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). According to the equation proposed by Freney *et al.* (1985), 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). 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). 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). 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), 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; Lea and Miflin, 2003).

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). The major forms of nitrogen in the phloem sap are again Gln and Asn (Hayashi and Chino, 1990). 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). In developing sink organs, the remobilized Gln is reutilized for many biosynthetic reactions, via the GS/GOGAT pathway (Lea and Miflin, 2003), which is mostly responsible for the metabolism of Gln in rice (Tobin and Yamaya, 2001).

As in other plants (Ireland and Lea, 1999; Yamaya and Oaks, 2004), a small gene family has been identified that encodes GS1 (Ishiyama *et al.*, 2004*b*; Tabuchi *et al.*, 2005) 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), and other GS/GOGAT species are important for normal growth and development (Yamaya *et al.*, 2002; Yamaya and Oaks, 2004). This is because mutants lacking either GS2 or Fd-GOGAT (Wallsgrove *et al.*, 1987; Kendall *et al.*, 1986; Leegood *et al.*, 1995) 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) 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), tomato (von Wirén *et al.*, 2000), and rice (Sonoda *et al.*, 2003*a*) has shown that the *AMT1* family in plants consists of at least three to five members. In *Arabidopsis* (Sohlenkamp *et al.*, 2000) and rice (Suenaga *et al.*, 2003), several genes with high homology to bacterial AMT and yeast AMTs (methylammonium permease: MEP) were also identified (Lea and Azevedo, 2006). 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*). 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). 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*), as in the case of up-regulation of *OsNADH-GOGAT1* in rice roots (Hirose *et al.*, 1997). 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). *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). 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*). 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*) with relatively high *V*max values, as compared with the major high-affinity isoenzyme in *Arabidopsis* (Ishiyama *et al.*, 2004*b*). Low-affinity forms of GS1 seen in *Arabidopsis* were absent in rice roots (Table 1).

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

**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*) and Ishiyama *et al.* (2004*b*), 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).