Nitrogen is quantitatively the most essential nutrient for plants and a major limiting factor in plant productivity. The major form of inorganic nitrogen that is available for the growth of rice plants in paddy soil is the ammonium ion (NH4+). The ammonia (NH3) gas molecule is a weak base that protonates rapidly to form the NH4+ ion with a dissociation constant of 10−9.25 ([Kleiner, 1981](javascript:;)). According to the equation proposed by [Freney *et al.* (1985)](javascript:;), 99.4% of the total ammonia in water is in the protonated form at pH 7.0 and 25 °C, and the NH4+ ion is the major molecular species in paddy soil, as well as in most of the compartments of rice plants. On the other hand, excess NH4+ can apparently be toxic to some plants ([Kronzucker *et al.*, 2001](javascript:;)). Thus, efficient NH4+ transport and subsequent assimilation systems should be highly regulated within the roots. The major forms of nitrogen in the xylem sap of rice plants are Gln and Asn ([Fukumorita and Chino, 1982](javascript:;)). Real-time monitoring of NH4+ transport by the positron emitting tracer imaging system (PETIS) showed that the signals of 13N taken up by rice roots were detected in the basal part of shoots within a short period, but the translocation was completely inhibited by methionine sulphoximine, an inhibitor of glutamine synthetase (GS) ([Kiyomiya *et al.*, 2001](javascript:;)). These results strongly suggest that most of the NH4+ taken up by the roots can be assimilated within the organ by the reaction catalysed by GS. Since the discovery of the GS/glutamate synthetase (GOGAT) cycle by [Lea and Miflin (1974)](javascript:;), it is now well established that this cycle is the only route for the primary assimilation of NH4+ in plants grown under normal conditions ([Ireland and Lea, 1999](javascript:;); [Lea and Miflin, 2003](javascript:;)).

In the top part of japonica rice, approximately 80% of the total nitrogen in the panicle arises from remobilization through the phloem from senescing organs ([Mae and Ohira, 1981](javascript:;)). The major forms of nitrogen in the phloem sap are again Gln and Asn ([Hayashi and Chino, 1990](javascript:;)). The synthesis of Gln in senescing organs is the essential step for this dynamic nitrogen recycling, as Asn is synthesized by the transfer of an amide group from Gln ([Lea *et al.*, 2007](javascript:;)). In developing sink organs, the remobilized Gln is reutilized for many biosynthetic reactions, via the GS/GOGAT pathway ([Lea and Miflin, 2003](javascript:;)), which is mostly responsible for the metabolism of Gln in rice ([Tobin and Yamaya, 2001](javascript:;)).

As in other plants ([Ireland and Lea, 1999](javascript:;); [Yamaya and Oaks, 2004](javascript:;)), a small gene family has been identified that encodes GS1 ([Ishiyama *et al.*, 2004*b*](javascript:;); [Tabuchi *et al.*, 2005](javascript:;)) and NADH-GOGAT (M Tabuchi *et al.*, unpublished results) in rice, in addition to the major species of GS2 and ferredoxin (Fd)-GOGAT in the chloroplasts of green tissues of this plant. These are *OsGS1;1*, *OsGS1;2*, and *OsGS1;3*, and *OsNADH-GOGAT1* and *OsNADH-GOGAT2*. It has been shown that the expression profile of each gene was different in terms of age and tissue specificity, and response to NH4+, suggesting that each gene product apparently has a distinct function in rice. As discussed in earlier reviews, the major function of GS2 and Fd-GOGAT in chloroplasts is in photorespiratory nitrogen metabolism ([Lea and Miflin, 2003](javascript:;)), and other GS/GOGAT species are important for normal growth and development ([Yamaya *et al.*, 2002](javascript:;); [Yamaya and Oaks, 2004](javascript:;)). This is because mutants lacking either GS2 or Fd-GOGAT ([Wallsgrove *et al.*, 1987](javascript:;); [Kendall *et al.*, 1986](javascript:;); [Leegood *et al.*, 1995](javascript:;)) were able to grow normally under non-photorespiratory conditions.

In this review, the functions of GS1 and NADH-GOGAT isoenzymes in rice are discussed. Because some of these genes are up- or down-regulated by exogenous NH4+ supply, possible mechanisms of the regulation are also discussed.

**GS1;2 and NADH-GOGAT1 could be key players in the assimilation of NH**4+**taken up by rice roots**

Rice is able to take up NH4+ ions when grown in a paddy field, through the action of ammonium transporters (AMTs). [Ninnemann *et al.* (1994)](javascript:;) first identified a gene encoding a high-affinity AMT from *Arabidopsis thaliana* (*AtAMT1;1*) using functional complementation of a yeast mutant defective in NH4+ uptake. Since then, the isolation of AMT1 homologues from *Arabidopsis* ([Gazzarrini *et al.*, 1999](javascript:;)), tomato ([von Wirén *et al.*, 2000](javascript:;)), and rice ([Sonoda *et al.*, 2003*a*](javascript:;)) has shown that the *AMT1* family in plants consists of at least three to five members. In *Arabidopsis* ([Sohlenkamp *et al.*, 2000](javascript:;)) and rice ([Suenaga *et al.*, 2003](javascript:;)), several genes with high homology to bacterial AMT and yeast AMTs (methylammonium permease: MEP) were also identified ([Lea and Azevedo, 2006](javascript:;)). Expression of *OsAMT* genes in rice showed distinct profiles, i.e. root-specific and NH4+-inducible expression for *OsAMT1;2*, constitutive expression in roots and shoots for *OsAMT1;1* and *OsAMT2;1*, and root-specific and NH4+-derepressed expression for *OsAMT1;3*. The up-regulation of *OsAMT1;2* gene expression occurred as rapidly as 30 min following the supply of NH4+ ions, when NH4+-responsive expression was observed in specific cell types in the root tips, for example exodermis, sclerenchyma, endodermis, and pericycle ([Sonoda *et al.*, 2003*a*](javascript:;)). There are two Casparian strips in rice roots, (i) between the exodermis and sclerenchyma and (ii) in the endodermis, requiring a symplastic system for solute transport in these regions ([Morita *et al.*, 1996](javascript:;)). The AMT1;2 protein could be responsible for cell-to-cell transport of NH4+ ions. Pharmacological studies suggested that the up-regulation of *OsAMT1;2* gene expression was caused by Gln rather than NH4+ ions ([Sonoda *et al.*, 2003*b*](javascript:;)), as in the case of up-regulation of *OsNADH-GOGAT1* in rice roots ([Hirose *et al.*, 1997](javascript:;)). It seems likely that OsAMT1;2 mainly functions in NH4+ uptake from NH4+-enriched soils. On the other hand, OsAMT1;3, which is down-regulated by NH4+, may be present to support OsAMT1;1 in taking up NH4+ ions, when the availability of NH4+ in the soil is low.

Among the three GS1 genes in rice, *OsGS1;1* was expressed in all organs, i.e. root, leaf blade, leaf sheath, and spikelet, with higher expression in the leaf blade during the vegetative stage of growth of the plants ([Tabuchi *et al.*, 2005](javascript:;)). *OsGS1;2* transcripts were also detected in all organs, with higher expression in the root following the supply of NH4+ at the seedling stage, while *OsGS1;3* was specifically expressed in the spikelet ([Fig. 1](javascript:;)). The *OsGS1;1* and *OsGS1;2* transcripts showed reciprocal responses to NH4+ supply in the surface cell layers of roots ([Ishiyama *et al.*, 2004*a*](javascript:;)). Transcripts of *OsGS1;1* accumulated in the dermatogen, epidermis, and exodermis under NH4+-limited condition. In contrast, *OsGS1;2* was abundantly expressed in the same cell layers under NH4+-sufficient conditions, replenishing the loss of *OsGS1;1*, 3–6 h after the supply of NH4+ ions. Within the central cylinder of the root elongation zone, both genes were up-regulated by NH4+. The kinetic properties of OsGS1;1 and OsGS1;2 that had been purified from the recombinant proteins overexpressed *Escherichia coli* showed that both enzymes could be classified as high-affinity subtypes for NH4+ ions ([Ishiyama *et al.*, 2004*a*](javascript:;)) with relatively high *V*max values, as compared with the major high-affinity isoenzyme in *Arabidopsis* ([Ishiyama *et al.*, 2004*b*](javascript:;)). Low-affinity forms of GS1 seen in *Arabidopsis* were absent in rice roots ([Table 1](javascript:;)).

|  |  | | |  | | |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

**Table 1.**

Kinetic properties of the GS1 isoenzymes in *Arabidopsis* and rice

| **Isoenzyme** | ***K***m | | | ***V***max**(nkat mg**−1**protein)** | | |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Glu (mM)** | **NH**4+**(μM)** | **ATP (μM)** | **Glu** | **NH**4+ | **ATP)** |
| OsGS1;1 | 1.9±0.0 | 27±5 | 450±20 | 190.0±12.3 | 186.3±18.4 | 170.2±15.1 |
| OsGS1;2 | 2.1±0.0 | 73±2 | 530±80 | 97.4±9.0 | 98.1±1.7 | 109.1±14.8 |
| AtGS1;1 | 1.1±0.4 | <10 | 300±20 | 29.3±1.6 | 27.4±0.7 | 21.4±0.4 |
| AtGS1;2 | 3.8±0.2 | 2450±150 | 1100±140 | 65.7±0.2 | 65.7±1.1 | 66.6±4.4 |
| AtGS1;3 | 3.9±0.1 | 1210±40 | 850±30 | 162±24 | 93.9±10 | 100.0±0.04 |
| AtGS1;4 | 0.6±0.1 | 48±6 | 400±50 | 79.2±1 | 65.7±1.5 | 73.9±4.2 |

Results for rice (OsGS1;1 and 1;2) and *Arabidopsis thaliana* (AtGS1;1 to 1;4) were adapted from [Ishiyama *et al.* (2004*a*)](javascript:;) and [Ishiyama *et al.* (2004*b*)](javascript:;), respectively. Purified recombinant enzymes were used for the assay. Kinetic values for the GS synthetic activities were determined by Eadie–Hofstee equations. One katal of enzyme activity is defined as 1 mol of Gln synthesized per second at 30 °C. Data are means ±SE (*n*=3).