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REVIEW

Evidence supporting distinct functions of three cytosolic glutamine synthetases and two NADH-glutamate synthases in rice

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Abstract

The functions of the three isoenzymes of cytosolic glutamine synthetase (GS1;1, GS1;2, and GS1;3) and two NADH-glutamate synthases (NADH-GOGAT1 and NADH-GOGAT2) in rice (*Oryza sativa* L.) were characterized using a reverse genetics approach and spatial expression of the corresponding genes. *OsGS1;2* and *OsNADH-GOGAT1* were mainly expressed in surface cells of rice roots in an NH_4^+-dependent manner. Disruption of either gene by the insertion of endogenous retrotransposon *Tos17* caused reduction in active tiller number and hence panicle number at harvest. Re-introduction of *OsGS1;2* cDNA under the control of its own promoter into the knockout mutants successfully restored panicle number to wild-type levels. These results indicate that GS1;2 and NADH-GOGAT1 are important in the primary assimilation of NH_4^+ taken up by rice roots. *OsGS1;1* and *OsNADH-GOGAT2* were mainly expressed in vascular tissues of mature leaf blades. *OsGS1;1* mutants showed severe reduction in growth rate and grain filling, whereas *OsNADH-GOGAT2* mutants had marked reduction in spikelet number per panicle. Complementation of phenotypes seen in the *OsGS1;1* mutant was successfully observed when *OsGS1;1* was re-introduced. Thus, these two enzymes could be important in remobilization of nitrogen during natural senescence. Metabolite profiling data showed a crucial role of GS1;1 in coordinating metabolic balance in rice. Expression of *OsGS1:3* was spikelet-specific, indicating that it is probably important in grain ripening and/or germination. Thus, these isoenzymes seem to possess distinct and non-overlapping functions and none was able to compensate for the individual function of another.

Key words: Glutamate synthase, glutamine synthetase, metabolic balance, nitrogen utilization, physiological function, rice.

Introduction

Inorganic nitrogen is quantitatively the most essential nutrient for plants and a major limiting factor for their growth and productivity. Although most plants grown in oxidative upland fields utilize NO_3^- as an inorganic nitrogen source (Andrews *et al.*, 2013), rice (*Oryza sativa* L.) plants grown in a reductive paddy soil prefer NH_4^+ (Tobin and Yamaya, 2001). The NH_4^+ is taken up by rice roots via NH_4^+ transporters (Sonoda *et al.*, 2003) and then assimilated into the amide residue of glutamine by the coupled reactions of glutamine synthetase (GS; EC 6.3.1.2) and glutamate synthase (GOGAT). Since the discovery of the GS/GOGAT cycle by Lea and Miflin (1974), it is now well established that this cycle is the only route for the primary assimilation of NH_4^+ in plants grown under normal conditions (Ireland and Lea, 1999; Lea and Miflin, 2003). The major forms of nitrogen in the xylem sap of rice are glutamine and asparagine (Fukumorita and Chino, 1982). The positron emitting tracer imaging system showed that the signals of ^{13}N taken up by the roots were detected in the basal

part of shoots within a short period, but the translocation of ^{13}N was completely inhibited by methionine sulfoximine, an inhibitor of GS (Kiyomiya *et al.*, 2001). These results suggest that the major part of NH_4^+ taken up by rice roots is assimilated within the roots.

In japonica rice, approximately 80% of the total nitrogen in the panicle arises from remobilization through the phloem from naturally senescing organs (Mae and Ohira, 1981). Thus, this process is very important in determining the productivity of rice. The major forms of nitrogen in the phloem sap are glutamine and asparagine (Hayashi and Chino, 1990). As asparagine is synthesized by the transfer of the amide group from glutamine (Lea *et al.*, 2007), the synthesis of glutamine in senescing organs is essential for this nitrogen recycling. The slight basic solution of the phloem sap (Nishiyama *et al.*, 2012) makes it possible to solubilize these amides at concentrations as high as 30 mM or more. In sink organs, such as developing leaves and grains, the remobilized glutamine is re-utilized for many biosynthetic reactions, via GS/GOGAT reactions (Lea and Miflin, 2003), which are responsible for the metabolism of glutamine in rice (Tobin and Yamaya 2001).

It is well known from work in barley mutants that GS2 and Fd-GOGAT in chloroplasts are responsible for the assimilation of NH_4^+ released during photorespiration (Wallsgrove *et al.*, 1987; Kendall *et al.*, 1986). However, mutants are able to grow normally under non-photorespiratory conditions. This suggests that other species of GS and GOGAT are apparently important in the normal growth and development of plants (Yamaya and Oaks, 2004). A small gene family has been identified that encodes cytosolic GS (GS1) in various plants (Goodall *et al.*, 2013; Ishiyama *et al.*, 2004; Martin *et al.*, 2006; Swarbreck *et al.*, 2011), including rice in which *OsGS1;1*, *OsGS1;2*, and *OsGS1;3* were identified (Tabuchi *et al.*, 2005). There is also a small gene family that encodes NADH-GOGAT (EC 1.4.1.14), i.e. *OsNADH-GOGAT1* and *OsNADH-GOGAT2*, in rice (Tamura *et al.*, 2010). This is unlikely to be the case in *Arabidopsis thaliana*, which possesses only a single gene for NADH-GOGAT (The Arabidopsis Genome Initiative, 2000).

Reverse genetic approaches are powerful for elucidating individual gene function. In rice, knockout mutants lacking *OsGS1;1* (Tabuchi *et al.*, 2005), *OsGS1;2* (Funayama *et al.*, 2013), *OsGS1;3* (Nakamura *et al.*, unpublished results), *OsNADH-GOGAT1* (Tamura *et al.*, 2010), and *OsNADH-GOGAT2* (Tamura *et al.*, 2011) have been successively isolated and characterized. Rice is the only example where knockout mutants lacking each individual gene for GS1 and NADH-GOGAT isoenzymes have been characterized.

In this review, distinct functions of each GS1 and NADH-GOGAT isoenzyme in rice plants are discussed. In the course of profiling of metabolites in a GS1;1 mutant, we found that GS1;1 is important in coordinating the global metabolic network in rice. This point is also discussed in this review.

The primary assimilation of NH_4^+ taken up by rice roots

Knockout mutants caused by homozygous insertion of an endogenous retrotransposon *Tos17* into *OsGS1;2* or *OsNADH-GOGAT1* genes (Fig. 1) were characterized phenotypically. Recent observations strongly suggest that both GS1;2 (Funayama *et al.*, 2013) and NADH-GOGAT1 (Tamura *et al.*, 2010) are important in the primary assimilation of NH_4^+ taken up by rice roots (Fig. 2). Both mutants showed severe reduction in active tiller number and hence panicle number, when those mutants were grown in paddy fields with normal fertilizer until harvest. Other yield

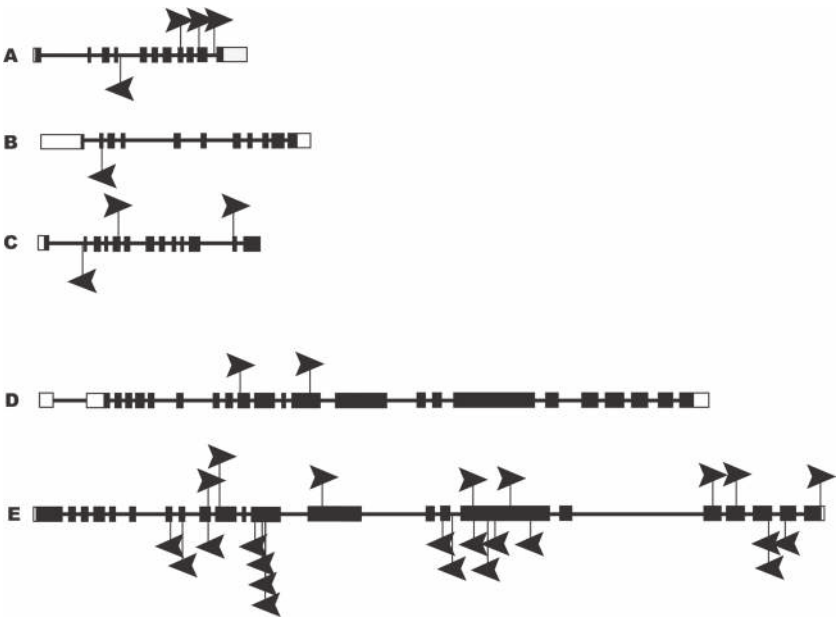

Fig. 1. Isolation of knockout mutants lacking either *OsGS1* or *OsNADH-GOGAT* in rice. Diagram of the insertion position of the retrotransposon *Tos17* (arrowhead) in *OsGS1;1* (A), *OsGS1;2* (B), *OsGS1;3* (C), *OsNADH-GOGAT1* (D), and *OsNADH-GOGAT2* (E). Exons are indicated as boxed regions, whereas lines represent introns and 5'- and 3'-untranscribed regions. The open boxes correspond to untranslated regions.

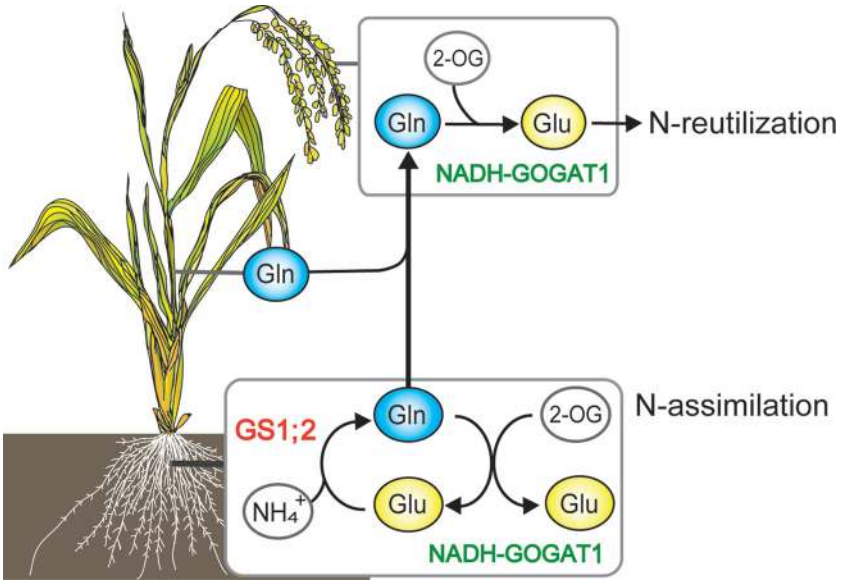

Fig. 2. Schematic model for the primary assimilation of NH_4^+ by GS1;2 and NADH-GOGAT1 in rice roots. NADH-GOGAT1 is apparently important in the reutilization of glutamine in sink organs transported from roots and senescing organs. Abbreviations: Gln, glutamine; Glu, glutamate; and 2-OG, 2-oxoglutarate.

components, such as spikelet number per panicle, 1000-spikelet weight, and proportion of well ripened-grains, were nearly identical between both mutants and wild-type plants. This phenotype is quite similar to N deficiency symptoms in rice (Mae, 1997). It has been suggested that the development of tillers is inhibited by a high concentration of a phytohormone, strigolactone (Luo *et al.*, 2012; Seto *et al.*, 2012). It was therefore hypothesized that the loss of GS1;2 may increase the concentration of strigolactone in the GS1;2 mutants. However, our preliminary analysis of strigolactone in roots of this mutant suggested that, unlike Pi deficiency (Seto *et al.*, 2012), there were apparently no big differences in content of this phytohormone between the mutants and wild type (Ohashi *et al.*, unpublished data). The GS1;2 mutants at the seedling stage showed that there were marked reductions in the content of glutamine, glutamate, asparagine, and aspartate, but a remarkable increase in free NH_4^+ ions in the roots when compared with those in the roots of wild-type plants. In xylem sap, concentrations of these amino acids and free NH_4^+ ions behaved in a similar fashion (Funayama *et al.*, 2013). Contents of amino acids and free NH_4^+ ions in roots of NADH-GOGAT1 mutants also fluctuated similarly to the GS1;2 mutants (Tamura *et al.*, 2010). Re-introduction of *OsGS1;2* cDNA under the control of its own promoter into the GS1;2 mutants successfully restored active tiller number, contents of amino acid and free NH_4^+ ions in the roots and in the xylem sap. The expression of *OsGS1;1* and of other genes involved in nitrogen metabolism is the same in the GS1;2 mutants as it is in the wild type, hence there is no indication that these compensate for the loss of GS1;2 in the mutants. Similarly, expression of NADH-GOGAT2 and Fd-GOGAT genes in the NADH-GOGAT1 mutant was identical with that in wild type, suggesting that these GOGATs are not able to compensate for NADH-GOGAT1 function.

Abundant expression of *OsGS1;2* was detected in the surface cell layers of rice roots, i.e. in the epidermis and exodermis cells, following the supply of NH_4^+ ions as a nutrient (Ishiyama *et al.*, 2004; Tabuchi *et al.*, 2007). Accumulation of *OsNADH-GOGAT1* transcript was also detected in the two cell layers of the root surface in an NH_4^+-dependent manner (Ishiyama *et al.*, 2003), similar to the expression of *OsGS1;2*. In rice, there is the Casparian strip between the second and third cell layers of the root surface (Morita *et al.*, 1996) in addition to the Casparian strip in the endodermis. This additional Casparian strip probably protects radial oxygen loss from the roots of rice plants grown in paddy fields (Watanabe *et al.*, 2013). As discussed in our previous review on localization studies, GS1;2 and NADH-GOGAT1, both located in cells outside the additional Casparian strip, are responsible for the assimilation of NH_4^+ within these cell types, and the resulting glutamine and/or glutamate could be transported through the Casparian strip into the cortex and central cylinder (Tabuchi *et al.*, 2007). It is likely that the occurrence of GS1;2 and NADH-GOGAT1 in the outer two cell layers of the root surface could protect these cells from potential toxicity of NH_4^+ (Hachiya *et al.*, 2012; Kronzucker *et al.*, 2001), even in rice plants. Knockout of either gene caused severe reduction in the primary assimilation of NH_4^+ and showed the N-deficiency-like phenotype. Free NH_4^+ unassimilated within the roots is probably transported to the aerial parts where other types of GS/GOGAT are operating.

There is little progress on understanding the regulation of *OsGS1;2* and *OsNADH-GOGAT1* in response to NH_4^+/glutamine status in rice, since our previous discussion (Tabuchi *et al.*, 2007). On the other hand, some of the genes for adenosine phosphate-isopentenyltransferase, an enzyme that synthesizes cytokinins that are involved in metabolic and signalling processes closely related to nitrogen availability, were found to be upregulated by glutamine or a related metabolite

in rice (Kamada-Nobusada *et al.*, 2013), as has been found for *OsGS1;2* and *OsNADH-GOGAT1* (Tabuchi *et al.*, 2007).

Remobilization and reutilization of nitrogen during natural senescence

Expression of both *OsGS1;1* and *OsNADH-GOGAT2* has been mainly detected in mature leaves of rice plants (Tabuchi *et al.*, 2007). Disruption of *OsGS1;1* caused very severe reduction of growth rate and grain filling (Tabuchi *et al.*, 2005). At the vegetative stage of the GS1;1 mutants grown with a normal supply of nitrogen, the plant height and leaf-blade elongation were strongly reduced. At the harvest stage, ripening was retarded, panicle size was very small, and filled spikelet number was severely reduced. This severe phenotype was successfully complemented by the re-introduction of *OsGS1;1* cDNA under the control of its own promoter (Tabuchi *et al.*, 2005). During natural senescence of rice leaf blades, total chlorophyll and soluble protein contents decline continuously (Kamachi *et al.*, 1991). A large proportion of nitrogen is remobilized, in the form of glutamine and asparagine, through the phloem (Hayashi and Chino, 1990) from senescing organs to sink organs (Mae and Ohira, 1981). For example, approximately 80% of the total nitrogen in the panicle arises from remobilization (Fig. 3). GS1 protein was detected in phloem companion cells and phloem parenchyma cells in the vascular bundle of mature leaf blades of rice (Sakurai *et al.*, 1996). Because *OsGS1;1* transcript was the major isogene product in mature leaf blade (Tabuchi *et al.*, 2007), the GS1 protein detected in phloem cells could be the translation product of *OsGS1;1* mRNA. Thus, GS1;1 should have a central function to generate glutamine, as the primary form of remobilized nitrogen, in senescing leaves for long-distance transport. Disruption of *OsNADH-GOGAT2* also caused marked reduction in spikelet number, and hence productivity of rice (Tamura *et al.*, 2011). Expression of an *OsNADH-GOGAT2* promoter::GUS gene was identical to the localization of GS1;1 protein in the fully expanded leaf blade (Tamura *et al.*, 2011). These results strongly suggest that NADH-GOGAT2 provides glutamate for the reaction of GS1;1 in vascular tissues of rice leaves during senescence. As large amounts of free glutamate were detected in the mature leaf blade of rice (Kamachi *et al.*, 1991), the phenotype of NADH-GOGAT2 mutants was probably not severe,

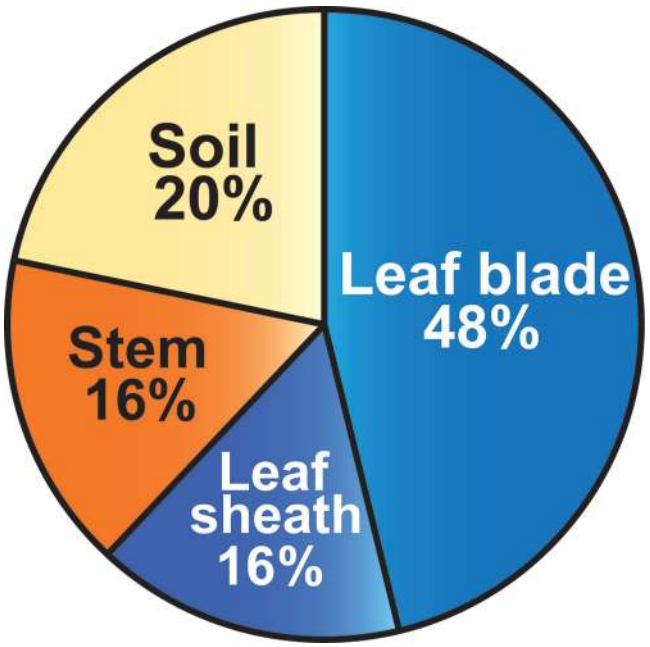

Fig. 3. Origin of nitrogen in spikelets from various organs and soils in rice during natural senescence. The results using ^{15}N-pulse-chase labelling studies, obtained by Mae and Ohira (1981), were drawn in this figure by permission of Oxford University Press (Japanese Society of Plant Physiologist).

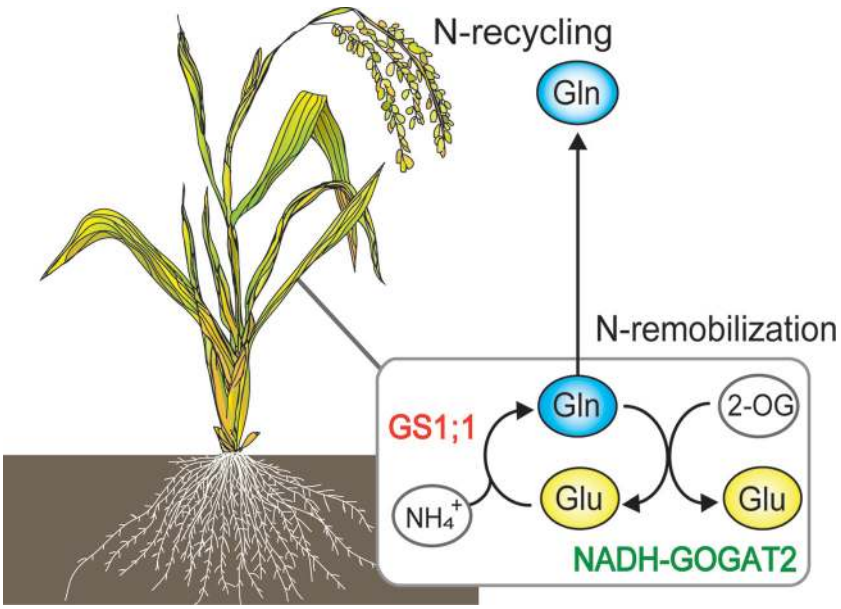

Fig. 4. Schematic model of the synthesis of glutamine by GS1;1 and NADH-GOGAT2 for the remobilization of nitrogen from senescing organs to sink organs via phloem. Abbreviations: Gln, glutamine; Glu, glutamate; and 2-OG, 2-oxoglutarate.

when compared with that of GS1;1 mutants. These results all indicate that GS1;1 and NADH-GOGAT2 are important in the process of nitrogen remobilization accompanying the generation of glutamine from catabolically degraded proteins, nucleic acids, and chlorophyll in senescing organs (Fig. 4). Although transcripts of GS1;2, GS2, NADH-GOGAT1, and Fd-GOGAT were all detected in mature leaves, our results indicate that these enzymes are not able to compensate for the

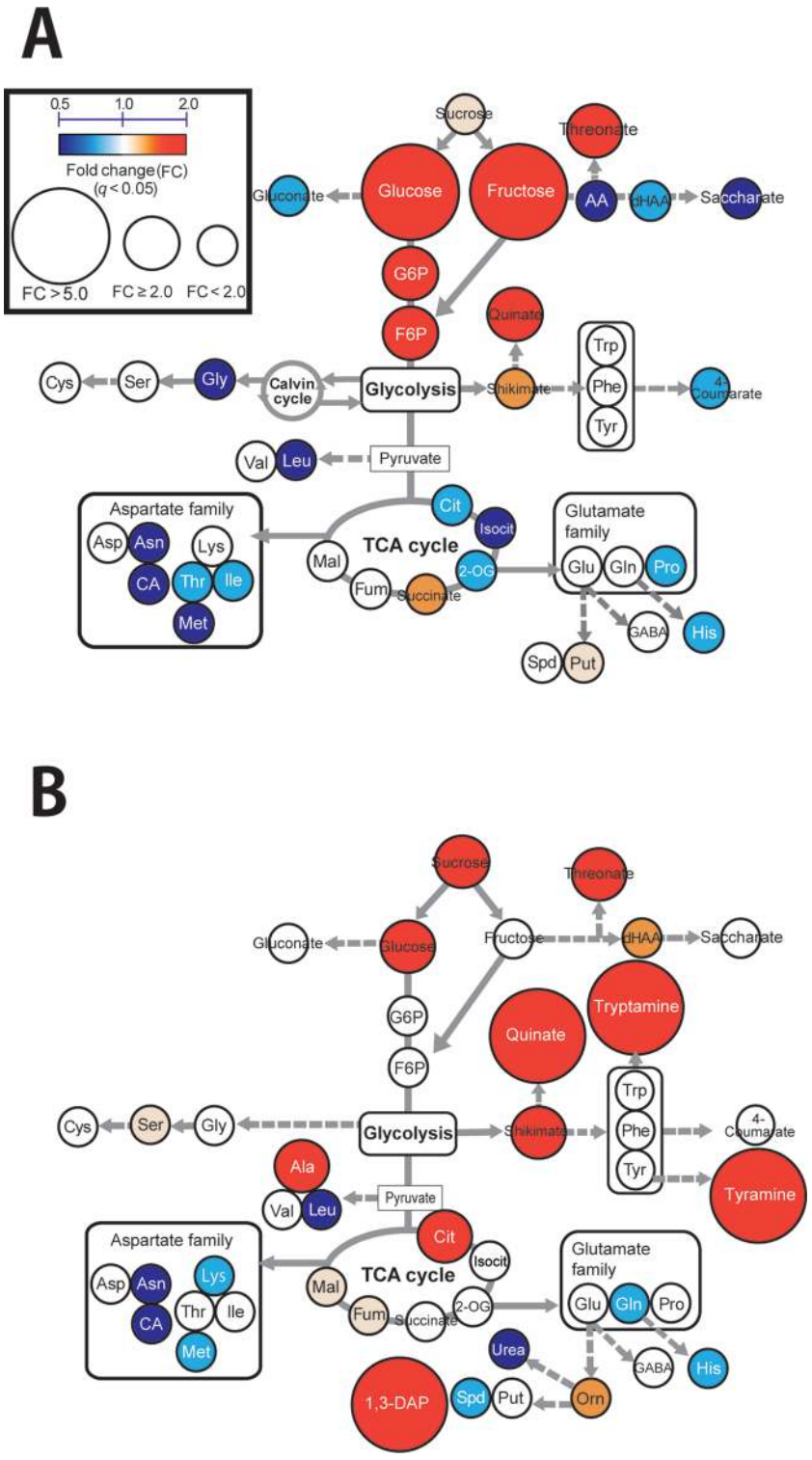

Fig. 5. Metabolite changes in the primary and secondary metabolic pathways in leaf blade (A) and roots (B) of *OsGS1;1* knockout mutant of rice. Fold changes in the metabolite level are represented by circle size and colour scale on the metabolite map. Abbreviations: AA, ascorbate; GABA, γ-aminobutyrate; dHAA, dehydroascorbate; 1,3-DAP, 1,3-diaminopropane; CA, β-cyanoalanine; Put, putrescine; and Spd, spermidine. Amino acids in three letter code and organic acids in the tricarboxylic acid (TCA) cycle are not listed here. The figure was modified from the work by Kusano **Tabuchi M, Fukushima A, Funayama K, Diaz C, Kobayashi M, Hayashi N, Tsuchiya YN, Takahashi H, Kamata A, Yamaya T, Saito K.** 2011. Metabolomics data reveal a crucial role of cytosolic glutamine synthase 1;1 in coordinating metabolic balance in rice. *The Plant Journal* **66,** 456–466; with permission.

function of GS1;1 and NADH-GOGAT2. Because NADH-GOGAT1 protein was detected in vascular tissues of developing leaves and spikelets (Hayakawa *et al.*, 1994), re-utilization of glutamine transported via the phloem could be catalysed by this enzyme. Reduction in the size of sink tissues (panicle number per plants) in the mutants could be the result of the lack of this reaction (Tamura *et al.*, 2010).

A crucial role for GS1;1 in coordinating metabolic balance in rice

In general, the metabolism of carbon and nitrogen in plants is well balanced under normal or suboptimal growth conditions (Reich *et al.*, 2006). GS and GOGAT are the crucial steps for nitrogen metabolism. GS catalyses the assimilation of inorganic NH_4^+ to generate the organic product glutamine, whereas GOGAT is the contact point between nitrogen (glutamine) and carbon (2-oxoglutarate) metabolites. The two reactions together provide the only route in plants for assimilation of ammonium into organic products (Ireland and Lea, 1999). When a single gene among multiple GS1 or NADH-GOGAT genes was mutated, rice plants seem to maintain the capacity to reproduce fully ripened seeds, although significant reduction of panicle or spikelet number was observed in knockout mutants of *OsGS1;2* (Funayama *et al.*, 2013), *OsNADH-GOGAT1* (Tamura *et al.*, 2010), or *OsNADH-GOGAT2* (Tamura *et al.*, 2011). On the other hand, a knockout mutant for *OsGS1;1* was nearly lethal and no production of the next generation was observed as long as inorganic NH_4^+ was supplied (Tabuchi *et al.*, 2005). This severe phenotype could be a good way to learn the coordination of metabolic balance in rice. For understanding the global coordination of carbon and nitrogen metabolism, metabolite profiling is a powerful tool. This relatively new technology has been applied to understand metabolic networks even with a silent phenotype (Weckwerth *et al.*, 2004) and in transgenic plants (Mattoo *et al.*, 2006). Because the GS reaction is the first step for inorganic nitrogen to enter into an organic nitrogen form in plants, GS1;1 knockout mutants provide a valuable resource for investigating whole-plant metabolic regulation. Even though flux in primary metabolism is complicated, metabolite profiling as well as metabolite-to-metabolite correlation analysis between the GS1;1 knockout mutant and wild-type rice provide us with a global picture of whole metabolic processes. According to our previous findings (Kusano *et al.*, 2011), the metabolite profiles of the GS1;1 knockout mutants showed (i) an imbalance in metabolites of sugars, amino acids, and organic acids in the TCA cycle, and (ii) over-accumulation of secondary metabolites with nitrogen atom(s) in their chemical structures, particularly in the roots under continuous supply of NH_4^+ nutrition (Fig. 5). The occurrence of mutant-specific networks between tryptamine and other primary metabolites was observed from the correlation analysis. These results clearly show that GS1;1 performs a crucial function in coordinating the global metabolic network in rice grown in the presence of NH_4^+ nutrition (Kusano *et al.*, 2011). It is interesting to note that the photorespiratory metabolites, such as serine, showed little change in the GS1;1 knockout mutants (Fig. 5A). This is probably caused by the fact that GS2 activity in the leaves of GS1;1 mutants was at the same level as in the wild type (Tachuchi *et al.*, 2005). On the other hand, rice mutants lacking *OsGS1;2* showed metabolite levels very similar to those in the wild-type plants (Kusano *et al.*, unpublished results). These contrasting results from metabolite profiling further indicate that there are distinct and separate functions for GS1;1 and GS1;2 in rice.

Conclusions

Approaches using reverse genetics and metabolite profiling clearly provide evidence that GS1;2 and NADH-GOGAT1 are important in the primary assimilation of NH_4^+ taken up by rice roots, whereas GS1;1 and NADH-GOGAT2 are important in the remobilization of nitrogen in senescing organs of rice plants. The two GS and two NADH-GOGAT isoenzymes apparently possess a distinct function and none of the isoenzymes is able to compensate for the specific function of another. This is the first opportunity to characterize the majority of the multiple GS1 isoenzymes and all NADH-GOGAT isoenzymes in plants. From the approach of metabolite profiling, GS1;1 in rice possesses a crucial role in coordinating metabolic balance. There is only one further GS1 isoenzyme, GS1;3, remaining to be characterized in rice. The *OsGS1;3* is specifically expressed in the spikelet (Tabuchi *et al.*, 2007), similar to *TaGSe* in wheat (Swarbeck *et al.*, 2011), *ZmGln1-2* in maize (Martin *et al.*, 2006), and *HvGS1_3* in barley (Goodall *et al.*, 2013). Several lines of knockout mutants lacking GS1;3 have already been obtained, and the mutants apparently exhibited a reduced rate of natural senescence in the paddy field, and also showed a relatively slow rate of germination (Nakamura *et al.*, unpublished results). However, there has been no strong evidence supporting its function from these phenotypic characterizations. This isoenzyme probably operates in the maturation of the spikelet in relation to the accumulation of storage protein, for example, or in the germination process. GS1;3 is probably associated with NADH-GOGAT1, because its gene expression was observed in the spikelets (Tabuchi *et al.* 2007). Metabolomics data, together with the reverse genetics approach, may give an answer for our last question in the near future.

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References

Andrews M, Raven JA, Lea PJ. 2013. Do plants need nitrate? The mechanisms by which nitrogen form affects plants. *Annals of Applied Biology* **163,** 174–199.

Fukumorita T, Chino M. 1982. Sugar, amino acid, and inorganic contents in rice phloem sap. *Plant and Cell Physiology* **23,** 273–283.

Funayama K, Kojima S, Tabuchi-Kobayashi M, Sawa Y, Nakayama Y, Hayakawa T, Yamaya T. 2013. Cytosolic glutamine synthetase1;2 is responsible for the primary assimilation of ammonium in rice roots. *Plant and Cell Physiology* **54,** 934–943.

Goodall AJ, Kumar P, Tobin AK. 2013. Identification and expression analyses of cytosolic glutamine synthetase genes in barley (*Hordeum vulgare* L.). *Plant and Cell Physiology* **54,** 492–505.

Hachiya T, Watanabe CK, Fujimoto M, Ishikawa T, Takahara K, Kawai-Yamada M, Uchimiya H, Uesono Y, Terashima I, Noguchi K. 2012. Nitrate addition alleviates ammonium toxicity without lessening ammonium accumulation, organic acid depletion and inorganic cation depletion in *Arabidopsis thaliana* shoots. *Plant and Cell Physiology* **53,** 577–591.

Hayakawa T, Nakamura T, Hattori F, Mae T, Ojima K, Yamaya T. 1994. Cellular localization of NADH-dependent glutamate-synthase protein in vascular bundles of unexpanded leaf blades and young grains of rice plants. *Planta* **193,** 455–460.

Hayashi H, Chino M. 1990. Chemical composition of phloem sap from the upper most internode of the rice plant. *Plant and Cell Physiology* **31,** 247–251.

Ireland RJ, Lea PJ. 1999. The enzymes of glutamine, glutamate, asparagine and aspartate metabolisms. In: Singh BK, ed. *Plant amino acids: biochemistry and biotechnology* . New York: Marcel Dekker, 49–109.

Ishiyama K, Inoue E, Tabuchi M, Yamaya T, Takahashi H. 2004. Biochemical backgrounds of compartmentalized functions of cytosolic glutamine synthetase for active ammonium assimilation in rice roots. *Plant and Cell Physiology* **45,** 1640–1647.

Ishiyama K, Kojima S, Takahashi H, Hayakawa T, Yamaya T. 2003. Cell type distinct accumulations of mRNA and protein for NADH-dependent glutamate synthase in rice roots in response to the supply of NH_4^+. *Plant Physiology and Biochemistry* **41,** 643–647.

Kamachi K, Yamaya T, Mae T, Ojima K. 1991. A role for glutamine synthetase in the remobilization of leaf nitrogen during natural senescence in rice leaves. *Plant Physiology* **96,** 411–417.

Kamada-Nobusada T, Makita N, Kojima M, Sakakibara H. 2013. Nitrogen-dependent regulation of de novo cytokinin biosynthesis in rice: The role of glutamine metabolism as an additional signal. *Plant Cell Physiology* **54,** 1181–1893.

Kendall AC, Wallsgrove RM, Hall NP, Turner JC, Lea PJ. 1986. Carbon and nitrogen metabolism in barley (*Hordeum vulgare* L.) mutants lacking ferredoxin-dependent glutamate synthase. *Planta* **168,** 316–323.

Kiyomiya S, Nakanishi H, Uchida H, *et al*. 2001. Real time visualization of ^{13}N-translocation in rice under different environmental conditions using positron emitting tracer imaging system. *Plant Physiology* **125,** 1743–1754.

Kronzucker HJ, Britto DT, Davenport RJ, Tester M. 2001. Ammonium toxicity and the real cost of transport. *Trends in Plant Science* **6,** 335–337.

Kusano M, Tabuchi M, Fukushima A, Funayama K, Diaz C, Kobayashi M, Hayashi N, Tsuchiya YN, Takahashi H, Kamata A, Yamaya T, Saito K. 2011. Metabolomics data reveal a crucial role of cytosolic glutamine synthase 1;1 in coordinating metabolic balance in rice. *The Plant Journal* **66,** 456–466.

Lea PJ, Miflin BJ. 1974. Alternative route for nitrogen assimilation in higher plants. *Nature* **251,** 614–616.

Lea PJ, Miflin BJ. 2003. Glutamate synthase and the synthesis of glutamate in plants. *Plant Physiology and Biochemistry* **41,** 555–564.

Lea PJ, Sodek L, Parry MAJ, Shewry PR, Halford NG. 2007. Asparagine in plants. *Annals of Applied Biology* **150,** 1–26.

Luo L, Li W, Miura K, Ashikari M, Kyozuka J. 2012. Control of tiller growth of rice by *OsSPL14* and strigolactones, which work in two independent pathways. *Plant and Cell Physiology* **53,** 1793–1801.

Mae T. 1997. Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis, and yield potential. *Plant Soil* **196,** 201–210.

Mae T, Ohira K. 1981. The remobilization of nitrogen related to leaf growth and senescence in rice plants (*Oryza sativa* L.). *Plant and Cell Physiology* **22,** 1067–1074.

Martin A, Lee J, Kichey T, *et al.* 2006. Two cytosolic glutamine synthetase isoforms of maize (*Zea mays* L.) are specifically involved in the control of grain production. *The Plant Cell* **18,** 3252–3274.

Mattoo AK, Sobolev AP, Neelam A, Goyal RK, Handa AK, Segre AL. 2006. Nuclear magnetic resonance spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions. *Plant Physiology* **142,** 1759–1770.

Morita S, Lux A, Enstone DE, Peterson CA, Abe J. 1996. Re-examination of rice seminal root ontogeny using fluorescence microscopy. *Japanese Journal of Crop Science* **65,** 37–38.

Nishiyama R, Kato M, Nagata S, Yanagisawa S, Yoneyama T. 2012. Identification of Zn–nicotianamine and Fe–2'-deoxymugineic acid in the phloem sap from rice plants (*Oryza sativa* L.). *Plant and Cell Physiology* **53,** 381–390.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JM, Naeem S, Trost J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature* **440,** 922–925.

Sakurai N, Hayakawa T, Nakamura T, Yamaya T. 1996. Changes in the cellular localization of cytosolic glutamine synthetase protein in vascular bundles of rice leaves at various stages of development. *Planta* **200,** 306–311.

Seto Y, Kameoka H, Yamaguchi S, Kyozuka J. 2012. Recent advances in strigolactone research: Chemical and biological aspects. *Plant and Cell Physiology* **53,** 1843–1853.

Sonoda Y, Ikeda A, Saiki S, von Wirén N, Yamaya T, Yamaguchi J. 2003. Distinct expression and function of three ammonium transporter genes (*OsAMT1;1-1;3*) in rice. *Plant and Cell Physiology* **44,** 726–734.

Swarbreck SM, Defoin-Platel M, Hindle M, Saqi M, Habash DZ. 2011. New perspectives on glutamine symthetase in grasses. *Journal of Experimental Botany* **62,** 1511–1522.

Tabuchi M, Sugiyama K, Ishiyama K, Inoue E, Sato T, Takahashi H, Yamaya T. 2005. Severe reduction in growth rate and grain filling of rice mutants lacking OsGS1;1, a cytosolic glutamine synthetase1;1. *The Plant Journal* **42,** 641–651.

Tabuchi M, Abiko T, Yamaya T. 2007. Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). *Journal of Experimental Botany* **58,** 2319–2327.

Tamura W, Hidaka Y, Tabuchi M, Kojima S, Hayakawa T, Sato T, Obara M, Kojima K, Sakakibara H, Yamaya, T. 2010. Reverse genetic approach to characterize a function of NADH-glutamate synthase1 in rice plants. *Amino Acids* **39,** 1003–1012.

Tamura W, Kojima S, Toyokawa A, Watanabe H, Tabuchi-Kobayashi M, Hayakawa T, Yamaya T. 2011. Disruption of a novel NADH-glutamate synthase2 gene caused marked reduction in spikelet number of rice. *Frontiers in Plant Science* **2, doi:** 10.3389/fpls.2011.00057

The Arabidopsis Genome Initiative. 2000. Analysis of genome sequence of the flowering plant *Arabidopsis thaliana. Nature* **408,** 796–815.

Tobin AK, Yamaya T. 2001. Cellular compartmentation of ammonium assimilation in rice and barley. *Journal of Experimental Botany* **52,** 591–604.

Watanabe K, Nishiuchi S, Kulichikhin K, Nakazono M. 2013. Does suberin accumulation in plant roots contribute to waterlogging tolerance? *Frontiers in Plant Science* **4, doi:** 10.3389/fpls.2013.00178

Wallsgrove RM, Turner JC, Hall NP, Kendall AC, Bright SWJ. 1987. Barley mutants lacking chloroplast glutamate synthetase: biochemical and genetic analysis. *Plant Physiology* **83,** 155–158.

Weckwerth W, Loureiro ME, Wenzel K, Fiehn O. 2004. Differential metabolic networks unravel the effects of silent plant phenotype. *Proceedings of the National Academy of Sciences, USA* **101,** 7809–7814.

Yamaya T, Oaks A. 2004. Metabolic regulation of ammonium uptake and assimilation. In: Amâncio S, Stulen I, eds. *Nitrogen acquisition and assimilation in higher plants* . Dordrecht/Boston/London: Kluwer Academic Publishers, 35–63.