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Pergamon

4.17 Improving Crop 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 of commercial or chemical nature.

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

QTLs Quantitative Trait Loci refers to the genetic loci that determine 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.

Phenotype The observable constitution of a trait in an organism.

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

4.17.1 Introduction

Modern cropping systems and practices rely heavily on the use of inorganic nitrogenous fertilizers at huge costs, both in economic and environmental terms. This is because plants are not capable of directly using N_2 gas abundant in the air and depend on more reactive inorganic forms such as nitrates, ammonium compounds and urea. The Food and Agricultural Organization (FAO) estimated the global use of N fertilizer at about 120 million metric tons in 2018 and is growing at a compounded annual growth rate of 1.4%.[13] The global average of crop nitrogen use efficiency is 30% in terms of harvested N, but there are further losses in transport, storage and food processing. Moreover, crops are also grown with fertilizers for animal feed and as there are further losses from the animal, the harvested N falls further from dairy products to non-vegetarian diets. Sutton et al.[31] estimated that the full chain NUE of the global food production system is as low as 15%. They have also shown that the environmental costs of reactive N compounds from unused fertilizers or animal excreta, due to water and air pollution, ill health, climate change and threats to biodiversity, are manifold larger than the direct economic costs of wasted fertilizer. For example, they argued that a relative improvement in the current NUE by 20% (e.g., from 15% to 18%) would translate into benefits worth around US $170 billion/year, of which US

☆*Change History*: August 2018. The authors have updated the entire article. The title has been made more focussed from plant to crop N-use efficiency. The entire text from title to acknowledgments and references has been thoroughly updated including new sections on agronomic NUE, microbiological nitrogen fixation and phenotyping for NUE . Table 1 has been thoroughly updated and tables (2 & 3) have been deleted. Figures 1 & 2 from the previous edition have been retained as such, as they are still relevant.

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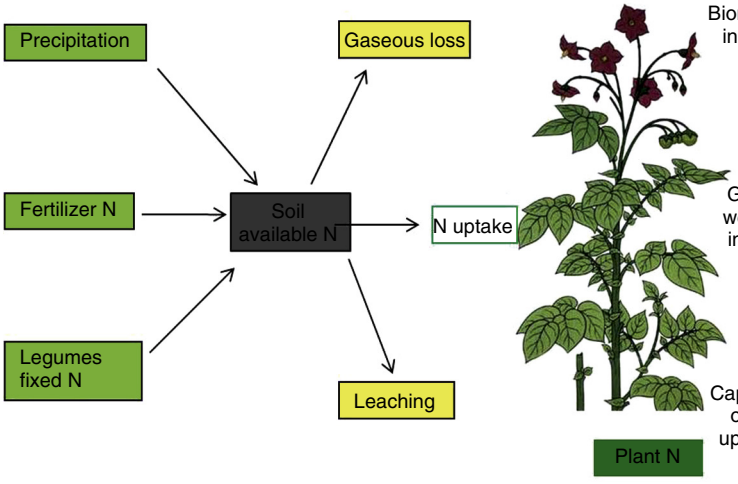
$23 billion/year would accrue from the saved fertilizer costs and the rest in terms of reduction in societal costs to health, ecosystems and climate[1,31] Globally, N losses from agricultural soils are the largest contributors of nitrous oxide, which is 300 times more potent greenhouse gas than carbon dioxide. Therefore, improving fertilizer NUE of crops has emerged as a major global need and hence the growing interest in reducing fertilizer-N inputs by improving plant N use efficiency (NUE).

While NUE improvement is needed for every crop, those that consume most of the N-fertilizer should naturally attract the top priority. As cereals constitute the bulk of global food grain production, global cooperation may be possible around important cereal crops, while individual countries may choose their own target crops in accordance with their consumption of N fertilizers. For example, bulk of the Indian N-fertilizer goes into cereal production,[2] whereas horticulture crops may dominate in China. While the amount of N available to the plant can be improved by adopting agronomic practices that optimize fertilizer-soil-water-air interactions, the innate efficiency of the plant to utilize this available N must be tackled through biological interventions. They need to be targeted to appropriate biological processes that include nitrogen uptake, distribution, assimilation and remobilization, as well as their optimal contribution towards chosen agricultural outputs such as grain/leaf/flower/fruit/seed. The identification of appropriate phenotypes, genotypes, molecular markers and target candidates for improvement of NUE continues to be a challenge despite some major advances in the last decade. This chapter discusses NUE as a concept and the progress on understanding its biological determinants, the different approaches used so far for enhancing crop N use efficiency and emerging avenues for future development.

4.17.2 What Is NUE?

As a concept, NUE is an outcome that 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 yield in terms of either grain per unit of applied N (NUE grain) or biomass per unit of applied N, many other interpretations of NUE have emerged (**Fig. 1**). One of them is the efficiency of extracting N from soil by the crop, ideally by using an unfertilized control for comparison. In agronomic terms, the product of physiological efficiency (PE) and apparent nitrogen recovery (AR) is used to arrive at the net agronomic efficiency (AE), which in combination with NUE grain (product of uptake efficiency and utilization efficiency) reflects the overall efficiency of the applied nitrogen in producing grain yield. AR reflects the efficiency of the crop in obtaining nitrogen-based fertilizer from the soil, whereas PE can be viewed as the efficiency with which crops use nitrogen in the plant for the synthesis of grain (reviewed in Ref. 25).

However, the appropriateness of a method to estimate NUE also depends on the crop, its harvest product (grain/fruit/flower/leaf/root/stem) and the specific physiological processes involved. For example, most internal estimates of NUE for monocot plants are represented as UtE or NUE grain to include grain yield and express yield in relation to N supplied. In most cases, UtE is considered to be better, since NUE grain is influenced by N uptake as well as internal utilization or partitioning, and considerable variation was found in uptake efficiency (UpE) and UtE in several important crop species including rice, maize, and barley. While crop breeding has typically focused on agronomic trends across cultivars/lines without addressing the biological or genetic basis for



	Nomenclature	Formula
Biomass index	NUE	$NUE = S_w/N$
	Usage index	$UI = S_w^*(S_w/N)$
Grain weight index	NUE grain	$NUE = S_w/N_s$
	Utilization efficiency	$UtE = G_w/N_t$
	Agronomic efficiency	$AE = (G_{w_f} - G_{w_c})/N_f$
	Physiological efficiency	$PE = (G_{w_f} - G_{w_c})/N_f$ uptake$-N_c$ uptake
Capacity of N uptake	Uptake efficiency	$UpE = N_t/N_s$
	Apparent nitrogen recovery	$AR = (N_t$ uptake $-N_c$ uptake$)/N_t$*100

Figure 1 The key events involved in uptake and utilization of nitrogen in plants. Sw: shoot weight, N: total nitrogen content of shoots, Gw: grain weight, Ns: nitrogen supplied in gram per plant, Nt: total nitrogen in plant, Gwf: grain weight with fertilizer, Gwc: grain weight without fertilizer (control), Nf: nitrogen fertilizer applied, Nf uptake: plant nitrogen with fertilizer, Nc uptake: plant nitrogen unfertilized control, PE: physiological N-use efficiency. Reproduced from Pathak et al., 2011.

NUE in the germplasm, molecular approaches have concentrated on the genes, proteins and their regulatory mechanisms or physiological factors such as amino acid pools and photosynthesis measurements. Very few studies have been able to investigate which of the genes/proteins or physiological factors contribute to yield per unit N, and to what extent. However, recent biotechnological interventions used transgenic NUE lines involving individual target genes to assess their contribution to the net improvement in NUE.

4.17.3 Agronomic Approaches for Improving NUE

The various sources of usable N include chemical fertilizers, biofertilizers, animal dung, residue burning, mulching, biochar, compost, manure etc., but the adverse consequences of their leakages into the environment are qualitatively similar, even if they vary in quantitative terms. Therefore, the overall N use efficiency in food grain production is also expressed as partial factor productivity of N (PFPN). It is an aggregate efficiency index that includes contributions to crop yield from native soil N, fertilizer N-uptake efficiency, and the efficiency with which the N acquired by the plant is converted to grain yield. Fertilizer N use efficiency can be improved by greater synchrony between crop N demand and N supply throughout the growing season by using a combination of decisions before planting (anticipatory) or during the growth season (responsive). Accurate decisions on N supply should consider the levels of mineral and organic soil N and the splitting of N applications according to phenological stages. Decision aids are available to diagnose soil and plant N status during the growing season (models, leaf color charts, sensors) or using controlled-release fertilizers or inhibitors.[2] Fertilizer developers have produced four major types of efficiency enhancing fertilizer formulations (EEFs): polymer-coated fertilizers (PCF), nitrification inhibitors, urease inhibitors, and double inhibitors, i.e., urease and nitrification inhibitors combined. A recent review systematically analyzed their effectiveness in increasing yield and NUE and reducing N losses and concluded that they are not a panacea, as their benefits vary from crop to crop, nature of EEF and farmers' practices.[19] A more detailed review of agronomic approaches is beyond the scope of this chapter, due to its focus on biotechnology.

4.17.4 Microbiological Avenues to Improve NUE Through N-Fixation

Prior to the invention of the Haber-Bosch process, which was used initially for the manufacture of explosives and was later adapted for chemical fertilizers, farmers only used manures, which were often not enough. Another approach of farmers was to exploit the available biological diversity through crop rotation involving a leguminous crop with a cereal or any other non-leguminous crop or mixed cropping involving legumes to exploit their N-fixing ability. It was demonstrated as early as 1888 that this ability to convert atmospheric N_2 gas into ammonium ions is unique to some free-living microbes and others that colonize the root nodules of legumes. Since then, consistent attempts have been made to extend this symbiotic association with nitrogen-fixing bacteria to non-legume crops, particularly cereals. In 1988, the discovery of *Gluconacetobacter diazotrophicus* (Gd), a non-nodulating, non-rhizobial, nitrogen-fixing bacterium in sugarcane provided a fresh impetus to this line of research. Strains of this organism were shown to intracellularly colonize the roots and shoots of the cereals: wheat, maize (corn) and rice, as well as crops as diverse as potato, tea, oilseed rape, grass and tomato.[10] Other N-fixing non-symbiotic organisms were found in cereals, such as, *Beijerinckia, Azotobacter, Azospirillum, Herbaspirillum, Gluconacetobacter, Burkholderia, Clostridium, Methanosarcina, and Paenibacillus*.[5] Such findings have led to the development of biofertilisers, though their efficacy depends on whether the soil conditions are appropriate for N-fixation.

Endophytic associations of some N-fixing microbes are also known in cereals and other non-leguminous crops, but their contribution to the overall plant N budget is not well known. Recently, it was also found that cereals like maize have mucilage-secreting aerial roots, which attract microbial associations that include diazotrophic N-fixers.[34] Such findings will trigger screening crop germplasms for traits that aid in plant association with N-fixing microbes. For the last 4 decades, molecular biologists have been trying to engineer crop plants that can self-fertilise by fixing atmospheric nitrogen. While a detailed review of this area is beyond the scope of this chapter, it is worth mentioning the recent progress in the expression of 16 nitrogenase proteins in the tobacco mitochondrial matrix.[3] A more recent review traces the major milestones in this line of research that rekindled the hopes of genetic engineering of N-fixing ability in crop plants to liberate them from fertilizers completely.[8]

4.17.5 Plant Biotechnological Interventions Through N-Uptake, Assimilation and Remobilisation

The main focus of the present chapter is with respect to the biotechnological interventions to improve the plant's inherent ability to efficiently use the usable forms of N available to it. The predominant usable N compounds in the soil are nitrate, ammonium compounds or urea, which are also the main constituents of inorganic N fertilizers. There are several transporters in the plant roots and other tissues for each of the above N-compounds. There are both high affinity and low affinity isoforms, and their genes are either constitutively expressed or induced by N or other factors.[18] Once inside the plants, their metabolism by the primary nitrate assimilation pathway involves nitrate reductase (NR), nitrite reductase (NiR), plastidic glutamine synthetase (GS2) and glutamate synthase (Fd-GOGAT). All other organic N compounds in plants are derived from glutamine-glutamate cycle and aminotransferases. Some of them also play important roles in the secondary N metabolism or remobilization of internal N from source to

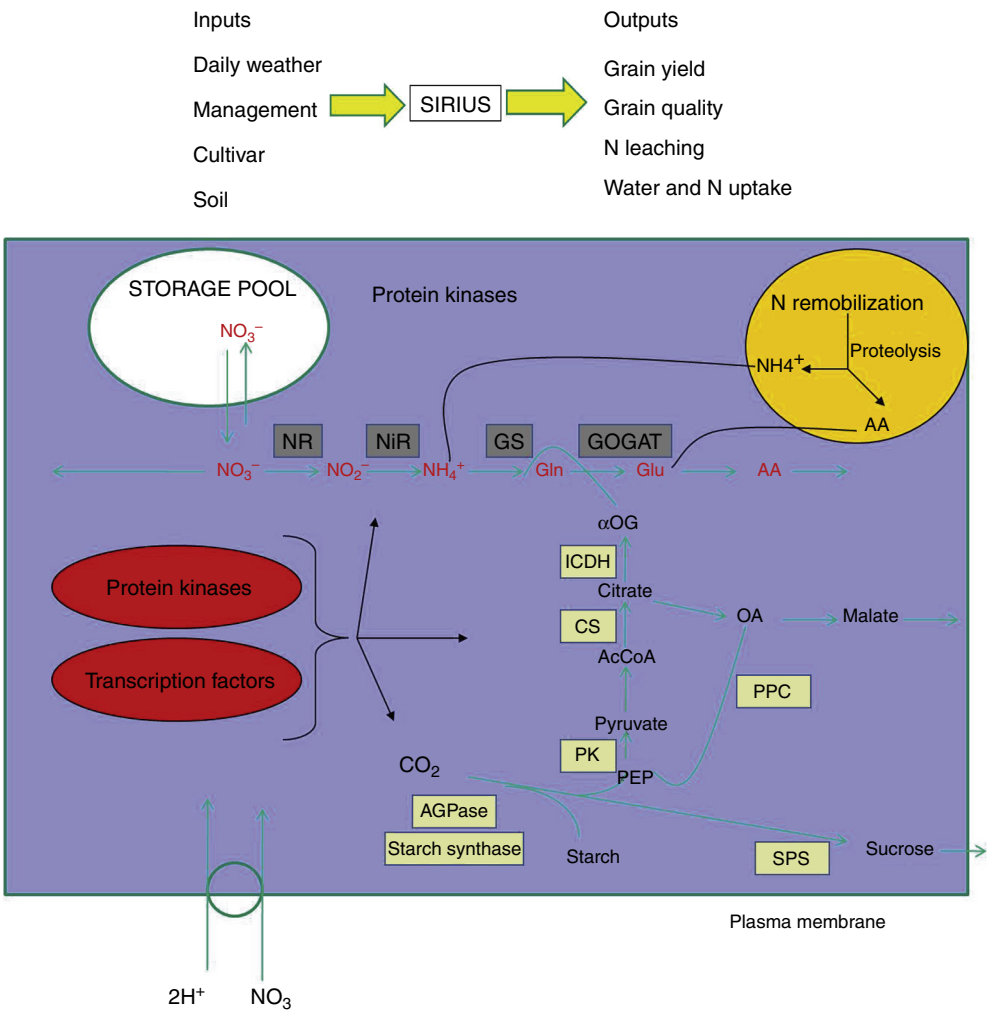


Figure 2 Schematic representation of key processes and enzymes involved in nitrogen metabolism in plants. Nitrate and ammonium ions are taken by transporters across the cell membrane, assimilated and incorporated into C metabolites to generate amino acids. The amino acids from degraded proteins in senescing 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, $NH4^+$: ammonium ion, Gln: glutamine, Glu glutamate, AA: amino acids, α OG: 2- oxoglutarate (α ketoglutarate dehydrogenase), ICDH: isocitrate dehydrogenase, CS: citrate synthase, PK: pyruvate kinase, AGPase: ADP – glucose phosphorylase, PPC: phosphoenol pyruvate carboxylase, SPS: sucrose phosphate synthase, PEP: phosphoenolpyruvate, OA: oxaloacetate, Ac CoA: acetyl coenzymeA. Reproduced from Pathak et al. 2011.

sink tissues, such as from senescing leaves to grains in the case of cereals. They include cytosolic GS1, NADH-GOGAT, glutamate dehydrogenase (GDH) and various aminotransferases and amino acid and peptide transporters.[25] All the three processes, uptake, assimilation and remobilization, offer opportunities for biological intervention to improve the inherent NUE of the plant[23] (Fig. 2). There have also been some attempts towards limiting N efflux or loss through volatilization to improve N retention for NUE.

4.17.6 Phenotype Development for NUE

The development of genetic avenues for crop improvement to enhance fertilizer NUE has long been hampered by a lack of understanding on what constitutes the phenotype and genotype for crop N response and NUE. There have been some attempts towards phenotypic characterization of the various traits associated with N response and NUE in rice. They include root length, density and surface area, dense and erect panicle, etc. Recently, we have reported additional phenotypic characters in rice such as N-responsive germination, oxygen consumption, seed urease activities, root growth and crop duration as potentially important for NUE.[28] In wheat, some more characters have been reported such as onset of post-anthesis senescence and plant height, etc. The most extensive focus so far has been on the N-responsive changes in root system architecture and its potential for NUE.[20] Arabidopsis and rice continue to be the most important model plants for dicots and monocots, respectively, to understand the phenotypic as well as

the molecular basis for N-response and NUE.[18] More extensive as well as intensive characterization of the phenotypic traits/components for NUE are required for different crops, so as to distinguish those that are common for all crops from those that are unique to particular crops, crop types (cereals, vegetables) or their outputs (grain/leaf/tuber/flower/fruit).

4.17.7 Transgenic Efforts to Improve NUE

NUE is a multigenic trait spread across hundreds of genes that extends beyond N uptake, primary nitrate assimilation and secondary remobilization. The determination of the key genes that contribute predominantly to the genotype of NUE is an area that had to rely on reverse genetics, mutants and transgenics to progress independently in the absence of a clearly defined phenotype. Naturally, transgenic efforts have concentrated on diverse targets that include genes belonging to uptake, primary assimilation, translocation, secondary remobilization and carbon metabolism, apart from their regulators and signaling intermediates. They include overexpression or knockout strategies to manipulate many critical candidate genes to assess their effects on biomass, plant nitrogen status and overall yield. They have been previously reviewed in Ref. 25 and summarized in **Table 1**. These efforts reveal the diversity of gene targets and the endpoints used to evaluate the effect of their manipulation in various plants. For example, in cereals, the total grain biomass or grain N-content might seem to be the obvious target, but studies also revealed the importance of targeting the distribution of N between canopy (leaves, stem) and roots, better photosynthetic rate/unit leaf N, reduced leaf senescence, photorespiration, modifying Rubisco, Rubisco activase, etc. The following paragraphs elaborate the various attempts in terms of the processes targeted in different plants.

4.17.7.1 N Uptake

N uptake is the first step in N acquisition which mainly involves transporters of nitrate and ammonium ions, though urea transporters and amino acid transporters are also known to exist. There have been many attempts to manipulate transporters to enhance NUE. Nitrate transporters can belong to the low affinity transport system (LATS), encoded by NRT1 gene family or the high affinity transport system (HATS), encoded by NRT2 gene family. Initial attempts of overexpression of nitrate/nitrite and ammonium transporters reported enhanced uptake, but increase in assimilation and NUE remained inconclusive.[25] More recently, transgenic overexpression of various nitrate transporters in rice such as OsNRT1.1b, OsNRT2.1, OsNPF2.4 and OsNRT2.3b,[11] OsNRT1.1A/OsNPF6.3,[37] OsNPF7. 2,[36] have been reported to have some benefits, subject to further validation. Similarly, the overexpression of ammonium transporter OsAMT 1.1 in rice has been shown to improve growth and yield and ammonium-potassium homeostasis.[17,27]

4.17.7.2 N Assimilation

Several attempts for transgenic manipulation of the genes encoding the enzymes of primary and secondary N assimilation have been made as described in **Table 1**, with little or no improvement in NUE. Nitrate reductase overexpression (nia1 and nia2) in Arabidopsis, tobacco, potato and lettuce did not lead to any specific improvement in NUE.[25] However, recent reports in rice suggest that transgenic overexpression of OsNAR2.1 enhances nitrogen uptake efficiency and grain yield,[9] indicating possible differences between monocots and dicots in this regard. Similarly, overexpression of NiR improved nitrite assimilation, but no enhancement in NUE was reported. Overexpression of plastidic GS (GS2) has been earlier reported to increase seedling growth in tobacco and more recently to increase nitrogen uptake and yield in wheat.[15] The potential of transgenic overexpression of Fd-GOGAT gene has not yet been evaluated for NUE improvement, even though it has been shown to be involved in the control of grain protein content (GPC) in durum wheat.[24]

The genes of secondary ammonia assimilation have also been overexpressed in several transgenic crops to enhance NUE. Cytosolic GS1 has emerged as a strong candidate gene, whose overexpression has been tried in several plants including cereals like rice and maize,[6] which resulted in higher grain yield and/or biomass with improved N content.

4.17.7.3 N Translocation and Remobilization

Nitrogen remobilization is one of the key steps in improving NUE in plants.[18] In cereals, 60%–92% of the requirement of nitrogen during grain-filling is remobilized from the senescing vegetative parts. The amount of N remobilized depends on source-sink relationship in terms of N remobilization efficiency and the amount of N available.[33] It is also known that genotype and environmental factors affect nitrogen translocation, which makes the genes involved in remobilization and translocation attractive targets for improvement of NUE. Overexpression of asparagine synthetase,[7] alanine aminotransferase or AAT[29] showed some early promise, though their actual performance varied in different crops. Recently, overexpression of a rice peptide transporter (OsNPF7. 3), which is induced by organic nitrogen, has been shown to contribute to nitrogen allocation and grain yield.[12] Similarly, blocking of amino acid transporter OsAAP 3 has been shown to improve grain yield by promoting outgrowth buds and increasing tiller number in rice.[22] Though the importance of glutamate dehydrogenase (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 tobacco, tomato, maize and wheat.[21]

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 (*Arabidopsis*)		CaMV 35S	*Arabidopsis*	Increase in constitutive nitrate uptake but not in induced
Nrt2.1-High affinity nitrate transporter (*N. plumbaginifolia*)		CaMV 35S, rol D	*N. tabacum*	Increased nitrate influx under low N conditions
Nrt2.1-High affinity nitrate transporter (*Chlamydomonas reinhardtii*)		CaMV 35S	*Arabidopsis*	Shoot dry weight increased, no effect on nitrate uptake
OsNPF7.2		*Ubi-1* promoter	Rice	Positively regulates tiller number and grain yield
OsNRT1.1A (Rice)		*ACTIN1 promoter*	Rice	High yield and early maturation in rice
OsNPF2.4		Ubiquitin	Rice	Low-affinity acquisition and long-distance transport
OSNRT 1.1a/b		Ubiquitin	Rice	Increased shoot biomass under low N
Os NRT 2.3 b		CaMV 35S/Ubi promoter	Rice	Improved growth, yield, and NUE
OsNRT2.1		*pOsNAR2.1*	Rice	Enhances nitrogen uptake efficiency and grain yield
OsNPF7.3 (Rice)		*35S promoter*	Rice	Contributes to nitrogen allocation and grain yield
OsNPF7.7 (Rice)		Ubiquitin	Rice	Regulates shoot branching and nitrogen utilization efficiency in rice
OsAMT1.1		CaMV 35S	Rice	Ammonium uptake and ammonium-potassium homeostasis
OsAMT1.1		Ubiquitin	Rice	Superior growth and higher yield under optimal and sub optimal conditions
NR - Nitrate reductase	*N. plumbaginifolia*	CaMV 35S	*N. tabacum*	3–4 fold drop in NR protein and activity, no change in NR transcript
	N. plumbaginifolia	CaMV 35S	*N. tabacum*	Increased NR activity, biomass, drought stress
Nia - Nitrate reductase	*N. tabacum*	CaMV 35S	*L. sativa*	Reduced nitrate content, chlorate sensitivity
	N. tabacum	CaMV 35S	*N.plumbaginifolia*	Nitrite accumulation in high nitrate supply
Nia2- Nitrate reductase (*N. tabacum*)		CaMV 35S	*S. tuberosum*	Reduced nitrate levels
Nia2- Nitrate reductase (*N. tabacum*)		CaMV 35S	Lettuce	NR and NO_3^- content increase in leaves
NiR - Nitrite reductase	*N. tabacum*	CaMV 35S	*N.plumbaginifolia, Arabidopsis*	NiR activity, no phenotypic difference
	S. oleracea	CaMV 35S	*Arabidopsis*	Higher NiR activity, higher nitrite accumulation, higher nitrite assimilation
GS2- Chloroplastic glutamine synthetase	*O. sativa*	CaMV 35S	*N. tabacum*	Improved photorespiration capacity, and increased resistance to photooxidation
	O. sativa	CaMV 35S	*O. sativa*	Enhanced photorespiration, salt tolerance
	N. tabacum	Rubisco small subunit	*N. tabacum*	Enhanced growth rate
Fd- GOGAT-Fd dependent glutamate synthase (*N. tabacum*)		CaMV 35S	*N. tabacum*	Diurnal changes in NH3 assimilation
GS1- Cytosolic glutamine synthetase	*G. max*	CaMV 35S	*L. corniculatus*	Accelerated senescence
	G. max	rol D	*L. japonicus*	Decrease in biomass
	P. vulgaris	Rubisco small unit	*T. aestivum*	Enhanced capacity to accumulate nitrogen
	M. sativa	CaMV 35S	*N. tabacum*	Enhanced growth under N starvation
	G. max	CaMV 35S	*M. sativa*	No increase in GS activity
	Pea	CaMV 35S	*N. tabacum*	Enhanced growth, leaf soluble protein, ammonia levels
	P. sylvestris	CaMV 35S	Hybrid poplar	Enhanced growth rate, leaf chlorophyll, total soluble protein
	G. max	CaMV 35S	*P. sativum*	No change in whole plant N
	Alfalfa	CaMV 35S	*L. japonicus*	Higher biomass and leaf proreins
	Oryza sativa Japonica	Ubiquitin	Rice	Spikelet yield↑ 29%–35% under HN, NUE↑30%–33% under HN
		Ubiquitin/T-DNA insertion	Maize	Grain yield increases 45% under low N Leaf TAA and TN↑, grain yield↓85% under LN

NADH-GOGAT- NADHdependent glutamate synthase	*O. sativa*	*O. sativa*	*O. sativa*	Enhanced grain filling, increased grain weight
	M. sativa	CaMV 35S	*N. tabacum*	Higher total C and N content, increased dry wt
	Alfalfa	Soybean leghemoglobin promoter (Ibc3)	Alfalfa	Shoot fresh mass decreases 29%–41%, N content decreases 37%–38%, total amino acid decreases 50%–70%
GDH- Glutamate dehydrogenase	*E. coli*	CaMV 35S	*N. tabacum*	Increased biomass and dry weight
	E. coli	CaMV 35S	*N. tabacum*	Increased ammonium assimilation and sugar content
	L. esculentum	CaMV 35S	*L. esculentum*	Twice GDH activity,higher mRNA levels AND twice glutamate concentration
	E. coli	OsUB1	*Z. mays*	Increased N assimilation, herbicide tolerance, biomass, grain aa content
	C. sorokiniana	CaMV 35S	*T. aestivum*	Schmidt and Miller, 2009 (patent no., 627,886)
	C. sorokiniana	CaMV 35S	*Z. mays*	Schmidt and Miller, 2009 (patent no., 627,886)
ASN1- Glutamine dependent Asparagine synthetase (*A. thaliana*)		CaMV 35S	*A. thaliana*	Enhanced seed protein
ASNI - Asparagine synthetase (Pea)		CaMV 35S	*N. tabacum*	Free asparagine in leaves↑, growth rate↑
ASNI - Asparagine synthetase (*P. sativum*)		CaMV 35S	*N. tabacum*	Reduced biomass and increased level of free asparagine
*Asp*AT - Mitochondrial aspartate aminotransferase (prosomillet)		CaMV 35S	*N. tabacum*	Increased AspAT, PEPCase activity
AlaAT -alanine aminotransferase (Barley)		btg26	*Brassica napus*	Good yields even with 50% less N fertilizer
OSAAP_ Amino acid permease		Rice 35S andUbi-1	Rice	Improves grain yield by increasing bud and tiller number
ANR1- MADS transcription factor (*Arabidopsis*)		CaMV 35S	*Arabidopsis*	Lateral root induction and elongation
GLB1- PII regulatory protein (*Arabidopsis*)		CaMV 35S	*Arabidopsis*	Growth rate, increased anthocyanin production in low N
MdATG18 (Apple)		CaMV 35S	*Arabidopsis and Apple*	Tolerance to nitrogen deficiency and anthocyanin accumulation
Dof1- Transcription factor (*Zea mays*)		35S C4PDK	*Arabidopsis*	Enhanced growth rate under N limited conditions, increase in amino acid content
ZmDof1 (Zea mays)		rbcs1	Wheat	Increase in biomass and yield
MADS-box transcription factor, Arabidopsis Nitrate Regulated1 (*ANR1*) (Arabidopsis)		CaMV 35S	*Arabidopsis thaliana*	Root plasticity in response to NO_3^-, promotes NRT1.1 dependent lateral root growth
OsENOD93-1 Nodulin gene		35S C4PDK	Rice	Increased shoot mass and seed yield
NAM, ATAF, and CUC transcription factor (TaNAC2-5A)		CaMV 35S	Wheat	Promotes root growth
NLP7 (*Arabidopsis*)		CaMV 35S	*Nicotiana tobaccum*	Improves plant growth under low N conditions
SnRK1 (Malus hupehensis Rehd. var. pinyiensis)		CaMV 35S	Rice	Increases carbon assimilation, nitrogen uptake and modifies fruit development
C_4-PEPC (Maize)			Rice	Better yield under low N condition
Gnp4/LAX2 (Nippobare)			Rice	Increases grain length and 1000 grain weight
AP2 TF-SOS1 (Rice)		Ubiquitin	Rice	Controls organ size
bHLH (*PGL1*:Rice)		Ubiquitin	Rice	Increased grain length and weight

Modified from Pathak et al. 2011.

4.17.7.4 C Metabolism or Its Regulators

Sugar metabolism not only provides carbon skeletons in the form of organic acids for amino acid synthesis but also influences seed germination, embryogenesis, flowering, senescence and hormone signaling. The genes involved in N metabolism and nitrate signaling are also tightly regulated by sugar signaling. SnRK1, a principle regulator in carbon signaling, is known to be linked to nitrogen and amino acid metabolism. Overexpression of a heterologous SnRK1 in tomato increased C assimilation, N uptake and modified fruit development.[38] Similarly, transgenic Arabidopsis plants overexpressing STP13, a member of sugar transporter family, showed improved N use, with the induction of a nitrate transporter and higher total N per plant. Recently, overexpression of maize PEPC in rice has been shown to confer better yield than wild type under low N conditions.[32]

4.17.7.5 Signaling Targets

Nitrate is a potent signal that affects plant N and C metabolism as well as growth and development. The genome-wide identification of nitrate responsive genes in several plants has prompted the search for nitrate response elements that could be the targets for regulators of N-response and/or NUE, but without much success.[7,25] However, there have been some reports on transgenic manipulation of transcription factors (TFs) and other signaling intermediates contributing to changes in N response and NUE. Transgenic plants overexpressing Dof1, a plant-specific maize transcription factor, showed some enhanced biomass and yield in wheat and sorghum.[26] Other transcription factors whose overexpression showed potential impact on N-response/NUE include ANR1 MADS-Box Gene, NAC Transcription Factor TaNAC2-5A, SMALL ORGAN SIZE1, NLP7 (reviewed in Ref. 24), an atypical bHLH named POSITIVE REGULATOR OF GRAIN LENGTH 1 (PGL1)[14] and AP2-type transcription factor.[4] A few other attempts to manipulate signaling/regulatory proteins have also been made, such as OsNPF7. 7 in rice,[16] Gnp4/LAX2 in rice[39] and MdATG18a overexpression in apple.[30] Transgenic inhibition of SLG by RNAi has been shown to control grain size and leaf angle by controlling brasinosteriod pathways.[33] While many such regulatory targets are being tried in many plants, their utility for NUE across different crops and cropping conditions remains to be validated.

4.17.8 QTL Mapping for New Targets

The advent of techniques to identify molecular markers facilitated subsequent evaluation of the inheritance of NUE using specific quantitative trait loci (QTLs). Despite the poor characterization of the phenotype for NUE, some QTLs have been identified in various model plants and crop species for N-response/NUE by comparing them at high and low N levels for various yield parameters. Some of the early QTL studies were responsible for targeting GS1 and NADH-GOGAT in cereals, but other targets have also been emerging. For example, OsNRT1.1B was identified as a critical QTL contributing to NUE divergence between rice subspecies. More recently, using automated phenotyping and phenomic techniques, QTLs are being investigated on a large scale. Some such QTLs include, JASMONATE RESPONSIVE 1 (JR1), CALCIUM SENSOR RECEPTOR, PhzC, ROOT SYSTEM ARCHITECTURE 1, and PHOSPHATE 1 (reviewed in Ref. 23). Several positive coincidences between QTLs for uptake of N and other nutrients and QTLs for root architecture traits indicate that a way of increasing water and nutrient use efficiencies is to simply breed for a root system architecture that enhances the surface area for nutrient absorption.

4.17.9 Improving NUE: A Systems Biology Approach

Systems biology studies on the interactions between the components of *biological systems*, fueled by the huge data generated by functional genomics, transcriptomics and proteomics, have greatly accelerated the application of systems biology. Such studies have enabled us to identify novel interacting partners and identified missing links in our knowledge regarding N sensing, signaling and metabolism.[35] The list of potential targets for NUE manipulation has expanded beyond primary N metabolism and secondary assimilation/remobilization as indicated by numerous patents that have been filed in the earlier decade. The increasing convergence of genomics, functional genomics, phenomics and genome-wide association studies (GWAS) and systems biology approaches are rapidly expanding the identification of newer target genes for the manipulation of nitrogen use efficiency. Due to these developments, the next decade may witness many more exciting new lines of various crops with improved NUE than ever before.

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