	CaMV 35S	<i>Arabidopsis</i>	Growth rate, increased anthocyanin production in low N
Dof1 – transcription factor ( <i>Zea mays</i> )	35S C4PDK	<i>Arabidopsis</i>	Enhanced growth rate under N-limited conditions, increase in amino acid content
<i>OsENOD93-1</i> nodulin gene	35S C4PDK	<i>Rice</i>	Increased shoot mass and seed yield



**Figure 3** Schematic representation of key processes and enzymes involved in nitrogen metabolism in plants. Nitrate and ammonium ions are taken by transporters across the cell membrane, assimilated and incorporated into C metabolites to generate amino acids. The amino acids from degraded proteins in senescing tissues are remobilized into the N pool of the cell. All these processes are controlled by signaling molecules and transcription factors. NR, nitrate reductase; NiR, nitrite reductase; GS, glutamine synthetase; GOGAT, glutamate synthase;  $NO_3^-$ , nitrate ion;  $NO_2^-$ , nitrite ion;  $NH_4^+$ , ammonium ion; Gln, glutamine; Glu, glutamate; AA, amino acids;  $\alpha$ OG, 2-oxoglutarate ( $\alpha$ -ketoglutarate dehydrogenase); ICDH, isocitrate dehydrogenase; CS, citrate synthase; PK, pyruvate kinase; AGPase, ADP glucose phosphorylase; PPC, phosphoenolpyruvate carboxylase; SPS, sucrose phosphate synthase; PEP, phosphoenolpyruvate; OA, oxaloacetate; Ac CoA, acetyl coenzyme A.

directly act on NR in plants. A mutant lacking GCN2 showed decreased expression of nitrate reductase (*nia1*) gene in *Arabidopsis*. However, whether this had any direct implication on improvement of NUE remains to be validated. Recently, transgenic *Arabidopsis* plants overexpressing STP13, a member of sugar transporter family, showed increased rates of glucose uptake, higher internal sugar levels, and more total C per plant. STP13OX seedlings also displayed improved N use, with the induction of a nitrate transporter and higher total N per plant (Figure 3).

#### 4.16.4.6 Manipulating Signaling Targets

Nitrate is a potent signal that affects N and carbon metabolism as well as organ growth and development in plants. These effects are mediated at least in part by changes in gene expression that are elicited by nitrate. Identification of nitrate-responsive gene expression profiles of plants would offer potential targets for improvement of NUE. The list of nitrate-responsive genes in *Arabidopsis* runs into several hundreds, as revealed by microarray analyses. Recently, we found more than 1000 genes in rice to be nitrate responsive in green and etiolated leaves, with 159 genes being exclusively nitrate responsive (Gene Expression Omnibus accession ID: GSE 12940). Transcription factors (TFs) are master regulators that coordinate the expression of entire response networks of target genes and a number of attempts have been made to identify TFs that regulate nitrate-responsive gene expression. Recently, bioinformatic approaches have been employed to search for TFs in nitrate-responsive genes identified by microarray in *Arabidopsis* and rice, although no consensus candidate has been revealed yet [8]. Transgenic *Arabidopsis* lines overexpressing Dof1, a maize protein that belongs to the Dof family of plant-specific TFs known to activate the expression of several C-metabolizing genes associated with organic acid metabolism have been generated. The genes upregulated by Dof1 overexpression clearly belong to the list of known nitrate-responsive genes, opening up attractive possibilities of improving NUE through coordinated expression of N and C metabolizing genes. A few other attempts to manipulate signaling/regulatory proteins have been made without significant advantage in terms of NUE. TFs such as AtNF-YB1 from *Arabidopsis thaliana* and ZmNF-YB2 from maize improved NUE and water use efficiency (WUE) in transgenic plants. (Other attempts, such as the one to manipulate a MADS box protein that controls nitrate-induced changes in root architecture, have not been assessed for their impact on NUE.)

#### 4.16.4.7 Transgenic Approaches for Improving NUE: A Critical Evaluation

The last decade has witnessed an explosion in transgenic approaches aimed at improving NUE. Transgenic overexpression of primary N assimilatory genes in model plants did not yield any significant improvement in NUE [13, 15], although physiological experiments comparing high-NR and low-NR cultivars of wheat in India indicated that the expression levels of primary N-assimilatory enzymes may matter for NUE in some cases. On the other hand, marker studies have indicated that genes of secondary N metabolism could be more critical for NUE than the genes of primary N assimilation, especially in cereals which remobilize internal N pools from senescing leaves during grain filling.

Apart from genes belonging to the the N-assimilatory pathway, various other candidates are being indentified through screening of different varieties for their NUE under limiting conditions [5]. Transgenic overexpression of carbon-assimilating genes such as PEPCase and PPDK, plant transcriptional factors like hap3 and Dof-1, and genes involved in N fixation and stress response are also being studied for potential genetic manipulations to enhance NUE. Identification of putative regulatory elements for plant adaption to low/limited N conditions has also suggested some novel candidates for transgenic studies.

As detailed above, these studies have remained largely inconclusive vis-à-vis its impact on various NUE assessment parameters. For instance, out of the many studies, only one reported improvement in NUE, more specifically UpE [17] Out of the remaining, a few provided data that facilitated calculation of NUE based on conventional indices, while a couple of field trials were conducted on transgenic varieties to assess on-field performance. Major revelations from these studies have been the marked difference in NUE-assessment indices for monocots and dicots. Although usage index (UI) and UpE have emerged as more reliable and sensitive-assessment factors in case of dicots, NUE has been measured in terms of UpE and UtE in monocot species by overexpressing N-assimilatory enzymes such as GS1, GOGAT, and AlaAT. Although NUE is addressed at various molecular levels, to date, neither transgenic products nor classically bred enhanced NUE material have been released for commercial use anywhere in world. Many biotech companies such as Pioneer Hi-Bred International, Monsanto, Evogene, Metanomics GmbH, and Arcadia Biosciences have numerous patents filed with promising claims of enhancing NUE in plants (Table 2 and Table 3), though these claims are yet to be validated.

However, recent reports of field trails of genetically modified crops with high NUE have been appearing in the public domain. An Australian organization, Commonwealth Scientific and Industrial Research Organisation (CSIRO) has applied for a licence for dealings involving the intentional release of 17 lines of wheat (*Triticum aestivum* L. em Thell.) and 10 lines of barley (*Hordeum vulgare* L.), which have been genetically modified for enhanced nutrient utilization efficiency on a limited scale and under controlled conditions. The genetically modified (GM) wheat and barley lines contain a metabolic enzyme gene (*Me1*) derived from barley which is expected to enhance the efficiency of N utilization resulting in an increased plant biomass and yield.

In addition, there is an increasing need to link the crop yield potential with the better adaptation to various stresses because many of the transgenic varieties with improved NUE parameters are also resistant to abiotic stresses such as drought. This is evident from recent efforts in crop-breeding practices that have been aiming to obtain nutrient efficient varieties by using a combinatorial strategy of screening for water-use efficiency and nutrient-use efficiency in plants.

#### 4.16.5 Quantitative Trait Loci Mapping to Find New Targets for Manipulation

Over the past century, increase in crop yields have been attributed to the selection of genotypes with a higher yield potential and to the parallel increase in the application of fertilizers, particularly N [1,3,16]. The advent of techniques to identify molecular markers facilitated subsequent evaluation of the inheritance of NUE using specific quantitative trait loci (QTLs). Over the last decade, QTLs have been identified in tropical maize for NUE, grain yield, and its components at high and low N levels that included a number of genes encoding enzymes involved in N and C metabolism,. QTLs for tolerance to low N have also been described in *Arabidopsis*, rice, and barley [12]. Several positive coincidences between QTLs for N-uptake and QTLs for root architecture traits, have suggested that a way of increasing NUE is to simply breed for a root system more efficient at taking up N [6]. QTL-based approaches offer exciting opportunities to discover factors that might control yield and its components directly or indirectly, thereby improving overall NUE.

#### 4.16.6 Improving NUE: A Systems Biology Approach

Systems biology deals with the study of the interactions between the components of biological systems, and how these interactions give rise to the function and behavior of that system (e.g., the enzymes and metabolites in a metabolic pathway). From the early general systems theory of Ludwig von Bertalanffy and the Hodgkin and Huxley's mathematical model that explained the action potential propagating along the axon of a neuronal cell; systems biology today encompasses multiple disciplines and interdisciplinary tools. Functional genomics and transcriptomics that are a part of the systems biology approach have made available large quantities of high-quality data, which along with a concomitant making development of computational and bioinformatic tools greatly accelerated the application of systems biology to study myriad biological phenomena.

The increasing availability of genomic and transcriptomic data has enabled segregation of biological responses to nutrients and signals that hint at a more pervasive interconnected mode of regulation that includes multiple pathways. For instance, the separation of light and nitrate signals in rice using microarray (described above) offers newer targets for improving NUE using



**Table 2** Patents granted in aspects related to NUE in various plants

Patent no.	Patent issue date	Description/claim	Assignee	Inventee
7,696,409	13 April 2010	Nucleotide sequences and polypeptides encoded thereby useful for modifying nitrogen use efficiency characteristics in plants	Ceres, Inc. (Thousand Oaks, CA)	Schneeberger R <i>et al.</i>
7,589,257	15 September 2009	Increased ferredoxin NADP+ reductase activity in transformed maize causing increased NUE by maintaining similar output with less fertilizer uptake	Pioneer Hi-Bred International Inc. (Johnston, IA)	Hershey Howard P <i>et al.</i>
7,692,067	6 April 2010	A transgenic dicot plant having greater tolerance to low nitrogen during germination relative to a control plant having better yield and stress tolerance	Mendel Biotechnology, Inc. (Hayward, CA) Monsanto Company (St. Louis, MO)	Creelman Robert <i>et al.</i>
6084153	7/2000	Stress inducible promoter btg-26, leading to enhanced N assimilation/metabolism	The Governors of the University of Alberta (Edmonton, CA)	Good, Allen G <i>et al.</i>
7,560,626	7/2009	Oryza sativa antiqutin (OsAnt1) promoter sequence, higher yields with less N	Arcadia Biosciences, Inc.	Good, Allen G <i>et al.</i>
6822079	11/2004	Transgenic plants over- expressing plant nitrogen regulatory P11 polypeptide	New York University (New York, NY)	Coruzzi Gloria M. <i>et al.</i>
6,177,275	1/2001	Plant nitrogen regulatory P-PII genes	New York University (New York, NY)	Coruzzi Gloria M. <i>et al.</i>
5998700	12/1999	Plants containing a bacterial GdhA gene and methods of use thereof	The Board of Trustee of southern Illinois University, Carbondale, IL	Lightfoot David A. <i>et al.</i>
6329573	12/2001	Plants containing the gdhA gene and methods of use thereof	The Board of Trustee of southern Illinois University, Carbondale, IL	Lightfoot David A. <i>et al.</i>
7485771	3/2009	Polypeptides and polynucleotides relating to the alpha- and beta-subunits of glutamate dehydrogenases and methods of use.	University of Florida Research Foundation, Inc. Gainesville, FL	Schmidt, R. R. and Miller, P.
7390937	6/2008	Plants with enhanced nitrogen utilization protein operably linked to a root-epidermis-specific promoter	The Governors of the University of Alberta (Edmonton, Alberta, CA)	Good, Allen G <i>et al.</i>

exclusively nitrate-regulated genes. The expanding list of potential targets for NUE manipulation moves beyond primary metabolism as indicated by numerous patents that have been filed recently (Tables 2 and 3). United States Patent and Trademark Office (USPTO) has also recently awarded patents that are related to increased WUE in plants by developing transgenics of signaling molecules such as protein kinases and vesicle trafficking proteins, vesicle trafficking protein stress-related polypeptides (VTSRPs) and protein kinase stress-related protein (PKSRP), scarecrow-like stress-related polypeptides (SLSRPs), casein kinase stress-related polypeptide (CKSRP) (Patent numbers: 7,608,759; 7,442,853; 7,423,196; 7,399,904; 7,399,903).

#### 4.16.7 Global Status of NUE

Global consumption of N fertilizers has averaged 83 Mt in recent years, of which about 47 Mt is applied to cereal crops. However, significant differences exist among world regions, particularly with regard to NUE that depend on which cereal crops are grown, their attainable yield potential, soil quality, amount and form of N application, and the overall timeliness and quality of other crop-management operations. In developing regions, N fertilizer use was comparatively lower in the early 1960s and increased exponentially during the course of the Green Revolution. Although the growth rate in N consumption has slowed substantially in recent years, it still averaged 1.45 Mt N yr<sup>-1</sup> (3.2% yr<sup>-1</sup>) during the past 20 years. In developed regions, excluding Eastern Europe/Central Asia, cereal yields have continued to increase in the past 20 years without significant increases in N fertilizer use.

The share of total N fertilizer consumption that is applied to cereals ranges from a low of 32% in Northeast Asia to more than 71% in SE Asia. At a worldwide level, cereal production, cereal yields, and fertilizer N consumption have increased in a near-linear fashion during the past 40 years, although significant differences exist among world regions, particularly with regard to NUE. Improved NUE in case of developed countries can be attributed to investments in research and extension on crop improvement, new fertilizer products, and better management technologies by both public and private sectors, at levels that greatly exceed those currently available in the developing world. However, interventions to increase NUE and reduce N losses to the environment must be accomplished at the farm level through a combination of improved technologies and carefully crafted local policies that promote the adoption of improved N management practices while sustaining yield increases.

**Table 3** Patents filed in aspects related to NUE in various plants

USPA no.	Date of publication	Description/claim	Assignee	Inventee
20100115667	6 May 2010	Novel At1g67330 gene involved in altered <i>nitrogen</i> utilization and/or uptake in plants	Pioneer Hi-bred International Inc. Johnston, IA	Frank Mary J. <i>et al.</i>
20100115662	May 6, 2010	Manipulation of glutamine synthetases (gs) to improve <i>nitrogen</i> use efficiency and grain yield in monocots	Pioneer Hi-bred International Inc. Johnston, IA	Gupta Rajeev <i>et al.</i>
20100107280	April 29, 2010	Modulation of glutamine synthetase activity in maize resulting in high yield an NUE	Alston and Bird LLP Charlotte, NC	Hirel Bertrand <i>et al.</i>
20100037350	February 11, 2010	More activities of phosphoribosyl pyrophosphate synthases (PRPP synthetase, PRS) increasing yield	BASF Plant Science GmbH Ludwigschafen DE	Zank Thorsten <i>et al.</i>
20090320156	December 24, 2009	Monocots with improved nitrate uptake and/ utilization efficiency	Pioneer Hi-bred International Inc. Johnston IA	Hershey Howard P <i>et al.</i>
20090300794	12/2009	Increasing the activity of ammonium transporters, G6PD, under conditions which permit the enhanced N assimilation, accumulation, utilization for increasing total NUE content	Metanomics GmbH, Berlin	Plesch, Gunnar <i>et al.</i>
20090183270	07/2009	Hap3 transcription factor protein with CAAT box binding domain	Monsanto	Adams, Thomas R. <i>et al.</i>
20090119804	05/2009	LNT2 polypeptide manipulated for agronomic traits like total plant N content, fruit N content, N content in vegetative parts and N uptake	El and Pioneer Hi-bred International Inc.	Aukerman, Milo <i>et al.</i>
20090049573	02/2009	A plant HAB3 transcription factor and a polypeptide (N-terminal domain of 14-3-3 protein) which improves tolerance to water deficit stress and increases N availability in rice	Monsanto	Dotson, Stanton B <i>et al.</i>
20090025102	01/2009	Glutamate receptor associated genes and proteins for enhancing NUE	Pioneer Hi-bred International Inc. and The George Washington university, Washington DC	Hershey, Howard P <i>et al.</i>
20080313775	12/2008	NR from red algae ( <i>Porphyra perforate</i> -PpNR) and <i>Porphyra yezoensis</i> - PyNR to increase yield or NUE under lower fertility	Pioneer Hi-bred International Inc	Loussaert F Dale <i>et al.</i>
20100050293	12/2009	Ammonium transporter (AMT) to improve NUE	Pioneer Hi-bred International Inc	Rajeev Gupta and Dhugga Kanwarpal S. Nadzan Gred <i>et al.</i>
20070169219	07/2007	Modulate Dof1 transcription factor gene expression while the 2 <sup>nd</sup> mechanism is to sequester or store N in times of abundance.	Birch Stewart Kolasch & Birch	Schneeberger, Richard <i>et al.</i>
20070044172	02/2007	Peptide transporters to sequester or store N	Birch Stewart Kolasch & Birch	Good, Allen G <i>et al.</i>
20070162995	7/2007	Nitrogen efficient monocot plants with increased yield and high alanine aminotransferase activity	Arcadia Biosciences, Inc.	Good, Allen G <i>et al.</i>
20090288224	11/2009	<i>Oryza sativa</i> antiquitin (OsAnt1) promoter sequence with improved NUE	Arcadia Biosciences, Inc.	Good, Allen G <i>et al.</i>
20090044297	2/2009	HAP3 transcription factor protein and 14-3-3 with improved agronomic and N traits	Monsanto	Andersen Scott E. <i>et al.</i>

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