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Nitrogen Nutrition, Its Regulation and Biotechnological Approaches to Improve Crop Productivity

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Abstract

Nitrogen is the most important macronutrient needed for plant growth and development. The availability of nitrogen in the soil fluctuates greatly in both time and space. Crop plants, except leguminous plants, depend on supply of nitrogen as fertilizers. Large quantities of nitrogen fertilizers are applied to crop plants, but only 33% of it is utilized by the plant. Plants have developed efficient mechanisms to sense the varying levels of nitrogen forms and uptake them. They also have well developed mechanisms to assimilate the incoming nitrogen immediately or translocate to different parts of the plant wherever it is needed. Maintenance of nitrogen homeostasis is essential to avoid toxicity. Apart from translocation and assimilation, plants have developed different mechanisms, nitrogen efflux; vacuolar nitrogen storage and downward transport of nitrogen from aerial parts to roots, for maintaining nitrogen homeostasis. In crop plants the "grain yield per unit of available nitrogen in the soil" is referred as the nitrogen use efficiency (NUE) for which remobilization of nitrogen, mediated by various transporters plays a crucial role. All these processes are tightly regulated by proteins and microRNA in response to both external and internal nitrogen levels, carbon status of the plant and hormones. As most crop plants are non-leguminous and depend on soil nitrogen, more production could be achieved if crop plants can be made to utilize the available nitrogen efficiently. The recent explosion of research information and the mechanisms behind nitrogen sensing, signaling, transport and utilization enables biotechnological interventions for better nitrogen nutrition of crop plants. This review discusses such possibilities in the context of recent understanding of nitrogen nutrition and the genomic revolution sweeping the crop science.

Keywords

Nitrogen, Nitrogen Use Efficiency, Genomics, Genetic Engineering, RNA-Seq, Nitrome, microRNA

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1. Introduction

Plants require many mineral elements for their growth and development. Elements like nitrogen, potassium, calcium, magnesium, phosphorus, and sulphur are required in large quantities (macronutrients), while boron, chlorine, copper, iron, manganese, molybdenum and nickel are required in smaller quantities (micro nutrients) [1] [2]. Each mineral is equally important to the plant and deficiency of one or more of these mineral elements affect the growth, development and yield of crop plants. Soil is the source of mineral nutrients for plants and for crop plants mineral nutrients are supplied in the form of fertilizers to increase the yield. Among macro nutrients, nitrogen is next to carbon in importance to plants. It is an important constituent of amino acids, proteins, enzymes, vitamins, alkaloids and some growth hormones. Though, molecular nitrogen constitutes 78% of the atmosphere, only some microbes and leguminous plants can fix atmospheric nitrogen [3]. All other plants depend on external source for nitrogen and nitrogen is a prominent limiting factor in these plants [4] [5]. By supplying nitrogen as fertilizers along with genetic improvement of crop plants, green revolution achieved substantial increase in crop productivity [6] [7].

Large quantities of nitrogen fertilizers are applied to crops worldwide each year. In 2010-2011, 57.5 million tons of nitrogen fertilizers (57.5% of global nitrogenous fertilizer consumption) were applied to cereals. Wheat was the main crop receiving nitrogen fertilizers, with 18.1% of global uses, followed by maize with 16.8% and rice with 15.4%. Other cereals accounted for 4.8% of the world total [8]. Though, nitrogen fertilizer application is the major factor responsible for huge increase in yield of crop plants, 2/3rds of the applied nitrogen is not utilized by the plants and is lost in the soil due to leaching, surface run-off, volatilization and de nitrification by microorganisms [9]-[16]. Huge quantities of energy (1.2% of total energy used globally) is used in the synthesis of nitrogen fertilizers, which significantly contributes to the production of greenhouse gases and the leached nitrogen fertilizers are the main contributors for eutrophication process [17]-[22].

As most crop plants are non-leguminous and depend on soil nitrogen, more production can be achieved if crop plants can be made to utilize the available nitrogen efficiently [23]-[25]. Efficient utilization of nitrogen (nitrogen use efficiency) is measured in terms of “the grain yield per unit of available nitrogen in the soil” for crop plants and “the fresh or dry matter yield per unit of available nitrogen in soil” for biomass plants. Nitrogen use efficiency depends on nitrogen uptake, translocation, assimilation, vacuolar storage and remobilization [26] [27].

Recently, an explosion of research information on the mechanisms behind nitrogen sensing, signalling, transport and utilization have occurred. Though a number of gaps still exist, the available information indicates that it is potentially possible to make biotechnological interventions for better nitrogen nutrition of crop plants. This paper aims at discussing such possibilities taking into consideration the voluminous information generated recently and the genomic revolution that is sweeping crop science.

2. Nitrogen in Soil

Plants predominantly acquire nitrogen from soil (rhizosphere) as inorganic nitrogen (nitrate, ammonium and urea) [28] [29]. But major part of soil nitrogen is present in organic form (organic matter) and plants cannot utilize organic nitrogen directly. Microorganisms present in the soil convert organic nitrogen (proteins and amino acids) into inorganic forms through the process of mineralization. Microbial utilization of inorganic nitrogen also depletes nitrogen available to plants. The balance among mineralization, nitrification and denitrification determines nitrogen availability to plants. The soil ammonium and nitrate concentration ranges from micro molar levels to hundreds of milli molar quantities [1] [30]. The diffusion coefficient of nitrate in soil is the consequence of nitrate available to plant roots and the nitrate lost through leaching (about 30% of inorganic nitrogen) [31] [32].

Soil ammonium and nitrate concentrations in agricultural soils are in micro molar (less than 1 milli mole) and milli molar (1 - 5 milli moles) ranges, respectively [33]-[35]. When compared to nitrate, leaching of ammonium is less which is due to the relative immobility of ammonium in soil. Urea is the predominant type of nitrogenous fertilizer applied to crop plants [36]. Natural soils usually contain lower concentrations of urea (~3 micro moles) but in the fertilized soils it is present up to 70 μM level [37]. Microbial urease enzyme converts soil urea into ammonium and CO_2. In addition to fertilizer application, arginine degradation (urea cycle) and ureide catabolism (catabolism of purines) are the sources of urea to plants [1] [36] [38] [39].

Plant roots receive nitrogen through transpiration dependent mass flow. Mass flow alone cannot meet the nitrogen demand of the roots and diffusion plays a complementary role in nitrogen uptake. Diffusion co-efficients of nitrate/ammonium and their concentration gradients determine their diffusion [1] [31]. Though the diffusion co-efficient of nitrate and ammonium in water is same, in soil it differs and is influenced by charge, ion size, soil moisture, temperature and water viscosity. Diffusion co-efficient of nitrate in soil is ca. 1×10^{-10} $m^2 \cdot s^{-4}$ and the diffusion co-efficient of ammonium is 10 - 100 folds less than that of nitrate [33]. The low diffusion coefficient of ammonium results in low leaching and less availability to roots for uptake [32] [40] [41].

3. Sensing Nitrogen Levels in Soil

As soil nitrogen levels vary greatly, to cope with this versatile sensing and adaptive mechanisms were evolved in plants (**Table 1**) [42]-[50]. The very first thing in nitrogen uptake is to sense the levels of nitrogen in soil and then activation of appropriate transport systems for uptake of nitrogen from soil [51].

3.1. Sensing of Soil Ammonium Levels

As ammonium concentration in the soil is highly variable, plants need to sense the level of ammonium and tightly regulate the NH_4^+ uptake and transport [52]-[54]. Plants have elaborate sensing and signaling mechanisms for ammonium in which ammonium transporting proteins AMT1; 1 and AMT1; 3 are involved [55]-[57]. The initial sensing process and the proteins involved in this are still not known. But it is clear that the unknown initial sensing mechanism changes the phosphorylation state of AMT1; 1 and AMT1; 3 by inducing the specific kinases involved in this [58]. They are expressed in the root hair rhizodermal cells [57] [59]. The trimeric form of these proteins has been shown to function as channels through which NH_4^+ is transported [60]-[62]. Opening and closing of these channels is controlled by phosphorylation of conserved threonine residues [51] [56] [63] [64]. In AMT1; 1 protein Thr460 located in the conserved C-terminal region is the target for phosphorylation [55] [65]-[67]. In the un-phosphorylated state, the channel is open and amenable for transport of NH_4^+ and when the concentration of ammonium is high in the soil it leads to phosphorylation of the AMT proteins which changes the conformation of these proteins leading to channel closure [57] [65]. Though the concentration dependent phosphorylation and closure of the channel has been proved, little is known about reversing this *i.e.* dephosphorylation and opening of the channel [68].

The involvement of AMT1; 3 in sensing ammonium and signaling is shown by its action on local ammonium concentration mediated lateral root growth. AMT1; 3 protein acts as a sensor but the signaling pathway is different [57]. Though a conserved threonine residue is present at 464th position, there is no evidence to support phosphorylation of this residue. High ammonium levels induce the oligomerization of AMT1; 3 and internalization of the oligomers [69]. This works as a shutoff mechanism to prevent ammonium toxicity. The internalization of AMT1; 3 oligomers is mediated by the clathrin-dependent and the membrane microdomain-associated endocytic pathways [70].

3.2. Sensing of Soil Nitrate Levels

In plants, sensing of soil nitrogen concentration is mediated by the nitrate transporting NRT1.1 and NRT2.1 proteins [47] [71]-[77]. Wide variations in external nitrate levels are sensed by the dual affinity transporter NRT1.1. Changes in soil nitrate concentrations regulate the phosphorylation level of NRT1.1. It is phosphorylated (at Thr101) by the protein kinase CIPK23 when the nitrate concentration is low. Higher nitrate concentration prohibits phosphorylation of NRT1.1 and the dephosphorylated NRT1.1 switches from high to low affinity mode [73] [78] [79]. The calcium binding CBL9 protein activates CIPK23 kinase but the exact mechanism is

Table 1. Proteins involved in sensing soil nitrogen levels.

S. No	Name of the protein	Mechanism	Reference
1.	NRT1.1	Phosphorylation/dephosphorylation	[47] [73]
2.	NRT2.1	Not known	[48] [83]
3.	AMT1; 1	Phosphorylation/dephosphorylation	[65]
4.	AMT1; 3	Oligomerization	[69]

not known [80] [81]. Once activated, CIPK23 directly interacts with NRT1.1 in the plasma membrane [82] [73]. CIPK8 kinase mediates the high-level primary nitrate response under high nitrate conditions. Nitrate induced CIPK8 acts at low affinity phase. Like CIPK23, CIPK8 has been suggested to be activated by another CBL protein but no further evidence is available now [78]. Based on the ability of NRT2.1 to modulate the root system architecture according to the available nutrients, NRT2.1 has been predicted to sense nitrogen levels. Further investigations are needed to understand the mechanism behind this sensing [48] [83].

4. Sensing Internal Nitrogen Levels

Plants possess multiple mechanisms for sensing the internal nitrogen levels. These include internal nitrogen sensing by nitrogen transporters, transcription factors, PII proteins, general amino acid control-based sensing, cytokinins and His-Asp phosphorelay-based sensing, inotropic glutamate receptors-based sensing and sensing by NIT2 proteins (**Table 2**) [84].

4.1. Nitrogen Transporters as Internal Nitrogen Sensor

Nitrate transporting NRT1.1, NRT2.1and ammonium transporting AMT1; 3 proteins also acts as internal nitrogen sensors [47] [57].

4.2. Sensing of Internal Nitrogen Levels by Transcription Factor Proteins

Nitrogen regulated transcription factors, MADS box transcription factor (ANR1), NLP7, LBD37/38/39 proteins, SPL9 and Dof1 are associated with internal nitrate sensing [85]-[87]. The localized stimulatory effect of nitrate on root growth requires ANR1 expression which is induced by nitrate. NRT1.1 acts upstream of ANR1 in this effect [48] [88].

Nitrate induction of many nitrate uptake and assimilatory genes is mediated by NLP7 protein which is highly expressed in vascular tissues, emerging secondary roots and root hairs. In the presence of nitrate NLP7 is retained or imported into the nucleus [89]. NLP7 is also expressed in stomata, and has a role in controlling stomata opening. The reduced expression or mutation in the NLP gene resulted in impaired nitrate uptake and assimilation. NLP7 protein has been found to negatively regulate nitrogen starvation inducible genes and positively regulate primary nitrate response (nitrate inducible) [78] [85]. Many genes associated with nitrate metabolism have NLP binding sites and co-coordinately regulated by NLP7 which helps the plant to adopt to different nitrogen levels [89]. Expression of LBD 37/38/39 genes was up regulated by nitrate, ammonium and glutamine while urea has no effect. LBD 37/38/39 transcriptionally regulates nitrate assimilatory genes, nitrate transporter

Table 2. Proteins involved in sensing internal nitrogen levels.

S. No	Name of the protein	Reference
1	NRT1.1	[47]
2	NRT2.1	[49]
3	AMT1;3	[57]
4	LBD37/38/39	[86]
5	ANR1	[87]
6	SPL9	[47]
7	Dof1	[91]
8	NLP7	[85]
9	P-II protein	[93]
10	General amino acid control-based	[116]
11	Cytokinins and His-Asp phosphorelay	[123]
12	Ionotropic glutamate receptors	[140]
13	NIT2-Protein	[144]

genes, and the internal N sensing PII protein genes [86]. Under low nitrogen concentrations, the growth and assimilation of plants is improved by over expression of DOF1 transcription factor [90] [91]. All these transcription factors act as nitrate signaling components and may have a role in internal nitrate sensing.

4.3. PII Protein-Based Sensing

Though PII proteins are known to regulate the nitrogen metabolism in bacteria, archaea and some of the eukaryotes, their function in plants is not understood and it has been suggested to play a role in sensing internal carbon/nitrogen ratio [92]-[96]. Plant PII proteins are nuclear encoded chloroplast proteins belonging to GlnB subfamily [97]. They are homotrimers of 12 - 13 KD and contain three conserved Loops (C loop, T loop and B loop). Though the trimeric PII protein has been found to regulate nitrogen metabolism at transcriptional level in bacteria and post-translational level in cyanobacteria, very little is known in plants [98] [99]. Both in bacteria and cyanobacteria PII proteins sense internal nitrogen status but the mechanism of sensing is different. Bacterial sensing of cellular nitrogen status involves covalent modification (uridylylation) of Try51 while cyanobacterial sensing of cellular nitrogen status involves phosphorylation of Ser-49 residue [100]-[102]. In plants, Tyr51 is replaced with Phenylalanine and the Ser52 residue is conserved [84] [103]. But, phosphorylation of the plant PII protein has not been proven yet [104].

The following research findings suggest a role for PII proteins in internal nitrogen sensing: Over expression of plant PII protein impaired the ability to sense glutamine when grown under high sucrose and glutamine as nitrogen source. PII protein was found to interact with the arginine biosynthetic enzyme, N-acetyl glutamate kinase. No equivalent of the two component NRI/NRII system (bacterial) or the *NtcA* system (cyanobacterial) has been found in plants. The mechanism by which PII proteins convert the nitrogen depletion signal into transcription control in plants remains to be elucidated [84] [103] [105]-[109].

4.4. General Amino Acid Control-Based Sensing

Yeast has a transcription regulation mechanism of amino acid biosynthesis under low nitrogen conditions (amino acid starvation) [110]. Uncharged tRNA, transcription factor GCN4, eIF2 and many other proteins are involved in this regulation [111]. Existence of a similar regulatory system has been implied in plants [112]-[115]. Although several homologs of the GAAC components have been found in plants, there is still no direct evidence linking up each component to verify their roles in the plant GAAC system [116]-[120]. No homolog to GCN4 protein, the main component of GAAC is found in plants [84] [103] [121].

4.5. Cytokinins and His-Asp Phosphorelay-Based Sensing

Cytokinins mediate long-range nitrogen signaling in plants. There is a direct correlation between nitrogen supply and level of cytokinins [122]-[125]. The growth limitation caused by nitrogen limitation was partially overcome when supplied with exogenous cytokinins. The action of cytokinins as long distance nitrogen signal was exemplified by the increase in xylem sap cytokinin level after nitrogen treatment in *Arabidopsis* and maize [126]. Not only there was increase in cytokinin levels under nitrogen conditions but also there was decrease in levels of cytokinins under nitrogen limiting conditions. Cytokinins regulate genes associated with nitrogen uptake and assimilation at transcriptional level [127] [128].

Expression of the cytokinin biosynthetic gene isopentenyl transferase 3 is induced by nitrate [129]. The root to shootnitrate translocation enhances xylem solute flux enabling root to leaf translocation of cytokinins, which in turn triggers His-Asp multi-step phosphorelay mediated cellular signals [125] [130] [131]. The His-Asp multi-step phosphorelay consists of histidine protein kinase, response regulator and histidine-phospho transfer protein [132]-[134]. Histidine protein kinase contains the conserved cytokinin receptor, the CHASE domain [135]. Both cytokinins and nitrate induce the expression of response regulator genes. Cytokinins His-Asp phosphorelay signal is induced upon sufficient supply of nitrogen that signifies a proper time for growth and development. The role of cytokinin as a systemic relay that integrates nitrogen status and the regulation of plant growth are not proven yet. Correlation between nitrogen signals and responses of cytokinins His-Asp phosphorelay has not yet been proven and functions of many response regulator proteins are not known. The mechanismthat distinguishesoverlapping signals from cytokinin and nitrate pathways is not clear but microarray studies have revealed that only a portion of genes responsive to cytokinin are nitrate inducible [136].

4.6. Ionotropic Glutamate Receptors-Based Sensing

Homologs of synaptic transmission associated animal inotropic glutamate receptorsare found in plants [137]-[139]. Plant ionotropic glutamate receptors regulate carbon and nitrogen metabolisms, using amino acids as the signaling molecules [140]. Transgenic *A. thaliana* under-expressing ionotropic glutamate receptor alters the C/N sensitivity of the plant during seed germination and alters accumulation of several enzymes/transporters associated with C/N metabolism [95]. Abscisic acid regulates nitrate mediated root growth in plants. Nitrogen signals (NO_3^- or amino acids) act as the positive signals stimulating glutamate receptors to inhibit the ABA biosynthesis and promoting root growth. Carbon signals (sucrose) act negatively toward ionotropic glutamate receptors to reduce the expressions of carbon and nitrogen metabolism genes and elevate ABA biosynthesis [140] [141]. Ionotropic glutamate receptors from *A. thaliana* are root specific and highly expressed in roots and they may take part in sensing the change of amino acid composition in soil, leading to the adjustment of root architecture for colonizing the nutrient-rich patch of soil [141] [142].

4.7. NIT-2 Protein-Based Sensing

The GATA transcription factor NIT-2 regulates many nitrogen metabolism genes in fungi, the homologs of which are found in plants [143]-[146]. In *Arabidopsis*, though a nitrate inducible GATA transcription factor has been identified, its role in nitrate regulation of gene expression is not clear [147]. Plant NIT2-like protein (NLT1) and NIT2 binding site (GATA element) on the promoter of plant genes have been identified. A single zinc finger domain is found in plant NIT2 proteins that showed high homology with fungal NIT2 proteins [144]. The NIT2-like regulatory system has been suggested to be involved in the regulation of nitrate and nitrite reductases. The exact N sensing mechanism of NIT2 proteins in plants is still unclear.

5. Nitrogen Transporters

Plants have two transport systems for nitrogen based on the nitrogen concentration (nitrate, ammonium and urea). The low affinity nitrogen transport system functions at high nitrogen levels (millimolar concentrations >1 mM) and the high affinity nitrogen transport is mediated by transporter proteins which function at sub-millimolar nitrogen concentrations (**Table 3**) [61] [148]-[156]. Further, these transporters are classified into constitutive and inducible transport systems. In constitutive transport system, the expression of transporter genes is independent of nitrogen while in the inducible transport system the expression of the transporter genes is induced by nitrogen. The transporters of all three nitrogen forms fall under the above classifications schemes [157]-[162]. The membrane localized nitrogen transporters, using the free energy provided by the proton motive force, rapidly depolarize the membrane. Ammonium transporters act as uniporters *i.e.* transport of ammonium along the electrochemical gradient or as NH_3/H^+ co-transporters while the nitrate transporters acts as symporters, transporting nitrate along with protons via a $2H^+$: NO_3^- symport [61] [152] [163] [164]. Urea transporting proteins act as urea/H^+ symporters [165] [166].

Different gene families coding for the transporters of nitrate, ammonium and urea exist in plants. Plant ammonium transport is mediated by transporter gene families, AMT1, AMT2, AMT3, AMT4 and AMT5. AMT1 proteins contribute to high affinity transport of ammonium into the plant roots; AMT2 and other AMT proteins function as low affinity transporters [167]-[171]. For transport of nitrate, five families of transporters, NRT1, NRT2, CLC, SLAC1/SLAH and ALMT1 (aluminium activated malate transporter1), are found in crop plants [153] [164] [172]-[176]. Members of NRT1 proteins, except NRT1.1 and NRT1.3 are low affinity transporters. Both NRT1.1 and NRT1.3 functions as a dual-affinity tranceptors. NRT2 proteins act as high affinity transporters and require an additional protein NAR2.1 and function as a 2 × 2 tetramer [177]-[179]. The tonoplast localized CLC (chloride channel family) proteins (CLCa and CLCb), mediate vacuolar nitrate accumulation by proton- NO_3^- exchange mechanism [180] [181]. SLAC1 (slow anion channel 1) and SLAH3 (SLAC1 homologue 3) proteins showed nitrate transport activity. ALMT12 is a novel class of anion transporter involved in stomatal closure and it is permeable more to nitrate than to chloride [175] [182] [183]. Like other Nitrogen transporters, Urea transporters are of two types, low affinity transporters (Major intrinsic proteins) and high affinity transporters (DUR3 proteins). The low affinity major intrinsic proteins are channel-like, allowing passive urea transport down the concentration gradient while the high affinity DUR3 transporters transport urea against concentration gradient actively using energy [36] [158].

Table 3. Nitrogen transport proteins.

S. No	Name of the proteins	Transporting form of Nitrogen	Type of transport	Reference
1	NRT1.1	Nitrate	Dual affinity	[75]
2	NRT1.2	Nitrate	Low affinity	[157]
3	NRT1.3	Nitrate	Dual affinity	[232]
4	NRT1.4	Nitrate	Low affinity	[289]
5	NRT1.5	Nitrate	Low affinity	[245]
6	NRT1.6	Nitrate	Low affinity	[327]
7	NRT1.7	Nitrate	Low affinity	[328]
8	NRT1.8	Nitrate	Low affinity	[286]
9	NRT1.9	Nitrate	Low affinity	[292]
10	NRT1.10	Nitrate	Low affinity	[434]
11	NRT1.11	Nitrate	Low affinity	[329]
12	NRT1.12	Nitrate	Low affinity	[329]
13	NAXT1	Nitrate	Low affinity	[246]
14	NRT2.1	Nitrate	High affinity	[76] [159] [225]
15	NRT2.2	Nitrate	High affinity	[232]
16	NRT2.3	Nitrate	High affinity	[287]
17	NRT2.4	Nitrate	High affinity	[227]
18	NRT2.5	Nitrate	High affinity	[477]
19	NRT2.6	Nitrate	High affinity	[172] [477]
20	NRT2.7	Nitrate	High affinity	[303].
21	CLCa	Nitrate	-	[180]
22	CLCb	Nitrate	-	[181]
23	SLAC1	Nitrate	-	[182]
24	SLAH	Nitrate	-	[182]
25	ALMT12	Nitrate	-	[210]
26	AMT1.1	Ammonium	High affinity	[168]
27	AMT1.2	Ammonium	High affinity	[67]
28	AMT1.3	Ammonium	High affinity	[58]
29	AMT2.1	Ammonium	Low affinity	[171]
30	AMT2.2	Ammonium	Low affinity	[265] [266]
31	AMT2.3	Ammonium	Low affinity	[220]
32	AMT3.1	Ammonium	Low affinity	[267]
33	AMT3.2	Ammonium	Low affinity	[268]
34	AMT3.3	Ammonium	Low affinity	[169] [220]
35	TIP	Urea Ammonium	Low affinity	[193] [242]
36	DUR3	Urea	High affinity	[158]
37	NIP	Urea	Low affinity	[241]

5.1. Ammonium Transporters

The ammonium transporting AMT proteins belong to ammonium transporter/methyl-ammonium permease/ rhesus family (AMT/MEP/Rh). These proteins are highly hydrophobic membrane proteins of 45 - 55 kDa size. They are trimers with substrate conduction channel within each monomer [60] [184] [185]. Ammonia channel proteins have either 11 or 12 transmembrane helices and divided into structurally similar two halves with a N-out, C-in topology (long cytoplasmic C-terminal) but organized with pseudo-two-fold symmetry [186] [187]. Ammonium is transported along a pore in between these two halves of the protein [188] [189]. A cytosolic loop connects the two halves (5th & 6th helices) and the residues of the helices are involved in recruitment and transport of ammonium, but the mechanism involved is not known [184]. Oligomerization is a general feature of Amt/Mep/Rh proteins and plant ammonium transporters have been found to form oligomers (homo or hetero) in the plasma membrane of roots. A functional pore is formed by each subunit of the oligomer [65]. The cytosolic C terminus (CCT) containing three conserved Ser/Thr residues regulate the function of AMT oligomers. When the external ammonium concentration is high, the AMT proteins are phosphorylated at the C-terminus (at Thr-460) leading to rapid shut down of ammonium transport into the cell [68]. Thr-460 is conserved among all AMT1 homologs except in AMT1; 5 [190]. Mutations or truncation of the C-terminus resulted in the inactivation of the AMT proteins [55] [56]. The interactions among CCT, the loop between 5 - 6 transmembrane helices and adjacent loops, serve as an allosteric switch that changes the conformational state of the monomers in the complex thereby controlling access to substrate or transport of ammonium through the pore [163]. This kind of allosteric regulation efficiently monitors ammonium transport capacities of AMT oligomer complexes in plant roots [69]. Vacuolar ammonium loading is mediated by tonoplastic intrinsic proteins (TIPs) TIP1; 2, TIP2; 1 and TIP2; 3 [191]-[193].

5.2. Nitrate Transporters

The low affinity NRT1 transporters belong to NPF family (previously called PTR/PTO family [194]. NRT1 transporters show sequence similarity to plant and animal peptide transporters (PTR). The earlier under standing off unctional distinction of NRT1 (nitrate transport) and PTR (peptide transport) members need to be revised asrecent investigations have shown that NRT1 proteins, in addition to transporting nitrate, also transporthormones, dipeptides and glucosinolates. Plant NRT1 proteins contain "12 transmembrane (TM) domains and a plant specific long hydrophilic loop between TM6 and TM7 domains". NRT1 transporters contain one or two MFS domains [47] [195]-[198].

Dual affinity nitrate transporters form dimer at the plasma membrane, this dimerization is depends on phosphorylation at Thr101 of the NRT1.1 transporter. Thr101 is closed by a pocket formed by transmembrane helices 2, 3 and 4, which is located at the homodimer interface. The substrate binding site is formed by Leu 49, Val 53, Leu 78 and Phe 511. The NRT1.1 contains His 356 at TMH7 which has a role in stabilization of nitrate in the binding site. The mechanism of dual affinity mode of action is mediated by oligomerization. In the un-phosphorylated state NRT1.1 transporters form a homodimer and function as low affinity nitrate transporter. When phosphorylated NRT1.1 homodimers are disrupted, each monomer functions as independent high affinity transporters. The long N-terminal structure of NRT1.1 forms a cleft between the NRT1.1 proteins in the dimer which provides the binding site for kinases and other enzymes [199].

The high affinity NRT2 transporters belong to Nitrate-Nitrite Porter family (NNP) under major facilitator super family [152] [200]. Like NRT1 transporters, NRT2 proteins also contain twelve transmembrane spanning domains and a cytosolic oriented loop in between TM6 and TM7 domains. Members of NRT2 have not been shown to transport anything else other than nitrate. Although both NRT1 and NRT2 proteins contained MFS domains, the NRT2 transporters contained an additional "nitrate motif" (A-G-W/L-G-N-M-G) located in the fifth transmembrane domain. The C terminal domain is long and is cytosolic. R87 and R459 located in transmembrane domains 2 and 8 respectively were identified as essential residues for substrate binding by mutation analysis.

CLC proteins, the vacuolar nitrate transporters, belong to secondary active transporters which is a subfamily of chloride channel proteins [201]-[203]. Plant CLCa proteins show selectivity in transporting nitrate than chloride. This transport is dependent on proton coupling which is a unique feature in plants when compared to animal and bacterial CLC proteins where it is not dependent on proton coupling [180]. The nitrate selectivity of CLC proteins is based on the presence of conserved amino acids [204] [205]. A serine residue (Ser107) is conserved in human and bacterial CLC proteins while in plants a proline residue (Pro160) is conserved in the conserved motif which selects nitrate over chloride [201] [206]. Along with proline there are 2 other conserved amino acids called gating glutamate and proton glutamate essential for the transport activity of CLCa protein. Seven and four members of CLC protein genes were found in *Arabidopsis* and in rice, respectively. Four of the *Arabidopsis* CLC proteins contained proline as the conserved residue while others contained serine as the conserved residue in the selectivity filter motif. Except for CLCa, b the physiological functions of other proteins are not known [180] [207]-[209].

SLAC1 are slow anion channel proteins located in guard cells capable of nitrate transport [182]. Homologues of SLAC1 located in other tissues are called SLAH proteins which are activated by the CPK21 kinase in a nitrate induced manner. The plasma membrane localized SLAC1 and SLAH member proteins contain 10 transmembrane helices. The aluminum activated malate transporter12 (ALMT12) is another protein found to transport nitrate to the guard cells. This guard cell plasma membrane localized protein contains six transmembrane α-helices with an N-out and C-out topology. The structural features determining the selectivity of these proteins is yet to be determined [210].

5.3. Urea Transporters

Plant urea transport proteins come under sodium solute symporter super family [36] [166]. Among the five urea transport proteins known in plants, DUR3 is the lone high affinity transporter while others are low affinity transporters. The membrane localized DUR3 contains 14 - 15 transmarine domains and N out and C in topology [211]. Domains 1 - 6 and 7 - 12 are conserved in most plants, but the length of C-terminal is variable. In between 8th and 9th transmembrane domains, an outside-oriented loop is located which contains the conserved Walker A motif (P-loop) [212]. The low affinity passive urea transport is carried out by aquaporins which are major intrinsic proteins (MIPs). They are of four types: Nodulin 26-like membrane intrinsic proteins, plasma membrane intrinsic proteins, small basic membrane intrinsic proteins and tonoplast intrinsic proteins [213].

Major intrinsic proteins contain “six transmembrane spanning helices, five interconnecting loops (A-E) with N out and C out topology” [214]. A, C and E loops are apoplastic oriented in plasma membrane intrinsic proteins and Nodulin 26-like membrane intrinsic proteins and vacuolar space oriented in tonoplast intrinsic proteins. The conserved NPA motif (asp-pro-ala sequence) is located in the partly membrane embedded small loops B and E. The NPA motif acts as the selectivity filter. The B and E loops bring together two highly conserved NPA-motifs (asparagine-proline-alanine) in the centre of the membrane and contribute to pore formation [215] [216]. The substrate specificity of these proteins can be due to size exclusion at two main pore constrictions, H-bonding mediated stereo specific recognition of the substrate and hydrophobic interactions within the pore. Along with urea, aquaporins which are able to transport ammonia were also found and they belong to the tonoplast intrinsic proteins family. The selectivity between urea and ammonia (NH_3) is due to the presence of the histidine instead of a hydrophobic aromatic amino acid in the pore constriction region [193].

6. Nitrogen Uptake

The most important function of nitrate, ammonium and urea transporters is the acquisition of different forms of nitrogen from the soil into the plant roots. Though many nitrogen transporters have been identified in plants, only few of them are found to be associated with soil nitrogen uptake (**Figure 1**). AMT1; 1, AMT1; 2, AMT1; 3, AMT1; 5, AMT2; 1 and AMT5; 1 proteins mediate ammonium uptake. Nitrate uptake is mediated by NRT1.1, NRT1.2, NRT2.1, NRT2.2 and NRT2.4. Urea uptake is mediated by DUR3 and MIP transporters (**Table 4**).

6.1. Ammonium Uptake

Ammonium uptake is carried out by AMT1:1, AMT1; 2, AMT1; 3 and AMT1; 5 proteins in *Arabidopsis* and other plants. However, in rice two additional proteins, AMT2; 1 and AMT5.1 were found to uptake ammonium [59] [163] [167] [171] [217] [218]. AMT1; 1, AMT1; 2, AMT1; 3 and AMT1; 5 are high affinity ammonium transporters while AMT2; 1 and AMT5; 1 are low affinity ammonium transporters. All these proteins show root specific expression. AMT1; 1 and AMT2; 1 are constitutively expressed but ammonium increases expression of these genes in roots. AMT1; 2 is an ammonium inducible protein while AMT1; 3, and AMT1; 5 are repressed by nitrogen [162] [218]. In *Arabidopsis* all AMT proteins are constitutively expressed but in maize, AMT1; 1 and AMT1; 3 are ammonium inducible [219]. AMT1; 1, AMT1; 3 and AMT1; 5 are expressed mainly in the

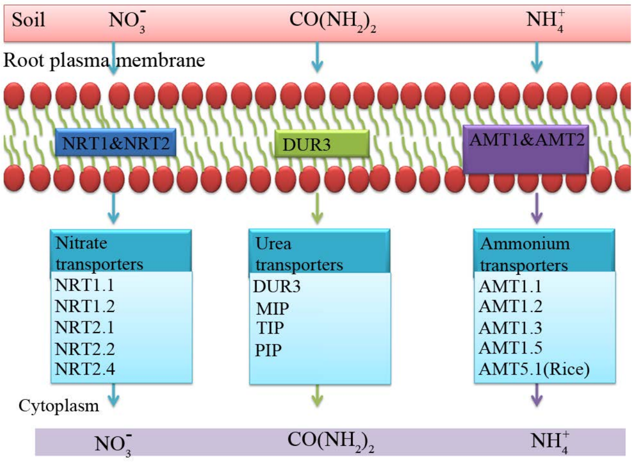

Figure 1. Nitrogen transporters involved in N uptake from the soil. NRT1.1, NRT1.2, NRT2.1, NRT2.2 and NRT2.4 are nitrate transporters, while AMT1.1; AMT1.2; AMT1.3; AMT1.5 & AMT5.1 are the ammonium transporters involved in uptake of nitrogen. Urea transporters DUR3, MIP, TIP and PIP proteins are involved in nitrogen uptake. All these proteins are plasma membrane localized and highly expressed in the roots.

Table 4. Proteins associated with nitrogen uptake.

S.No	Name of the protein	Mechanism	Reference
1	NRT1.1	NO_3^-/H^+ symport	[75] [161]
2	NRT1.2	NO_3^-/H^+ symport	[157]
3	NRT2.1	NO_3^-/H^+ symport	[159]
4	NRT2.2	NO_3^-/H^+ symport	[232]
5	NRT2.4	NO_3^-/H^+ symport	[227]
6	AMT1.1	NH_4^+ uniporters/NH_3/H^+ co-transporters	[59]
7	AMT1.2	NH_4^+ uniporters/NH_3/H^+ co-transporters	[170]
8	AMT1.3	NH_4^+ uniporters/NH_3/H^+ coransporters	[162]
9	AMT1.5	NH_4^+ uniporters/NH_3/H^+ coransporters	[162]
10	AMT2.1	NH_4^+ uniporters/NH_3/H^+ coransporters	[171]
11	AMT5.1	NH_4^+ uniporters/NH_3/H^+ coransporters	[167]
12	DUR3	Urea/H^+symport	[158] [165]
13	NIP	Urea transport through a channel	[191]

root epidermal cells and root hair regions [59] [67] [218]. AMT1; 2 is expressed in outer cortical cells, central root cylinder, endodermis cells, sclerenchyma cells, root hairs and pericycle cells of primary root tip [67] [220] [221].

Among all the AMT proteins AMT1; 1 has the highest substrate affinity and responsible for efficient ammonium uptake from the soil even under very low concentrations (nano molar). Under high ammonium conditions over-expression of OsAMT1; 1, increased the ammonium uptake and reduced the total biomass leading to impaired growth and development. In nitrogen deficient roots AMT1; 1 and AMT1; 3 carryout bulk of high affinity transport (1/3rd of) When the availability of NH_4^+ in the soil is low, AMT1; 3 acts in an additive manner with AMT1; 1 in taking up NH_4^+ ions About 90% of root ammonium uptake is mediated by AMT1; 1, AMT1; 2 and AMT1; 3 transporters [59] [160] [162] [217] [222] [223]. AMT transporters act either as NH_4^+ uniporters or NH_3/H^+ co-transporters. Structural studies with AMT1; 1 have shown that ammonium is taken up from the soil and it is recruited at the external pore by a coordinated interplay of highly conserved Trp178 and tyr133 residues. Then ammonium passes through the pore residues during which it is de-protonated (NH_3) by transfer of the protons to the two histidine residues (His168 and His318) within the pore. The NH_3 moves down the pore and again re-protonated before release into the cytoplasm using the proton transferred from histidine residues [61] [62] [150] [163].

6.2. Nitrate Uptake

Nitrate uptake is mediated by NRT1.1, NRT1.2, NRT2.1, NRT2.2 and NRT2.4 proteins. The dual affinity NRT1.1 transporter acts as an inducible low affinity transporter (except in rice and tomato where it was found to be constitutive) [161] [222] [224]. NRT1.2 is a low affinity constitutive nitrate transporter (except in tomato where it is inducible). While NRT2.1 and NRT2.2 proteins are inducible high affinity nitrate transporters [159] [225] [226]. The constitutive NRT2.4 transporter gene is induced by N limiting conditions [227].

All nitrate uptake transporters show expression in the root tissue. NRT1.1 isexpressed in primary and secondary root tips, pericycle cells, epidermal cells, root hairs and root meristem quiescent centre [72] [157]. NRT1.1 uptakes nitrate both at low and a high soil nitrate condition which is made possible by the phosphorylation based switching (see nitrogen sensing for details about this switching). The low affinity nitrate uptake by NRT1.1 is pH dependent [73] [79]. NRT1.2 is a root dominant gene, expressed in rhizodermis and this expression is independent of root age [228]. In tomato it has been found to express in root hairs [222]. Antisense lines of NRT1.2 in *Arabidopsis* showed decreased nitrate uptake indicating the functional role of NRT1.2 [157]. NRT2.1 gene showed a root specific expression [178] [229]-[231].

The role of NRT2 proteins in high affinity nitrate transport was identified from null mutant studies of NRT2:1 and NRT2:2. About 80% reduction in nitrate uptake was observed when genes coding for these two proteins are disrupted [148] [159] [232]-[234]. These two proteins need NAR2 protein for their functional activity. However, NRT2.4 does not need this additional protein for nitrogen uptake. The expression of NAR2 protein is induced by nitrate and T-DNA insertion of NAR2 also decreased the high affinity nitrate uptake. NAR2 directly interacts with the NRT2 proteins to form a hetero oligomer and are localized to the plasma membrane [179] [226]. NRT2.4 gene is expressed in the root primordium and epidermis of young lateral roots [152]. Mutational analysis of *Arabidopsis* NRT2.4 gene proved its role in nitrate uptake under N limiting conditions [227].

Nitrate uptake into the plant roots, depolarizes the plasma membrane and coupled with proton gradient. The depolarization is counteracted by the nitrate inducible plasma membrane H^+-ATPase [235] [236]. Both high and low affinity transporters mediate nitrate transport as 1 $NO_3^-/2H^+$ and the energy is provided by proton motive force. The internal nitrogen status and the root age determine the contribution of each transporter in nitrate uptake. Genes coding for NRT1.1 and NRT2.4 are expressed in the young roots while NRT2.1 is specific for mature roots. The nitrate binding site is closely bound both extra cellular (TM1-TM2 and TM7-TM8) and intracellular gates (TM4-TM5 and TM10-TM11). Proton coupled nitrate uptake is mediated by, protonation of His 356 followed by nitrate binding. Nitrate binding closes the extracellular gate and conformational changes occur in the protein, and opens up intra cellular gate to transport nitrate and protons in to the cell [237].

6.3. Urea Uptake

Urea uptake in plants is mediated by the high affinity DUR3 and low affinity NIP proteins [238]. It is expressed mainly in the root hairs, rhizodermis, root cortex and root xylem and localized to the plasma membrane. Nitrgen starvation and urea up regulates DUR3 gene expression while ammonium and nitrate repress the gene [158] [165] [239] [240]. DUR3 mediated urea uptake involves proton and membrane potential dependent 1 urea/1H^+ symport the details of it is yet to be understood [158] [165]. Arabidopsis DUR3 T-DNA insertion mutants showed impaired growth. Low affinity urea transporters NIP2; 1, NIP2.4 and NIP6.1 are associated with uptake of urea [241]-[243]. NIP2; 1 and NIP2.4 are expressed constitutively in the root epidermal cells. NIPs function as channels through which urea can pass and is independent of pH [191]. NIP6; 1 contained alanine instead of tryptophan in helix 2 and this creates a much wider pore at the ar/R region which is permeable to urea [244].

7. Nitrogen Efflux

For some unknown reasons, plants excrete nitrate outside and is induced by acidic conditions. The excretion may be passive or mediated by transporters NAXT1 and NRT1.5 (**Table 5**) [245] [246]. NAXT1 gene is specifically expressed in the mature root cortical cells and this protein is localized to the plasma membrane [246].

Table 5. Proteins associated with nitrogen efflux.

S. No	Name of the protein	Type	Reference
1	NAXT1	Nitrate efflux only	[246]
2	NRT1.1	Bidirectional nitrate transporter	[400]
3	NRT1.4	Bidirectional nitrate transporter	[400]
4	NRT1.5	Bidirectional nitrate transporter	[245]

NAXT1 is excretes NO_3^- to the external medium in response to acid load or medium acidification [164].

Ammonium accumulation in plants leads to toxicity and reduced nitrogen uptake efficiency [247]-[249]. For maintaining ammonium homeostasis, plants efflux ammonium into soil. Plants respond differently towards ammonium levels and based on this plants are classified as ammonium sensitive and tolerant species [250]. Under high ammonium conditions ammonium efflux occurs mainly at root elongation zone and depends on root respiration leading to repression of root growth and plant development [248]. The proteins associated with ammonium efflux are not yet identified. Efflux may be a mechanism in plants to protect from toxicity and to maintain root growth under excess ammonium conditions.

8. Nitrogen Translocation and Assimilation

Nitrogen (nitrate, ammonium and urea) taken up into the roots can either be transported to other parts of root and the shoot through xylem/phloem or stored in the vacuoles or assimilated (**Figure 2** & **Figure 3**) (**Table 6**). Nitrogen assimilation depends on nutrient availability, diurnal cycle and carbon availability [95]. Among the three nitrogen sources, ammonium is toxic, so assimilated immediately after uptake into the roots and transported to shoots in an organic form. The non-toxic nitrate and urea is either assimilated immediately in the roots or assimilated after translocation to shoots.

8.1. Translocation and Assimilation of Ammonium

Sources of ammonium includes direct ammonium uptake from the soil by AMT1 and AMT2 transporters, nitrate reduction, protein degradation and photo respiration [251]-[253]. Photorespiration produces large amounts (20 fold higher than nitrate reduction) of ammonium. As high ammonium is toxic, it is assimilated immediately into organic nitrogen forms [254]. Assimilation of ammonium into glutamine is mediated by glutamine synthetase enzyme. Glutamate synthase converts glutamine into two glutamate molecules (Glutamine and glutamates are used for synthesis of other amino acids [255] [256].

Glutamine synthetase exists as two isoforms, GS1 and GS2.GS1 isoform found in leaf cells and root phloem is associated with the root primary nitrogen assimilation. The GS2 isoform occurring in plastids is involved in re assimilation of ammonium released via photorespiration. Photorespiration plays an important role in maintaining the nitrogen status of the plant [257] [258]. The GS/GOGAT cycle incorporates photo respiratory and non-respiratory ammonium and provides nitrogen for transport and maintaining nitrogen status in the plant [259] [260]. Assimilation of one NH_4^+ molecule generates 1.33 H^+ molecules which are excreted out to maintain pH of the cytoplasm [261] [262]. Ammonium assimilation is essential for preventing ammonium toxicity which affects a number of processes in the plant [250] [263].

Though ammonium is assimilated immediately after uptake or generation, there are specific instances in plants where ammonium is translocated. Transport of ammonium to pollen is essential for its development, germination and growth of pollen tube. This transport is mediated by the constitutively expressed pollen grains and pollen tube localized AMT1; 4 ammonium transporter [264]. The expression pattern of AMT2; 2 and AMT2; 3 in shoots and roots indicate their role in translocation of ammonium but the exact mechanism of the translocation process is not known [171] [220] [265]-[267].

AMT3; 1, weakly expressed in root but more in the stem and the new leaves is involved in translocation of ammonium from roots to the leaves [171] [267]. N starvation induces the expression of AMT3; 1 in roots, AMT3; 2 is expressed in roots and shoots, is an inducible ammonium transporter involved intrans location of ammonium from the roots through the xylem. AMT3; 3 is an inducible ammonium transporter expressed in old leaves and in the stems and is associated with translocation of ammonium [169] [220] [267].

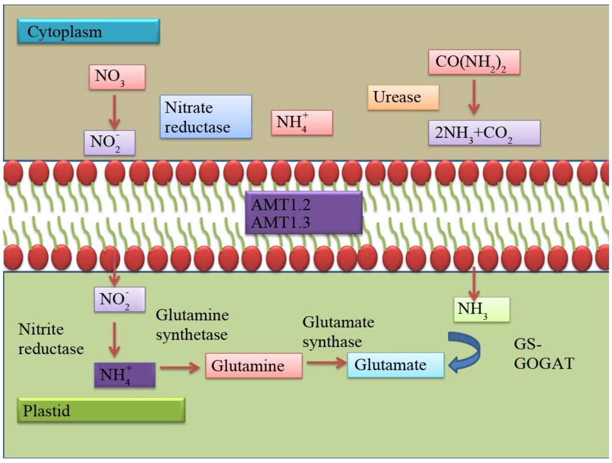

Figure 2. Role of nitrogen transporters in nitrogen assimilation in plants. Once taken inside nitrate, ammonia and urea are assimilated into different forms. Nitrate is assimilated to nitrite then to ammonia finally to amino acids. Urea is immediately converted to ammonium and then to amino acids. Nitrate transporter NRT1.1 and Ammonium transporters AMT1.2, AMT1.3 are involved in the N assimilation.

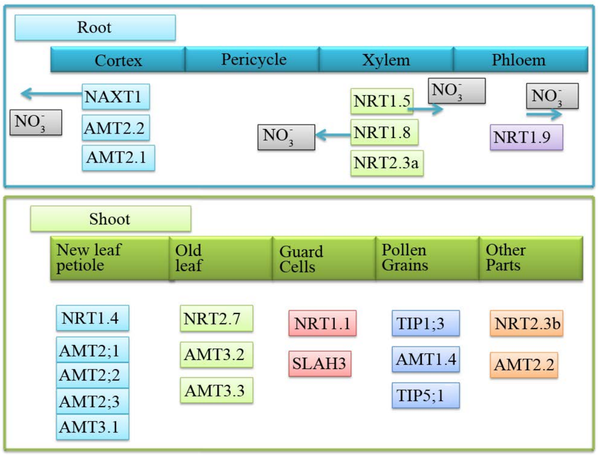

Figure 3. Nitrogen translocation. Nitrogen once taken inside the root is distributed to different parts of the plant and involves different N transporters. Different nitrogen transporters are involved in different organs of the plant.

Table 6. Proteins involved in nitrogen translocation and assimilation.

S. No	Name of the protein	Function	Reference
1	AMT1.2	Ammonium retrieval xylem	[268]
2	AMT1.4	Pollen development	[67] [264]
3	AMT2.2	Ammonium translocation	[265] [266]
4	AMT2.3	Ammonium translocation	[220]
5	AMT3.1	Root to leaf translocation of ammonium	[267]
6	AMT3.2	Ammonium translocation	[169]
7	AMT3.3	Ammonium translocation	[169] [220]
8	NRT1.3	Nitrate translocation	[232]
9	NRT1.4	Petiole nitrate storage	[289]
10	NRT1.5	Long distance nitrate transport	[245]
11	NRT1.8	Long distance nitrate transport	[286]
12	NRT1.9	Phloem nitrate transport	[292]

An ammonium transporter AMT1; 2 is involved in ammonium retrieval (**Figure 3**). AMT1; 2 is induced by both ammonium and nitrate [222] [268] [269]. AMT1; 2 contains serine rich plastid transit peptide. Two T-DNA insertions of AMT1; 2 mutant analysis showed that, the accumulation of ammonium is not altered showing that it has major function in the intracellular ammonium transport than uptake. AMT1; 2 is involved intransfer of ammonium to the vascular tissues [56]. Nitrate assimilation produces ammonium in both roots and shoots and AMT1; 2 is required to maintain these ammonium pools in the plastids. It is involved in efflux ammonium from the plastids to cytosol for further assimilation or transport into the chloroplast for further re assimilation [30]. AMT1; 2 is expressed root apoplast, endodermal and cortical cells and in the leaves and expression is regulated by whole plant nitrogen status. Further work required for the functional characterization of AMT1; 2 in nitrogen assimilation [162] [218] [270].

8.2. Translocation and Assimilation of Nitrate

The extent to which nitrate is assimilated in the roots and how much nitrate is transported to shoots is not clear and is influenced by various factors like the species of plant, developmental stage of the plant, light intensity and temperature [95] [271]-[273]. In the root, highest assimilation is found behind the root tip. Nitrate reductase reduces the incoming nitrate to nitrite immediately in a NAD (P) H-dependent manner in the cytosol and it is a rate limiting step in assimilation of nitrate [274] [275].

Then nitrite reductase reduces the nitrite transported into the chloroplast (by diffusion or by the nitrite transporter (Nitr)) into ammonium and is finally converted to amino acids by GS/GOGAT pathway [276]. Nitrate assimilation responds to internal and external signals (nitrate, light, sucrose, circadian rhythms, glutamine, auxin and pentose phosphate pathway metabolites) integrating nitrate assimilation with carbon and energy metabolism, ensuring availability of sufficient carbon and energy for the assimilation of inorganic nitrogen. They also influence nitrogen uptake by regulating root development. The nitrogen status of the plant regulates genes coding for nitrate transporters and N-assimilatory enzymes at transcriptional, translational and post-translational levels [276] [277]. Expression of nitrogen assimilatory genes nitrate reductase, nitrite reductase, glutamate synthase and carbon metabolism genes are induced by nitrate [136] [278]-[283]. Assimilation of one molecule of NO_3^- generates 0.67 molecule of OH^- which is effluxes into the soil from the roots. The OH^- generated in the shoots due to nitrate assimilation is neutralized using organic acids [261] [262] [284]. Along with nitrate, DNA-binding protein NLP7 (NIN-like protein-7) regulates nitrate assimilation [85]. After uptake into the cell NO_3^- is assimilated into amino acids that can directly feedback regulate the uptake of nitrate.

Translocation of nitrate from roots to shoots is mediated by transporters which load and unload nitrate into xylem and phloem by which nitrate is redistributed to different parts of the plant. Xylem is the major route of nitrate transport in plants. The low NRT1.5 and the high affinity NRT2.3 a load nitrate into xylem. Unloading of nitrate from xylem is mediated by NRT1.4 and NRT1.8. NRT1.5 is a nitrate inducible bi-directional transporter regulated by pH. It transports nitrate from root through xylem [173] [245]. Abiotic stresses down regulate expression of NRT1.5 by which the root nitrate is retained during stress contributing to stress [285] [286]. NRT2.3 a is another transporter that loads nitrate into xylem for transport to shoots. It is a vascular specific transporter expressed in the root stele and the scutellum tissue of rice [287] [288].

Unloading of nitrate from xylem is mediated by both NRT1.4 and NRT1.8 proteins. NRT1.4 is localized to petiole and it retrieves nitrate from the xylem and translocate to other parts of the leaf [228] [289]. It is mainly responsible for accumulation of nitrate in the petiole by which it regulates leaf nitrate homeostasis [164]. NRT1.8 is the main xylem nitrate unloaded and is expressed mostly in parenchyma cells of xylem [286]. Nitrate is also transported through phloem and the level of nitrate in phloem was found to be 1.9 to 8.1 mM under N non-limiting conditions [290] [291]. NRT1.9 and NRT2.4 load nitrate into phloem. NRT1.9 is a phloem loader, predominantly expressed in the companion cells of root phloem. It facilitates downward movement of nitrate which is considered to be a mechanism regulating shoot nitrate transport [292].

8.3. Translocation and Assimilation of Urea

Like nitrate, urea assimilation also occurs both in roots and shoots, but the mechanism is not known for this partition [293]. Urea is assimilated to NH_3 and CO_2 by the enzyme urease [157]. The GS/GOGAT pathway enzymes assimilate the NH_3 generated into amino acids. Urease is a nickel metalloenzyme requiring accessory proteins, UreD, UreF, and UreG, for activation [294]. Sources of urea are including urea transport by DUR3 and

NIP proteins, (Urea cycle) arginine degradation and ureides from purine catabolism [295]. Urea cycle derived urea is produced in mitochondria and then exported to the cytoplasm for hydrolysing into ammonium by urease [36] [219]. Assimilation of one urea molecule produces 0. 33H^+ ion [296].

9. Vacuolar Nitrogen Storage

In plants, vacuole occupies the major volume of mature cells and is used as a buffering system for maintaining homeostasis of cytoplasm. It is a major storage site for different forms of nitrogen. Vacuoles are acidic and contain hydrolytic enzymes. Vacuolar solute transport is driven by the proton-motive force generated by vacuolar ATPase/vacuolar pyrophosphatase [297] [298]. Vacuolar ion movement is mediated by aquaporins (water channel proteins) and other specific ion channels (**Figure 4**) (**Table 7**).

Vacuole (the nitrate storage pool) stores about 30 - 50 mol·m^{-3} nitrate when compared to just about 3 - 5 mol·m^{-3} nitrate present in the cytoplasm (the metabolic pool) and the stored vacuolar nitrate is transported into the cytoplasm for utilization or mobilization [299] [300]. Nitrate transporters like CLC family proteins and NRT2.7 are involved in vacuolar nitrate transport. Vacuolar nitrate storage is genotype and variety dependent regulated by photosynthates and energy [81] [176] [301] [302].

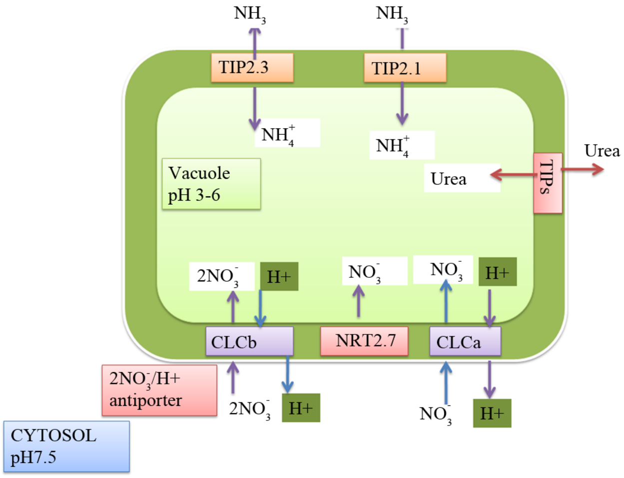

Figure 4. Vacuolar nitrogen storage. Vacuolar nitrogen storage is carried out by nitrate transporters NRT2.7 and CLCa, b proteins which are tonoplast localised. Vacuolar ammonium loading is driven by TIP2.1 and TIP2.3 proteins and vacuolar urea loading is mediated by TIPs.

Table 7. Proteins associated with vacuolar storage of nitrogen.

S. No	Name of the protein	Function	Reference
1	CLCa	proton- NO_3^- exchange into vacuole	[180]
2	NRT2.7	Seed vacuolar nitrate storage	[303]
3	TIP1;2	Maintanance of ammonium homeostasis	[307]
4	TIP2;1	Vacuolar ammonium loading	[309]
5	TIP2;3	Vacuolar ammonium loading	[193]
6	TIPa	Maintenance of urea homeostasis	[242]
7	TIP1;1	Vacuolar loading of urea	[242]
8	TIP2;1	Vacuolar loading of urea	[241]
9	TIP4.1	Urea accumulation	[314]
10	TIP4.4	Vacuolar loading of urea	[241]

In cereals, CLC proteins are the major vacuolar nitrate transporters [173]. Though absent in cereals, NRT2.7 is an important vacuolar nitrate transporter in Arabidopsis and is important for vascular nitrate loading during seed development [303]-[305]. The tonoplast localized CLCa and CLCb proteins possess high nitrate selectivity over chloride. They transport nitrate by the proton-nitrate exchange mechanism. About 50% of vacuolar nitrate storage is mediated by the mesophyll localized CLCa protein. Nitrate up regulates expression of CLCa and CLCb genes which are also diurnally regulated [180] [181]. NRT2.7 is expressed in the cotyledon tonoplasts during late embryonic developmental stage and in roots in the tonoplast of the main vacuole of mature roots [303].

Vacuolar ammonium concentration ranges from 2 - 45 mol·m^{-3} [306]. Vacuolar ammonium transport is mediated by ammonium inducible aquaporins, TIP1; 2, TIP2; 1 and TIP2; 3 proteins [307]-[310]. The ammonium in root cytoplasm brought in by AMT transporters are deprotonated immediately leading to increase in vacuolar pH. The differential pH in cytoplasm and vacuole generates an electro chemical gradient that drives the transport of ammonia into the vacuole by the TIP proteins. The ammonia entering the vacuole is reprotonated and stored as ammonium (NH_4^+) [247]. Vacuolar partitioning of ammonium by these proteins prevents excess ammonium build up in the cytoplasm thereby preventing ammonium toxicity. Stored ammonium is remobilized into cytoplasm during nitrogen starvation [192] [193] [216] [311] [312].

Vacuolar urea transport is mediated by the low affinity urea transporters, TIPa, TIP1; 1, TIP1; 2, TIP2; 1, TIP4; 1 and TIP4.4 [193] [242] [243] [313]. These transporters are constitutively expressed in tonoplast and involved in vacuolar loading and unloading of urea. Vacuolar urea transport is independent of pH gradient and is a passive transport [165]. Cytoplasm nitrogen depletion up regulates the expression of urea transporting TIP proteins leading to immediate remobilization of the vacuolar urea into the cytoplasm [241]. Over expression of TIP4; 1 gene increased not only vacuolar accumulation of urea, but also increased the urea uptake from soil [314]. Storage of urea in vacuoles avoids toxicity and prevents saturation of N-assimilation and stored urea is remobilized to cytoplasm when needed.

10. Nitrogen Remobilization

Remobilization is the process of moving nitrogen from old senescing plant parts to younger parts during vegetative phase or to the storage organs during reproductive phase [280] [315]-[319]. Remobilization facilitates efficient utilization of available nitrogen in the plant and also meets the inadequacy of the uptake system in providing enough nitrogen for storage in seeds. There is substantial quantity of nitrogen available in the senescing part of the plant, specially the leaves. Chloroplasts in leaves are one of the main sources of nitrogen for remobilization. Rubisco enzyme alone constitutes 50 - 56 percent of the total soluble proteins [320] [321]. The major contributor for seed nitrogen content is the leaf nitrogen remobilization and it varies from 50 - 90 percent in different crop plants [322]-[325]. So, the grain yield is influenced by the nitrate stored in the vegetative tissue and efficiency of remobilization of this stored nitrogen to the reproductive tissues. For efficient remobilization of leaf nitrogen there should be tight co-ordination between remobilization and senescence induced protein degradation. Fusion protein studies with rubisco and GFP/RFP showed that immediately after the onset of senescence the rubisco is taken inside the vacuole for processing. In autophagy deficient mutant this was not happening indicating the contribution of autophagy for degradation of substantial quantity of rubisco during senescence [326].

Nitrate transporters like NRT1.6, NRT1.7, NRT1.11, NRT1.12 and NRT2.4 are involved in N remobilization (**Figure 5**, **Table 8**) [227] [327]-[329]. Nitrogen starvation induces expression of NRT1.7 in shoots and the protein is targeted to the plasma membrane of mature leaf vascular tissue. It loads the excess nitrate available in mature leaves into phloem and remobilizes to younger leaves. Excess nitrate in older leaves is the vacuolar stored nitrate or the nitrate produced by senescence induced degradation of proteins. This vacuolar nitrate is likely to be transported by vacuolar nitrate transporters, which in turn is loaded into phloem by NRT1.7 [164] [328].

NRT1.6 and NRT2.7 proteins transport nitrate for seed development, the former during early stages and the latter during late stages of seed development [303] [327] [328]. Nitrogen starvation induces expression of NRT1.6 in flowers, vascular bundle, and the epidermis of silique, seed funiculus and embryo sac wall [327]. NRT2.4 is involved in remobilization of nitrate from leaves by phloem loading [227]. NRT1.11 and NRT1.12 are involved in remobilization of nitrate from mature to young leaves by transferring nitrate from xylem to phloem. Expression of NRT1.11 gene is shoot specific, while NRT1.12 is expressed both in roots and shoots [329]. High affinity urea transporter DUR3 and low affinity urea transporters TIP1; 3 and TIP5; 1 are also asso-

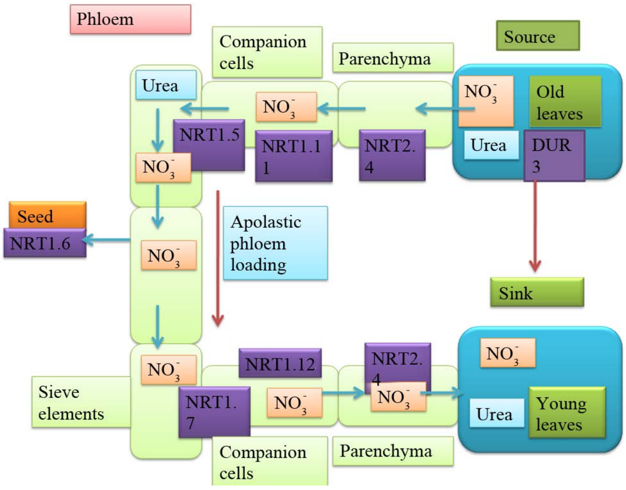

Figure 5. Nitrogen Remobilization. Source to sink nitrogen remobilization is mediated by nitrate and urea transporter proteins. Nitrate is remobilized from old leaves to young leaves by NRT1.7, NRT1.11, NRT1.12, and NRT2.4. For seed development nitrate is remobilised from source tissues to developing seeds by NRT1.6 protein. Urea transporting DUR3 proteins also participated in nitrogen remobilization.

Table 8. Proteins associated with nitrogen remobilization.

S. No	Name of the protein	Function	Reference
1	NRT1.6	Seed nitrate storage	[327]
2	NRT1.7	Nitrogen remobilization	[328]
3	NRT1.11	Nitrate remobilization from xylem to phloem	[329]
4	NRT1.12	Nitrate remobilization from xylem to phloem	[329]
5	NRT2.4	Nitrogen remobilization	[227]
6	DUR3	Urea remobilization	[239]
7	TIP1; 3	Pollen development	[330]
8	TIP5; 1	Mitochondrial urea transport	[330] [331].

ciated with nitrogen remobilization [239] [330] [331]. TIP1; 3 and TIP5; 1 are expressed in the mature pollen. TIP1; 3 is tonoplast localized while TIP5; 1 is mitochondria localized. These proteins remobilize nitrogen from source to sink tissues. Proteolysis of proteins during senescence results in production of free amino acids and the level of asparagine and glutamine increases in the phloem sap [332]-[334]. Amino acid transporting AAP-family proteins load free amino acids into phloem. In legumes ureides are used for translocation of nitrogen in the form of urea to seeds [335]-[337].

11. Nitrogen Transport and Root Development

Plant root development is a complex process influenced by multiple genes/QTLs and the influence of nitrogen on root development is significant as it affects the nutrient and water absorption from the soil [338] [339]. The root surface area and the location of transporters in the roots determine nitrogen uptake. Nitrogen supply affects primary root growth, LR initiation and elongation. Nitrogen has a local stimulatory and systemic inhibitory effect on root development [340]-[344].

11.1. Local Stimulatory Effect

Presence of local concentration of nitrate has a stimulatory effect on initiation of lateral root primordial and growth of lateral roots [48] [74] [345] [346]. This localized stimulatory effect is mediated by ANR1, NRT1.1 and auxin. NRT1.1 which can sense external nitrate has been suggested to stimulate localized lateral root growth via an ANR1-mediated pathway. ANR1 (Arabidopsis Nitrate Regulated1) is a nitrate inducible transcription factor which mediates induction of LR elongation in response to local supply of NO_3^- and acts downstream of the NRT1.1 [47] [48] [88]. Auxin induces NRT1.1gene expression. Further NRT1.1 protein was found to transport auxin [74] [345] [347] [348]. Local high nitrate concentrations inhibit NRT1.1 mediated auxin transport resulting in accumulation of auxin in the tips of lateral roots. Accumulation of auxin stimulates emergence and growth of LR. When external nitrogen concentration is low, root to shoot transport of auxin is mediated by NRT1.1 resulting in inhibition of LR growth [74] [349].

Ammonium, unlike nitrate, promotes lateral root initiation than elongation and local supply of ammonium inhibits elongation of existing roots and initiate new lateral roots leading to branching of existing roots. Nitrate and ammonium play complementary roles in promoting initiation and elongation of lateral roots, respectively. The ammonium sensing tranceptor AMT1; 3 mediates this local stimulatory effect [48] [58] [345] [347] [350]. The ammonium induced formation of new lateral roots is repressed by glutamine/glutamate. In the presence of ammonium as sole N source plants developed thinner and longer roots, with high root density [351].

11.2. Systemic Inhibitory Effect

A uniform high soil nitrogen concentration inhibits root growth and development this is a reversible process. When the plants are transferred to normal nitrogen concentrations, this inhibitory effect can be relieved.

The high nitrate mediated systemic inhibitory effect on root development involves auxin, abscisic acid (ABA), ethylene and plant growth promoting bacteria. High nitrate condition leads to either suppression of auxin synthesis or prevention of shoot to root auxin movement. High shoot nitrate concentration suppresses lateral root development and involves root to shoot signaling [174] [345]. Auxin response factors and microRNAs are involved in this inhibition. Auxin induces expression of auxin receptor AFB3 gene and AFB3 has been found to regulate nitrate responsive primary and lateral root development [341]. The gene coding for miR393 is induced by nitrogen metabolites. The miR167/ARF8 regulatory module regulates the ratio between initiation and emergence of lateral roots [352]. Auxin response factor 8 (ARF8) is up regulated by high nitrate, while the microRNA that targets ARF8 was down regulated. The ARF8/miR167 module inhibits the repressing effect of high nitrate on lateral root elongation [352]. ABA plays an important role in high nitrate mediated lateral root growth inhibition [353]. Auxin and ethylene regulate lateral root development synergistically [354]. High nitrate stimulates ethylene production, which in turn regulates nitrate transporters (NRT1 and NRT2), resulting in alteration of root branching [355]. High nitrate mediated inhibition of lateral root deployment is also influenced by soil bacteria. Plant growth promoting bacteria protect plants from high nitrate mediated inhibition of lateral root growth by altering expression pattern of nitrate transporter genes. The exact mechanism behind this is not known [356].

Under excess ammonium conditions, primary and lateral root growth is inhibited by decreasing cell elongation. Plant hormones auxin and ethylene have no role in this inhibition [250] [357] [358]. A direct contact of the root tip with external ammonium is needed for perceiving the inhibitory effect of excess ammonium and is mediated by GMPase (GDP-mannose pyrophosphorylase). This inhibition is due to altered ammonium metabolism and defect in N-Glycosylation of proteins in the root [58] [357] [359] [360].

Along with the above dual effects, nitrogen also regulates the root growth by different ways. Under high carbon to nitrogen ratio, lateral root initiation is inhibited, while under external L-glutamate root branching is stimulated and primary root growth is inhibited. The suppression of LR initiation in response to high carbon to nitrogen ratio is due to prevention of shoot to root auxin translocation [83]. Due to high metabolic activities under high C/N conditions, there is a depletion of nitrate levels which is sensed by the nitrate transporter NRT2.1, leading to repression of LR initiation. NRT2.1 acts as the integrator of carbon and nitrogen metabolism and is regulated by both carbon and nitrogen signals [83] [154] [361]-[363]. Under N-limiting conditions, mutations in NRT2.1 reduced the number of lateral root primordia. Glutamine represses emergence of lateral root resulting in low level of third-order lateral root formation. In contrast, glutamate even at low concentrations inhibited growth of primary root but stimulated lateral root initiation and growth resulting in highly branched short roots [49] [83] [141] [246] [352].

Nitrogen limitation increases the auxin content of roots by promoting shoot to root auxin translocation thereby inducing initiation and elongation of lateral roots [343] [364]. Low nitrogen induces lateral root growth by stimulating the auxin biosynthesis gene TAR2 (tryptophan aminotransferase related 2), which is needed for enhanced auxin accumulation in LR primordia [365].

12. Regulation of Nitrogen Nutrition

Regulation of Nitrogen levels inside the plant is a necessity as limitation in nitrogen availability affects many developmental processes and excess nitrogen is detrimental to plants because of direct or indirect toxicity. Regulation occurs at uptake, assimilation, translocation, storage and remobilization steps (**Figure 6**). The N sources (NO_3^-, NH_4^+ and Urea), N-metabolites, N deficiency, hormones, diurnal changes, sugars, cations (sodium, potassium, and cadmium), biotic stress and abiotic stress regulate nitrogen nutrition.

Imbalance in nitrate levels may lead to generation of superoxide, reactive nitrogen species (nitric oxide (NO); peroxynitrite (ONOO-)) resulting in oxidative stress [366]-[368]. So plants have multiple mechanisms to ensure nitrogen homeostasis. The nitrogen homeostasis is the result of balance among the following process: nitrogen uptake mediated by transport proteins, nitrogen assimilation, and storage in vacuole, translocation to other parts of the plant from roots, nitrogen efflux and downward transport of nitrogen into roots. The balance is achieved by regulating each of these processes individually and coordinated regulation of these processes. Fragmentary knowledge is available on individual regulation of these processes in which many transcription factor proteins (**Table 9**), small RNAs and kinases are involved. For understanding the co-ordinated regulation of these processes more detailed research is necessary.

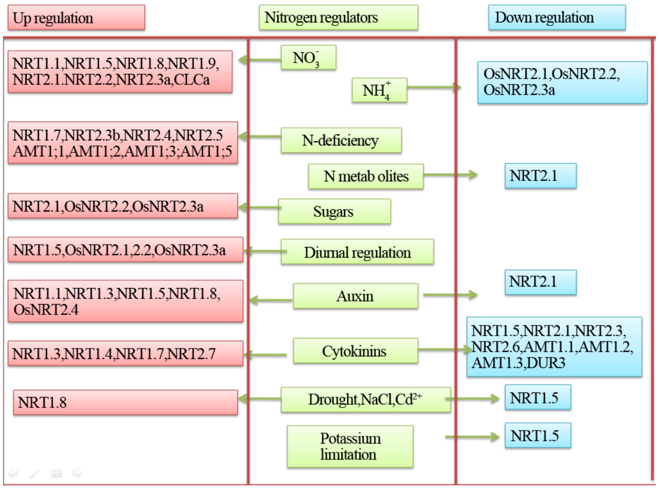

Figure 6. Regulation of nitrogen transporters. Nitrogen metabolism is regulated at many stages of plant development. The nitrogen transporting proteins are regulated by many factors like nitrate, ammonium, sugars, light, hormones, biotic and abiotic stresses, N-limitation and N metabolites.

Table 9. Transcription factors involved in regulation of nitrogen nutrition.

S. No	Transcription factor	Target gene	Reference
1	ANR1	MADS transcription factor	[87]
2	ARF8	miR167	[352]
3	AFB3	miR393	[45]
4	bZIP1	Glutamine synthetse, 1, 3 glutamine dehydrogenase	[455]
5	CCA1	Integratation of nitrogen nutrition and circadian regulation of gene expression	[402]
6	DOF1	Transcription factor and activator associated with carbon metabolism	[91]
7	GLB1	PII regulatory protein	[93]
8	HY	Nitrate reductase	[416]
9	LBD37/38/39	NRT1.1, NRT2.5	[86]
10	NLP7	Nitrate reductase, NRT1.1, NRT2,1	[85] [89]
11	NLA	the RING-type ubiquitin ligase	[443]
12	NF-YB1	NRT1.1; NRT2.1	[393]
13	SPL9	Nitrate assimilatory genes	[74]

12.1. Regulation of Nitrogen Uptake

The first layer of regulation of nitrogen uptake is at the level of different N forms which induce or repress expression of nitrogen uptake genes. Nitrogen uptake is controlled by many factors, such as nitrogen level, energy status of the plant, assimilation status of imported nitrogen, nitrogen demand, and involves mobile signals between shoots and roots as well as between different parts of the root system [369]. Nitrate induces expression of genes coding for nitrate transport associated proteins, NRT1.1, NRT2.1, NRT2.2, NAR2.4 and H^+-ATPases [178] [231] [232] [235] [288] [370] [371]. In rice, NAR2.1, NRT2.1, NRT2.2, and NRT2.3a genes are up regulated by nitrate and suppressed by ammonium. High temperature up regulates the expression of NRT2.2, NRT2.3b, and NRT2.4, but longer exposure to high temperature reduced the expression of NRT2.1, NRT2.2 and NRT2.3a [288] [372]. Both exogenous supply and internal increase of amino acids inhibit expression of nitrate transporters and nitrate uptake while decrease in level of root amino acids stimulated uptake of nitrogen [234] [373] [374].

Though NRT2.1 gene is nitrate inducible, very high nitrate concentrations inhibited the expression of NRT2.1 and this inhibition was found to be mediated by NRT1.1 [76] (Munos, 2004). Promoter histone methylation mediated by HIN9/AtIWS1 has been found to be associated with this repression. Expression of NRT2.1 gene is suppressed by reduced nitrogen metabolites and ammonium [375] [376]. The N & C-terminals of NRT2.1 contain many protein kinase recognition motifs. Like NRT1.1, NRT2.1 is regulated by a phosphorylation/dephosphorylation mechanism under high nitrogen conditions [377] [378] NRT2.1 gene expression is induced by light and sugars. Sugar and nitrate are coupled by glucose metabolism by oxidative pentose phosphate pathway (OPPP) [379]. Increased glucose levels and metabolites of OPPP pathway stimulate the expression of NRT2.1. The expression of different enzymes involved in OPPP pathway also directly regulates the expression of nitrogen transporters. Reduced expression of phosphogluconate dehydrogenase reduced glucose-mediated induction of NRT1.1, NRT1.5, NRT2.1 and AMT1; 3 [380] [381]. Antisense RNA mediated suppression of Hexokinase1 (HXK1) expression reduced NRT2.1 expression also. Sugar mediated nitrate uptake regulation involves glucose-6-phosphate and OPPP metabolites [382].

The basic factor determining the regulation of ammonium uptake is the plant nitrogen status. Expression of genes coding for ammonium transporters is up regulated by low tissue nitrogen levels, while high tissue nitrogen down regulates the expression of these genes [150] [383] [384]. The negative membrane potential of roots drives ammonium uptake leading to high cytosolic ammonium concentration. As ammonium can be stored in vacuole, it indirectly influences the ammonium uptake by regulating the level of ammonium in tissues [192] [385] [386]. In wheat the ammonium uptake by AMT1; 1 was found to be dependent on extracellular pH [387] [388].

Though ammonium represses most ammonium transporter genes, it has been found to up-regulate expression of AMT1; 1 of rice and AMT1; 2 of rice and tomato. In tomato AMT1; 2 gene expression has been found to be strongly up-regulated by nitrate [30] [218]. Genes coding for AMT1; 2 and AMT1; 3 transporters were found to be induced by sucrose and diurnal changes [382]. Nitrogen starvation derepressed nitrate, ammonium and urea uptake transporter genes in the roots [158] [168] [231] [361].

Nitrogen starvation conditions in roots induce expression of genes coding for urea transporters, DUR3, TIP4; 4 and PIP1; 5b [166] [241] [242]. Presence of nitrate represses expression of DUR3 gene, but induces the low affinity urea transporter PIP1; 5b [158] [313]. Genes coding for low affinity NIP2; 1 and NIP2; 4 urea transporters are expressed constitutively and not affected by nitrogen starvation and availability [241].

Post transcriptional regulation of proteins regulating expression of nitrate transporters is mediated by micro RNAs [389]. Micro RNA miR169 targets nuclear factor Y-A (NF-YA) subunits of the heterotrimeric NF-Ys transcription factors that directly regulate NRT1.1 and NRT2.1 expression [390] [391]-[393]. Nitrogen levels regulate expression of miR169 gene expression, nitrogen limitation down regulates expression of miR169 multigene family [394] [395]. Under nitrogen-sufficient conditions over expression of miR169a resulted in reduced expression of NRT1.1.

Nitrogen uptake is also regulated by hormones. Genes coding for uptake transporters, NRT1.1, NRT2.1, AMT1; 1, AMT1; 2 and AMT1; 3 and DUR3, were found to be repressed by cytokinin. The genes involved in cytokinin biosynthesis are induced by nitrate which is mediated by NRT1.1 [73] [75] [77] [396]. The cytokinin effect on nitrogen transporters is mediated by His–Asp phosphorelay system [397] [398].

Phosphorylation/dephosphorylation of proteins have been found to regulate nitrogen uptake. The NRT1.1 transporter is phosphorylated at Thr101 position by CIPK23 kinase when the nitrate concentration is low and the phosphorylated NRT1.1performs high affinity transport of nitrate. High concentration of nitrate prohibits phosphorylation and the dephosphorylated NRT1.1 performs low affinity transport of nitrate [73] [78] [79].

The ammonium channel made of trimeric form of AMT1; 1 protein is open and amenable for transport of NH_4^+ when AMT1; 1 protein is not phosphorylated. When the concentration of ammonium is high in the soil it leads to phosphorylation of the AMT proteins at Thr460 position of the conserved C-terminal region and change of conformation of these proteins leading to closure of the channels [57] [65]-[67]. High ammonium levels induce the oligomerization of AMT1; 3 and internalization of the oligomers leading to shutting off the ammonium channel which prevents ammonium toxicity [69] [70].

NLP7 protein is involved in NO_3^- signaling. This protein induces both nitrate uptake and assimilation genes. NLP7 induces the expression of NRT2.1 and NRT2.2 genes [85]. NRT1.1 and NRT2.1 nitrate transporter genes are induced by the protein kinase CIPK8 [87].

12.2. Regulation of Nitrogen Efflux

Only nitrate and ammonium efflux mechanisms are known in plants and there are no known efflux mechanisms for urea. NO_3^- efflux in barley has been found to be inducible requiring both RNA and protein synthesis. However, the turnover of the efflux system is much slower than that of the uptake system [399]. Xenopus oocyte experiments have shown that NRT1.5 can efflux nitrate and is regulated by the external pH and nitrate concentrations. Higher external nitrate concentrations inhibited the efflux [245]. NAXT1, the plasma membrane localized efflux protein is expressed in cortex of mature roots [246]. External acidity increases NAXT1 mediated nitrate efflux leading to depletion of internal nitrate levels Recently two other nitrate transporters NRT1.1 and NRT1.4 were proven to have nitrate efflux activity [400]. The efflux activity of NRT1.1 was found to be inhibited by CIPK23/CBL9 pair of kinases.

Like nitrate, excess ammonium is secreted out, to overcome ammonium toxicity and maintain the growth of the plants but the efflux mechanism involved in this is not known [247]. Ammonium can be effluxed in the form of NH_3 and it is an energy dependent process. Intracellular ammonium concentrations range from 1 - 10 millimoles [250]. Ammonium efflux is linked to high ammonium mediated inhibition of primary root development [359]. Under high nitrogen to carbon conditions some of the uptake transporters are down regulated and nitrogen effluxed to maintain homeostasis [246]. In plants the transport of NH_3 from leaves to outside contributes to overall ammonium efflux which is enhanced by high tissue ammonium concentrations [401].

12.3. Regulation of Nitrogen Translocation and Assimilation

Nitrate induces genes coding for assimilatory enzymes, nitrate reductase and nitrite reductase, while glutamine repress these enzymes [276]. NLP7 transcription factor is involved in induction of these genes [85]. Transcription factor, 14-3-3 regulates nitrate reductase and glutamine synthetase in a N-dependent manner. The circadian clock regulating CCA1 transcription factor coordinates the response of nitrate assimilatory genes [402].

There is a direct correlation between plant ammonium levels and expression of genes coding for assimilatory enzymes GS1.1 (shoots), GS1.2 (roots) GS2.1 (shoots and roots), Fd-GOGAT (shoots) NADH-GOGAT (roots) and GDH (shoots and roots). Ammonium assimilation genes are up regulated in the roots when there is an increase in ammonium levels due to over expression of AMT1; 1 [223]. Increased assimilation of nitrogen leads to increases in total amino acid content of the plant [403] [404].

There are two glutamine synthases, GS1 (cytosolic) and GS2 (plastidic), induced by low and high nitrate conditions, respectively. These enzymes are regulated by phosphorylation and dephosphorylation. Phosphorylated enzymes are active and protected from degradation while dephosphorylated forms are inactive and degraded when bound with 14-3-3 proteins. GS1 isoforms 1 and 2 have high and low affinities for ammonium, respectively. GS1-2 isoform is involved in nitrogen remobilization [405]-[408].

NRT1.5, the translocation and efflux protein, is involved in loading and unloading of nitrate in xylem. Gene coding for this protein is nitrate inducible but down regulated by abiotic stresses and mutation of this gene provided abiotic stress tolerance in *Arabidopsis* [245] [285]. The unloading of nitrate from xylem is mediated by the low-affinity transporter NRT1.8 and the expression of the gene coding for this protein is nitrate and cadmium stress inducible [286]. NRT1.9 is a phloem loader transporting nitrate downwards into roots. *Arabidopsis* plants with NRT1.9 mutation were found to transport more nitrate from root to shoot indicating a role for NRT1.9 in regulating nitrate levels in the shoot [292]. Cytokinin induces expression of nitrogen translocating

NRT1.3, NRT1.4 genes and auxin upregulates the expression ofNRT1.3, NRT1.5, NRT1.8 genes [124] [409]. Ammonium translocation proteins are induced by nitrogen starvation. AMT3; 1 is induced by N starvation while AMT3; 2 and AMMT3; 3 are ammonium inducible. The pollen tube localized TIP1; 3 and TIP5; 1 urea transporters are associated with nitrogen translocation in pollen tubes and are regulated by pH and nitrogen limitation [331].

12.4. Regulation of Vacuolar Nitrogen Transport

Different forms of nitrogen are stored in vacuoles with the twin aim of preventing toxicity and serving as a reserve so that it can be remobilized into cytoplasm when there is a limitation in nitrogen availability. Vacuolar nitrogen transporting CLCa and CLCb are diurnally regulated and up regulated by nitrate. Expression of vacuolar ammonium transporters TIP2; 1 and TIP2; 3 was found to be diurnally regulated in Arabidopsis roots. TIP1; 2 TIP2; 1 and TIP2; 3 proteins are ammonium inducible [309]. Ammonium supply increased the expression of these genes in a concentration dependent manner. Both nitrogen starvation and NH_4^+ supply induced expression of TIP2; 1 gene [193] [313] [386]. In Arabidopsis expression of TIP1; 2, TIP2; 1, and TIP4; 1 genes was upregulated by nitrogen depletion in roots. However, they are constitutively expressed in shoots [242]. Urea transporting TIP1; 2, TIP2; 1, and TIP4; 1 genes was up regulated by nitrogen depletion in roots. However, they are constitutively expressed in shoots [241].

12.5. Regulation of Nitrogen Remobilization

Expression level of NRT1.7 gene, in the phloem tissues of older leaf, is correlated with the nitrate levels in these leaves. This expression was found to be induced by nitrogen starvation NRT1.6 gene is also induced under nitrogen starvation [327] [328]. NRT1.11 and NRT1.12 are companion cell localized low-affinity nitrate transporters which transport nitrate into phloem of mature leaves for redistribution to young tissues. Expression of these genes is not affected by levels of nitrate and ammonium [329].

13. Genetic Engineering for Better Nitrogen Nutrition

Making the plant to utilize nitrogen efficiently is the primary goal of research on nitrogen metabolism which is aimed at reducing the fertilizer consumption and alleviating the environmental impact of excess nitrogen leached out. Nitrogen use efficiency is the "yield of grains per unit of available soil nitrogen" [410]. Though voluminous data has been generated in the past on nitrogen metabolism and nitrogen use efficiency, the genomic studies on nitrogen metabolism have shown that the nitrogen use efficiency is much more complex and more genes are involved than what was expected. Nitrogen use efficiency is influenced by the efficiency of the plant nitrogen uptake, efficiency of the plant in transporting nitrogen, efficiency of the plant in assimilating nitrogen, efficiency of the plant in storage (vacuolar) and efflux of nitrogen and efficiency of the plant in remobilization of nitrogen. Factors like soil nitrogen availability, photosynthetic carbon fixation, respiration and root architecture of the plant have a direct bearing of nitrogen use efficiency. Due to involvement of multiple processes in nitrogen use efficiency, it can be targeted at different levels depending on which process is limiting. Though nitrogen use efficiency is controlled by large number of genes, works carried out with individual pathways/genes have shown that targeting individual genes can alter nitrogen use efficiency of plants substantially (**Table 10**).

With respect to nitrogen uptake, it is not considered to be a limiting factor for improving nitrogen use efficiency because over expression studies with a number of nitrate uptake proteins did not alter the nitrogen use efficiency greatly. Over expression of *Nicotiana* NRT2.1 increased nitrate uptake but did not increase the overall NUE [411]. Over-expression of NRT1.1 improved growth of rice plant, but did not alter nitrogen uptake [288] [412]. Manipulation of nitrate assimilation genes also did not alter nitrogen use efficiency much. Though over expression of nitrate reductase and nitrite reductase enzymes increased the N uptake, no effect on growth and yield was seen [413]-[416]. Over expression of a hexose transporter STP13 resulted in increased expression of NRT2.2 leading to increased nitrate uptake and plant growth [417].

Though manipulation of nitrate uptake and assimilation genes did not have serious effect on NUE, engineering ammonium uptake genes and ammonium assimilation genes have significant effect on nitrogen use efficiency. Over expression of lotus AMT1; 1 and AMT1; 3 genes in Nicotiana increased ammonium uptake but decreased the growth of the plant [57]. Over-expression of AMT1; 1 gene in rice under nitrogen-limiting condi-

Table 10. Genetic engineering for nitrogen nutrition in plants.

S. No	Name of the protein	Organism	Mechanism	Reference
1	NRT1.1	Rice	Over expression	[288] [412]
2	NRT2.1	*Nicotiana*	Over expression	[411]
3	NRT2.2	*Arabidopsis*	Over expression	[417]
4	NRT2.3b	Rice	Over expression	[287]
5	NRT2.7	*Arabidopsis*	Over expression	[303]
6	AMT1.1	*Nicotiana, rice*	Over expression	[57] [217] [404]
7	AMT1.3	*Nicotiana*	Over expression	[57]
8	GS1 gene	*Arabidopsis*	Over expression	[410]
9	Alanine amino transferase	*Arabidopsis*	Over expression	[423]
10	ASN1	*Arabidopsis*	Over expression	[136] [282]

tions increased NH_4^+ uptake, biomass accumulation and grain yield [217]. AMT1; 1 gene over expression enhanced the root permeability for ammonium ions resulting in enhancement of plant growth and yield. It also enhanced overall plant growth, especially under low NH_4^+ levels, thereby increasing grain filling and total grain yield by >30% [404]. Over expression of GS1 gene resulted in higher grain yield (30% increase), better growth and biomass with improved N content at low N concentrations [410] [418]-[422]. Over expression of rice NADH-GOGAT1 enhanced the weight of spikelets by 80%. Engineering alanine amino transferase gene involved in assimilation increased the nitrogen use efficiency (40% reduction in fertilizer usage) resulting in enhanced yield and biomass. It also enhanced the nitrogen uptake and improved the root system [423]-[426]. Over expression of AS gene also increased the growth and total protein content under N limiting conditions.

The nitrogen entering the plant through the uptake route has to be utilized immediately or translocated to other parts of the plant to meet the needs of the physiological and developmental processes. Assimilated nitrogen is also transported as amino acids [427]. Nitrogen is translocated mostly as nitrate and ammonium is translocated at very low concentrations [54]. Very few studies are available on manipulating the genes associated with translocation of nitrogen. In rice, over expression of NRT2.3b has been found to increase the nitrogen uptake and plant yield.

Vacuolar nitrogen storage is used by the plant as a buffer to balance the nitrogen level, prevent toxicity and as a source for remobilization for seed formation. Over expression of vascular H^+-pyrophosphatase enhanced the enzyme activity and improved plant growth [428]. Over expression of Arabidopsis NRT2.7 resulted in enhanced seed nitrate content and improved seed germination.

The efficiency of nitrogen remobilization from vegetative tissues to reproductive tissues influences the seed development. Remobilization is a normal process happening at all stages of plant growth by which nitrogen in older leaves are mobilized to younger leaves. A huge quantity of nitrogen is stored in vegetative tissues in photosynthetic proteins (rubisco and PEFc) and GS. Rubisco alone accounts for close to 50% of store proteins in leaves [45] [95] [429]. Plants store substantial quantity of nitrate in their vacuoles and it is mobilized to seeds through phloem. Proteins associated with transport of nitrate through the tonoplast of plasma membranes to apoplast are yet to be identified.

Microarray and transcriptome studies on senescence have shown that there are many genes induced upon senescence some of which are associated with senescence induced remobilization. These genes include some isoforms of GS1, GDH, AS and proteases [427]. Over-expression of GS1 gene in lotus induced remobilization and early onset of senescence [430]. Nitrogen is remobilized as amino acids, glutamine, asparagine, glutamate and aspartame. Asparagine synthetase and aspartate aminotransferase are involved in conversion of glutamine to aspartame and glutamate to aspartate, respectively [228] [431]. Over expression ASN1 gene in Arabidopsis enhanced the soluble and total protein content of the plant, and facilitated better growth on N-limiting medium [136] [282].

14. Genomics of Nitrogen Metabolism

14.1. Plant Nitrome (Nitro (Gen)ome)

The first important contribution of genomics to nitrogen nutrition is that it revealed the number of genes associated with different aspects of nitrogen metabolism (**Table 11**). Analysis of complete genomes revealed that *Arabidopsis* and rice contain 66 and 84 nitrate transporter genes, respectively [164]. Among the 66 Arabidopsis genes, 53 code for NRT1 transporters, 7 genes code for NRT2 transporters and 6 genes code for CLC transporters. In rice 80 genes code for NRT1 transporters and NRT2 transporters are coded by 4 genes. Though 5 genes coding for CLC transporters have been found in rice, they are found to be incapable of nitrate transport due to absence of conserved proline residues [154] [288] [432].

Some of the NRT1 transporters are present in multiple copies. For example, three NRT1.1 genes are found in rice and sorghum, four in maize and *Brachypodium*. Single genes code for NRT1.2 transporter in Arabidopsis and grasses, three genes in cucumber and two genes in *V. vinifera* and Poplar. NRT1.3 is present in single copy in Arabidopsis, poplar and grass species but rice, maize and grape contains two copies of NRT1.3 in their genomes. NRT1.4 is present in two copies in rice maize, grape and cucumber genomes. NRT1.4 like gene is absent in poplar. Both NRT1.5 and NRT1.8 are coded by multiple genes in Brachypodium, cucumber, grape, maize, rice and sorghum NRT1.6 and NRT1.7 are coded by single genes in Brachypodium, poplar and rice. No NRT1.6 and NRT1.7 genes were found in Cucumber, grape, maize and sorghum genomes [305] [433] [434].

There are six, twelve and fourteen genes coding for AMT proteins in Arabidopsis, rice and *P. trichocarpa*, respectively [167] [169] [435]. Presence of multiple genes coding for multiple forms of ammonium transporting proteins facilitate functional specialization through regulatory flexibility [436]. *P. trichocarpa* consists of 6 AMT1 and 8 AMT2 proteins [171]. Rice DUR3 gene codes for a 721 residue long membrane-protein and showed similar expression and regulation patterns as that of *Arabidopsis* DUR3 gene [239] [240].

14.2. Genes Influenced by Nitrogen

The second contribution of genomics to nitrogen nutrition is that it revealed for the first time the number of genes

Table 11. Nitrome composition of plants.

S. No	Organism	Nitrate transporter	Ammonium transporter	Urea transporters
1	*Arabidopsis thaliana*	66	6	1
2	*Arabidopsis lyrata*	55	7	-
3	*Cucumber*	50	6	-
4	*Glycine max*	99	10	-
5	*Hordeumvulgare*	5		-
6	*Lotus japonicus*	41	5	-
7	*Maize*	54	8	1
8	*Nicotiana*	4		-
9	*Poplustrichocarpa*	76	14	-
10	*Prunuspersica*	51	7	-
11	*Rice*	84	12	1
12	*Ricinuscommunis*	45	7	-
13	*Sorghum bicolor*	71	8	-
14	*Soybean*	10	16	-
15	*Tomato*	4	4	-
16	*Vitisvinifera*	44	2	-
17	*Wheat*	19	4	1
18	*Medicagotruncatula*	84	7	-

influenced by nitrogen in crop plants. Plant transcriptome in response to nitrogen nutrition, level of nitrogen and tissue specific effects have been studied which identified large number of genes modulated by nitrogen (**Table 12**) [47] [283] [352] [437]-[439]. Microarray based gene expression profiling and RNA-seq analysis has been carried out in a). Nitrogen starvation conditions, b). Nitrogen limitation conditions c). In the presence of one or more nitrogen sources and d) different nitrogen and carbon combinations.

14.2.1. Nitrogen Starvation Conditions

Under nitrogen starvation conditions in Arabidopsis 509 genes were significantly differentially expressed. In roots up to 159 genes were significantly up regulated and up to 166 genes were significantly down-regulated. In shoots up to 104 genes were significantly up regulated whereas up to 80 genes were significantly down-regulated. These genes are associated with molecular transport, molecular metabolism, regulation and modification, organism development, stress stimuli and electron transport. In rice, 3518 genes are differentially expressed upon nitrogen starvation of which 462 genes showed differential expression in roots and shoots. In the same study a comparative study has been done between rice and Arabidopsis and shown that seventy three genes are expressed similarly in Arabidopsis and rice upon nitrogen starvation. Genes coding for miR399 and miR530 are differentially expressed upon nitrogen starvation [440]. RNA-Seq analysis of nitrogen-deficient cucumber plants showed that 196 genes associated with photosynthesis and cell wall metabolism are down regulated while 168 genes associated with antioxidant synthesis, flavonoid and phenylpropanoid biosynthesis are up regulated [441].

14.2.2. Nitrogen Limitation Conditions

Both microarray and recent RNA-Seq experiments have shown that under nitrogen-limiting conditions increased expression of high affinity nitrogen transporter genes were seen while under nitrogen-sufficient conditions expression of low affinity nitrogen transporters was increased [442].

Arabidopsis plants were grown in N-limiting (3 mM) and N-sufficient nitrate conditions (10 mM). In this study wild type plants and NLA mutant transcriptomes under different nitrate conditions were compared (nla mutants are plants with mutation in *NLA* gene which helps to cope with nitrogen limiting conditions). At early developmental stages wild type plants and NLA mutants under 3 mm and 10 mm nitrate conditions exhibited same transcriptome profiles but late developmental stages the expression profiles are different. Under nitrogen

Table 12. Transcriptome studies on nitrogen nutrition of in plants.

S. No	Name of the plant	Type of study	Nitrogen condition	Reference
1	*Arabidopsis*	Microarray	N-deficiency	[440]
2	Cucumber	RNA-Seq	N-deficiency	[441]
3	*Arabidopsis*	Microarray	N-deficiency and N-addition	[282]
4	*Arabidopsis*	Microarray	N-deficiency and N-resupply	[377]
5	*Arabidopsis*	Microarray	N-deficiency and N-resupply	[474]
6	Maize	Microarray	N-deficiency and N-resupply	[219]
7	*Arabidopsis*	Microarray	Nitrogen and Carbon	[469] [46]
8	*Arabidopsis*	Microarray	N-limitation	[443]
9	Maize	RNA-Seq	N-limitation	[447]
10	*Arabidopsis*	Microarray	N-addition	[136] [282] [283] [439] [445] [446]
11	*Arabidopsis*	Microarray	N-addition	[455]
12	*Arabidopsis*	Microarray	N-addition	[352]
13	*Arabidopsis*	Microarray	N-addition	[78]
14	*Arabidopsis*	Microarray	N-addition	[438]
15	*Arabidopsis*	RNA-Seq	N-addition	[449]
16	*Oryza sativa*	RNA-Seq	N-addition	[480]

limitation conditions in wild type plants the expression 629 genes is altered. Among 629 genes, 340 genes involved in protein degradation, ubiquitin pathway gens and the biosynthesis of anthocyanin and phenylpropanoids are up-regulated and 289 genes involved in photosynthesis, signal transduction, hormone response, aminoacid and nucleotide biosynthesis genes and transcriptional regulation are down-regulated [443] (Under N-limiting conditions, NLA mutants altered the expression of 1744 genes, 1122 genes were up-regulated and 622 repressed. Nitrogen limitation conditions up regulates the expression of CLC-c protein while down regulates nitrate transporting CLC-a proteins in wild type plants [443]. Rice plants were transferred from high nitrogen (1.44 mM NH_4NO_3) to low nitrogen (0.24 mM NH_4NO_3) conditions to check the effect of nitrate on gene expression [444]. 471 genes showed differential expression upon nitrogen stress in the rice at seedling stage out of which 115 genes are up regulated and 358 genes are down regulated. Among down regulated genes are those associated with photosynthesis and energy metabolism while genes coding for some transcription factors, early stress response and signal transduction are up regulated. Expression of genes associated nitrogen uptake and assimilation is not altered [444].

14.2.3. Nitrogen Addition Conditions

In Arabidopsis transcriptome study with 1 mM nitrate has induced 550 genes within 20 minutes. Nitrate addition induces genes associated with ribosomal proteins and oxidative pentose phosphate pathway genes and many other metabolic process associated genes. Ribosomal proteins and oxidative pentose phosphate pathway genes, regulate both nitrogen transport (NRT1.1, NRT1.5, NRT2.1, NRT2.4 and AMT1; 1) and assimilation. Among 550 genes, there are 146 genes which are constitutively induced upon nitrate addition, 120 genes are repressed by nitrate treatment and rest all genes are induced at different time courses. The early nitrate response on gene expression is mainly because of nitrate itself, but the delayed or prolonged nitrate response on the gene expression is due to the interaction between nitrate signalling and hormone signalling pathways [381]. In another study of Arabidopsis, plants were grown under both low (0.25 Mm) and high nitrate (5 Mm) condition and their transcriptome profiles were compared. At low nitrate concentration the expression of nitrogen transport, assimilation and metabolism genes were transiently increased compared to high nitrate conditions. In tomato resupply of nitrate to the nitrate starved plants increased the expression of 97 genes [445] [446]. In Arabidopsis nitrate addition to the nitrate starved plants significantly alters the expression of many genes involved in several metabolic processes within 30 min [282].

RNA-Seq analysis of nitrogen deprived/treated plants have identified additional genes associated with nitrogen metabolism [441] [447]-[450]. Arabidopsis root transcriptome sequencing has revealed 40 novel nitrate responsive genes and a unique nitrate responsive microRNA5640/PPC3 module which regulates carbon and nitrogen balance and nitrate assimilation [449]. Hybrid vigour in terms of increased nitrogen transport and content under nitrogen limiting conditions in maize when compared to the parental strains is attributed to differential expression of 643 and 725 genes in leaves and roots, respectively [447].

The number and type of genes influenced by nitrogen and their expression levels depend on the genotype of the plant. RNA-Seq analysis of two parental lines and the hybrid line in maize showed that the response of the plant to nitrogen starvation is genotype dependent. For example under nitrogen limiting conditions there was reduction in carbohydrate metabolic process due to reduction in expression of photosynthesis associated genes in SRG100 parental line but in the hybrid (SRG150) and the second parental line (SRG200), photosynthetic genes are not significantly down-regulated and they maintained significantly higher sugar content. Similarly, increased nitrogen transport under nitrogen limiting conditions was seen in both hybrid and SRG200 parental line which was not there in the other parental line [447]. Comparative RNA-Seq analysis of low nitrogen-tolerant and nitrogen-sensitive genotypes of Sorghum showed that they respond differently to N-limitation. Tolerant genotypes showed enhanced expression of genes coding for high affinity nitrate transporters and nitrate assimilation genes to enhance the plant nitrogen levels. While the sensitive genotypes showed enhanced expression of genes associated with flavonoid biosynthesis, cell wall metabolism, protein kinases and different transcription factors associated with auxin metabolism which is a sign of adaptation to low nitrogen levels.

14.3. Link between Nitrogen and Carbon Metabolism

The carbon and nitrogen metabolisms are complex and regulated by nitrate, ammonium, amino acids and signals from C metabolism. Nitrogen availability influences plant photosynthesis rate and yield [451] [452]. The integration of carbon metabolism with nitrogen mainly occurs at nitrogen assimilation and regulation of nitrogen metabolism. The nitrogen assimilation produces glutamine which is the primary source of nitrogen and carbon for amino acids [255]. C/N balance is regulated by senescence, which is dependent on nitrogen and carbon interactions [453] [454]. Maintaining an adequate photosynthetic rate under nitrogen starvation is necessary for keeping reduced C levels which favours efficient use of nitrogen [447].

Proteins which can sense intracellular carbon and nitrogen levels are associated with maintenance and regulation of the integration of carbon and nitrogen metabolism [455]. PII protein and glutamate receptors are involved in nitrogen sensing. NRT2.1, TOR pathway, SNF1/AMP-dependent kinase, the ubiquitin ligases ATL31 and ATL6 and trehalose-6-phosphateare specific to internal carbon status sensors [456]-[459]. Transcription factors like Dof1, methyl transferase OSU1, GABA (γ-amino butyric acid), CCA1 and bZIP1 and plant hormones cytokinin and abscisic acid also play roles in signaling carbon and nitrogen status [402] [460]-[464]. GNC and CGA1 protein genes are inducible by nitrate and cytokinin and are also involved in chloroplast biogenesis [465]-[467]. Cytokinin mediates the enhanced photosynthesis by stimulation chloroplast biogenesis through GNC and CGA1 in a nitrate induced manner. The C/N balance influences plant growth in many ways, regulating lateral root growth, seed germination and cell wall biogenesis. NRT2.1 acts a sensor of high carbon to nitrogen response during formation of lateral roots. Methyltransferase (oversensitive to sugar (OSU1) negatively regulates both high and low carbon to nitrogen responses [468].

Genome wide expression studies under different C/N condition have shown that, carbon sources such as sugars regulate the expression of nitrate, ammonium and urea transporters and also enzymes involved in nitrogen assimilation [361]. Even nitrogen sources (such as nitrate) have been shown to affect the expression of carbon metabolism genes [439]. Nitrogen modulates the expression of 8% of the glucose regulated genes. Some genes are up regulated by nitrogen and some other genes are down regulated by nitrogen. Carbon and nitrogen alone can repress or induce specific genes but when they are together they cannot do the same [455] [469]. Carbon/nitrogen balance is maintained by genes associated with hormone pathways, metabolism, signal transduction and protein synthesis and degradation.

14.4. Link between Senescence and Nitrogen Availability

Many earlier studies have shown that there is a direct connection between nitrogen depletion and early leaf senescence and the reversal of senescence upon resupply of nitrogen [470] [471]. But the actual mechanism behind this was not known until detailed transcriptome analysis was carried out, which revealed the participation of large number of genes [472] [473]. Senescence down regulates photosynthetic genes and up regulates genes associated with degradation and recycling of metabolic resources and regulatory genes [474] [475].

14.5. Evolution of Nitrogen Metabolism Genes

Genome wide analysis for nitrogen transporters have revealed that the high affinity transport is mediated by fewer numbers of transporters and the genes coding for them are present in fewer copies when compared to low affinity nitrogen transporters. For example in rice and *Arabidopsis* there are only four and seven genes coding for NRT2 proteins (high affinity) while NRT1 proteins (low affinity) are coded by 53 and 80 genes in *Arabidopsis* and rice respectively. Compared to nitrate transporters, the ammonium transporters are coded by fewer genes. In Arabidopsis and rice there are only six and twelve ammonium transporters respectively when compared to 66 and 84 nitrate transporter genes in Arabidopsis and rice respectively. But there is no clear trend with respect to genes coding for low and high affinity ammonium transporters. In Arabidopsis there are five high affinity AMT1 transport genes and in rice three genes code for AMT1 protein. In rice the low affinity AMT2 is coded by nine genes while in Arabidopsis AMT2 is coded by a single gene. With respect to urea transporters, the high affinity DUR3 is coded by a single gene in rice, what, maize and Arabidopsis, while low affinity transporter coding genes are many.

Gene(s) coding for different nitrate transporters show different levels of functional specialization in different hosts. Some of them are coded by single genes in some plants and multiple genes in some other plants. Multiple genes coding for nitrate transporters allow a greater regulatory flexibility and tissue specificity. In some plants additional regulatory flexibility is enabled by the presence of multiple transporter proteins performing similar function. The multifunctional nitrate transporter NRT1.1 is coded by multiple genes in rice (3), Sorghum (3), maize (4) and Brachypodium (4) but the expression pattern of individual copies is not known [305]. Though rice

NRT1.1A and NRT1.1B genes are expressed throughout the plant, OsNRT1.1A is predominantly expressed in the root. Though NRT1.2 is coded by single genes in Arabidopsis and grasses, it is coded by multiple genes in cucumber (3), grape (2) and poplar (2). In poplar the two copies, NRT1.2A and NRT1.2B show functional specialization and express specifically in leaves and roots, respectively. In Cucumber though all three copies are expressed only in the shoots (cotyledons and old leaves), NRT1.2B is additionally found to express specifically in young petioles [305].

NRT1.3 is coded by single genes in Arabidopsis and many grass species and two genes in rice, maize, poplar and grape. But the expression pattern of multiple genes is not known. NRT1.4 gene is present in two copies in rice maize, grape and cucumber genomes. No NRT1.4 homolog could be found in poplar. In cucumber NRT1.4A is specifically expressed only in young and old petioles, while NRT1.4B is expressed in cotyledons and leaves [305] [434]. NRT1.5 is coded by multiple genes in poplar (3), rice (2), maize (2), sorghum (2), Brachypodium (2), grape (2) and cucumber (3) (Both in rice and cucumber NRT1.5A gene is expressed all over the plant while NRT1.5B in rice is expressed in panicle. In cucumber both NRT1.5B and NRT1.5C genes are expressed only in the reproductive tissues. The functional specialization of multiple copies of NRT1.8 genes in cucumber (3) and poplar (2) is not known [434].

In addition to nitrate remobilization specific transporters, NRT1.6 and NRT1.7 were found to be involved in-nitrate remobilization and seed development in some plants (Arabidopsis, Brachypodium, poplar and rice) but absent in others (maize, sorghum, grape and cucumber) [305] [433]. Absence of these transporters in some plants indicates that they represent species specific functional specialization. *Arabidopsis thaliana* contains additional nitrate transporters NAXT1 (nitrate efflux), NRT1.9 (downward transport of nitrate through phloem), NRT1.10 (seed growth), NRT1.11 and NRT1.12 (nitrate remobilization from leaves). Cucumber also contains NRT1.9 and NRT1.10 genes. Poplar contains 4 genes coding for NRT1.9 transporter [433]. As there is no information regarding the presence of these transporters in other plants, probably these transporters represent Arabidopsis specific functional specialization.

Genome-wide analysis of NRT2 genes in monocots and dicots have revealed that NRT2 genes of dicots are intronic while the grass NRT genes are devoid of introns indicating that NRT2 genes must have developed after divergence of monocots and dicots [234]. NRT2.1 and NRT2.2 proteins are coded by single genes in many plants. No homologs to these genes could be found in poplar genome [432]. In rice NRT2.3 is coded by 2 genes, OsNRT2.3a and OsNRT2.3b are single exonic while in Arabidopsis a single copy of NRT2.3 is present and contains three exons. Though NRT2.3a and NRT2.3b genes of rice shared the same coding sequences, their untranslated regions (UTRs) are different. NRT2.3 coding genes have shown rice specific functional specialization, NRT2.3a gene showed root specific expression while NRT2.3b showed shoot specific expression [287] [288]. NRT2.3 showed nitrate inducible expression in shoots while in roots it is constitutive [232].

NRT2.4 high affinity nitrate uptake and remobilization transporter is coded by single genes in Arabidopsis and rice. In Poplar NRT2.4 is coded by 2 genes. Poplar NRT2.4A is expressed in roots, leaves and wood, while the expression pattern of NRT2.4B is not known [433]. Grass NRT2 transporters are intron less but rice NRT2.4 has an intron and similar to Arabidopsis NRT2.7 gene [154] [432]. NRT2.4 and NRT2.3b proteins are functional without NAR2 [288].

In Arabidopsis, Maize and Barley NRT2.5 is coded by a single gene while in poplar NRT2.5 is coded by 3 genes. Poplar NRT2.5A is expressed in the bark, NRT2.5B is expressed in leaves and the expression of NRT2.5C is not known [433] [476]. Nitrogen starvation induced the expression of NRT2.5 gene while supply of nitrogen (ammonium/nitrate) repressed the gene [232]. NRT2.5 is involved in plant growth promotion under the rhizospheric bacterium STM196 [477]. NRT2.6 is constitutive high affinity transporter involved in pathogen resistance in plants by producing reactive oxygen species and is coded by a single gene in Arabidopsis and barley [478]. NRT2.7 like transporters is specific to dicots and not found in grass species. NRT2.7 is coded by a single gene in Arabidopsis and poplar and is involved in vacuolar nitrate accumulation in the seeds and also oxidation of proanthocyanindin in seeds [303] [479]. In poplar NRT2.7 is expressed in the wood and leaves and maintains nitrate homeostasis in the leaves [433].

15. Biotechnological Options for Improving Nitrogen Nutrition in Crop Plants

Extensive research data is available on the genes/proteins associated with nitrogen nutrition of crop plants and their regulation and these data can be used for generating inter plant species/crop variations which have little practical significance for biotechnological interventions as differences are known to exist between plant species which are totally different genetic entities. What is needed is generations of high throughput data using modern genomic approaches which address inter cultivar variations on nitrogen use efficiency. Research has just entered this imported arena and by next few years we should have enough data on inter cultivar variations at genomic/proteomic levels. Availability of the genomes of cultivars and cultivar specific transcriptome and microRNA profiling data will enables us to understand the variations/lack of variations that facilitate better nitrogen utilization by specific high yileding crop cultivars. If such data are available we can employ biotechnological options like genetic engineering/gene silencing etc to improve the nitrogen utilization efficiency of crop plants. Another area of interest for biotechnological options would be to delink the root growth from nitrogen concentration in soil. Root growth has been found to be influenced by the nitrogen content of soil, inhibition/induction of root growth by high soil nitrogen content is a problem for crop plants which are grown in wide range of nitrogen concentrations. If higher nitrogen content inhibits lateral root growth it will have serious implications on mild drought conditions as the roots are shallow and cannot harvest waters from deep layers. More data is needed for understanding the link between soil nitrogen content and root growth which will facilitate delinking the two processes.

16. Conclusions

Having achieved substantial production increase in crop plants by applying nitrogenous fertilizers, it is essential for assessing the fertilizer use and the ability of the plant to utilize the applied fertilizer. Though more than 57.5 million tons of nitrogen fertilizer is applied each year for cereals alone, only 33 percent of it is utilized by the plant and the rest is lost. A deep understanding of the nitrogen nutrition is necessary for overcoming this loss.

Recent progress in genomics and genetic engineering studies of crop and other plants have revealed the composition of nitrome and the genes influenced by presence or absence of different forms of nitrogen.

Sensing the levels of nitrogen, external and internal, influences nitrogen nutrition greatly as expression/action of many genes involved in nitrogen nutrition is influenced by the level of nitrogen. Though the mechanism of sensing by the dual affinity transporter NRT1.1 is understood, the mechanism of sensing by NRT2.1 is not known. External ammonium sensing by AMT1; 1 and AMT1; 3 are understood but very little information is available with respect to sensing external urea levels. Despite the clear understanding of mechanisms of internal nitrogen sensing in bacteria and fungi and existence of homologues of these proteins in plants, their mechanism of sensing is not understood in plants.

Though plants face severe problems when nitrogen level is low, to overcome this plants have not developed more number of low affinity transporters when compared to high affinity nitrate transporters, indicating that the high affinity transporters are very efficient. The high number of low affinity transporters may not be due to selection of crop plants to suit high nitrogen conditions as non crop plants also have more number of low affinity transporters. This shows that nitrogen uptake may not be the limiting factor and the processes downstream to uptake need to be looked into for addressing efficiency in nitrogen nutrition.

Plants do possess mechanisms which favour efflux of nitrogen outside the plant for which evidences are there for nitrate and ammonium but so far no proof exists for efflux of urea. The mechanism of nitrate efflux by NAXT1 is understood, the role played by bidirectional transporters NRT1.1, NRT1.4 and NRT1.5 in efflux is not clear. Though ammonium efflux is known, the proteins involved and their mechanism of action are yet to be understood. Though the mechanisms of translocation and assimilation of different forms of nitrogen is understood, the regulation of these processes under differing nitrogen levels is yet to be understood. Over expression of nitrogen assimilation genes (GS, NADH-GOGAT, ASN and alanine amino transferase) increased the nitrogen use efficiency. This shows that probably the nitrogen assimilation is the limiting factor for understanding the nitrogen nutrition. Storage of different forms of nitrogen in vacuole might be playing an important role in maintaining nitrogen status in different tissues. The transporters associated with this have been studied in detail but the existence of variation in levels of accumulation of different forms nitrogen in different crop varieties and their implication on overall nitrogen use efficiency needs detailed examination.

Recent genomic studies have established the correlation between remobilization and senescence. Integrated regulation of these two processes merit detailed investigation. Correlation between soil nitrogen status and root growth is well understood. Further understanding of this process may facilitate delinking root growth from soil nitrogen level which is desirable for developing crop varieties to suit different soil nitrogen conditions. The influence of carbon level in relation to nitrogen level on nitrogen nutrition is revealed by recent transcriptome studies, understanding the regulation of which requires further investigation.

Overall, we have understood the machinery (nitrome) associated with nitrogen nutrition and the mechanism of functioning. But the major lacunae is the poor understanding of regulation of nitrogen nutrition in which many transcription factors and small RNAs are likely to be involved. Comparative genomics and transcriptomics studies in crop plants of differing genetic background are essential for this purpose.

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Abbreviations

ALMT1: aluminium activated malate transporter-1;
AS: asparagine synthase;
CLC: chloride channel proteins;
Dof1: DNA binding with one finger;
GACC: General amino acid control;
GS/GOGAT: Glutamine synthetase/glutamate synthase;
HATS: High affinity transport system;
LATS: Low affinity transport system;
LBD: Lateral organ boundary domain;
MFS: Major facilitator super family;
NLP: NIN like protein;
NNP: Nitrate-nitrite porter;
NPF: Nitrate protein family;
NUE: Nitrogen use efficiency;
SLAC1: Slow anion channel-like;
SLAH: Slow anion channel-like homologue;
SPL9: Squamosa promoter binding protein.