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The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops

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Abstract

This short review outlines the central role of glutamine synthetase (GS) in plant nitrogen metabolism and discusses some possibilities for crop improvement. GS functions as the major assimilatory enzyme for ammonia produced from N fixation, and nitrate or ammonia nutrition. It also reassimilates ammonia released as a result of photorespiration and the breakdown of proteins and nitrogen transport compounds. GS is distributed in different subcellular locations (chloroplast and cytoplasm) and in different tissues and organs. This distribution probably changes as a function of the development of the tissue, for example, GS1 appears to play a key role in leaf senescence. The enzyme is the product of multiple genes with complex promoters that ensure the expression of the genes in an organ- and tissue-specific manner and in response to a number of environmental variables affecting the nutritional status of the cell. GS activity is also regulated post-translationally in a manner that involves 14-3-3 proteins and phosphorylation. GS and plant nitrogen metabolism is best viewed as a complex matrix continually changing during the development cycle of plants. Along with GS, a number of other enzymes play key roles in maintaining the balance of carbon and nitrogen. It is proposed that one of these is glutamate dehydrogenase (GDH). There is considerable evidence for a GDH shunt to return the carbon in amino acids back into reactions of carbon metabolism and the tri-carboxylic acid cycle. Results with transgenic plants containing transferred GS genes suggest that there may be ways in which it is possible to improve the efficiency with which crop plants use nitrogen. Marker-assisted breeding may also bring about such improvements.

Key words: Glutamate dehyrogenase, glutamine synthetase, nitrogen metabolism, QTL, regulation, transgenic plants, *Triticum aestivum*, wheat.

Introduction

The improvement of nitrogen use efficiency, particularly in cereals, is a major goal of crop improvement. Such improved crops would make better use of the nitrogen fertilizer supplied; they would also produce higher yields with better protein content. This might be achieved, at least in part, by a better understanding of nitrogen metabolism and its regulation, and by identifying likely target genes for manipulation by either direct gene transfer or marker-assisted breeding.

Ammonia assimilation and reassimilation

Glutamine synthetase (GS; EC 6.3.1.2) was first purified and characterized from plants in 1956. One particular important characteristic is its high affinity for ammonia and thus its ability to incorporate ammonia efficiently into organic combination. Originally, glutamine was considered to donate its amide N only into a limited number of compounds. However, the discovery of NAD(P)H glutamate synthase in bacteria (Tempest *et al.*, 1970) and later ferredoxin-dependent glutamate synthase in plants (Lea and Miflin, 1974) established a route, the glutamate synthase cycle, for NH_3 [2] to enter into organic compounds via its assimilation by GS. Evidence based on labelling

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[2] The term NH_3 refers to both NH_3 and NH_4^+ and the precise equilibrium depends on the pH.

kinetics, use of inhibitors, *in organello* studies, and genetics established that this was the major route of primary nitrogen assimilation in plants (Miflin and Lea, 1980).

During the growth and development of plants, nitrogen is moved into and out of proteins in the different organs and transported between organs in a limited number of transport compounds. Some of the organic nitrogen is moved between compounds via the activity of transaminases and glutamine-amide transferases, but a significant portion is released as NH_3 and reassimilated via GS. For example, asparagine is a significant component of seed storage proteins in legumes and a major transport compound in cereals. It is metabolized to ammonia and aspartate via the action of asparaginase. Similarly ureides, such as allantoin, play an important role in N transport in legumes and their organic N is released as NH_3 via the action of urease. Thus, over the life of a plant, nitrogen is released as NH_3 and refixed several times (for a detailed description of these processes see Lea and Miflin, 1980). This flux through NH_3 and GS is dwarfed in C_3 plants by the flux of NH_3 released by glycine decarboxylase during photorespiration. This could be an order of magnitude more than the rate of primary assimilation. Biochemical and genetic experiments have shown that this NH_3 is also refixed via GS (Keys *et al.*, 1978; Somerville and Ogren, 1982; Wallsgrove *et al.*, 1987).

Overall GS acts at the centre of nitrogen flow as depicted by the scheme in the centre of Fig. 1. This central position of GS raises a number of crucial questions: How does the plant maintain C/N balance? How is GS distributed? How is GS activity regulated? Does glutamine regulate metabolism? Does GS regulate development? Is our knowledge of GS sufficient to be useful? Can we improve plants agronomically by modifying GS?

Carbon–nitrogen balance and the GDH-shunt

Plants have evolved to capture available carbon and nitrogen and to store and transfer it efficiently. To achieve this, they use individual amino acids and proteins that differ widely in their C/N ratio. Which compounds are used probably depends on the nutrition available to the plants. Thus, nitrogen-fixing legumes, which are rich in N, use transport compounds with a low C/N ratio such as allantoin (C/N ratio of 1) and have storage proteins with a high content of amides and basic amino acids. In times of stress, particularly carbon shortage, there is probably a strong demand to obtain carbon from amino acids to feed into the tricarboxylic acid (TCA) cycle. To achieve this the plant needs enzymes that intra-convert ureides, amides, amino acids, and keto-acids. The current view of how this might occur is shown in Fig. 2 and reflects the schemes published previously (Lea and Miflin, 1980; Miflin *et al.*, 1981). The proposed role of glutamate dehydrogenase (GDH; mitochondrial NADH dependent EC 1.4.1.2) is particularly noteworthy. This has been

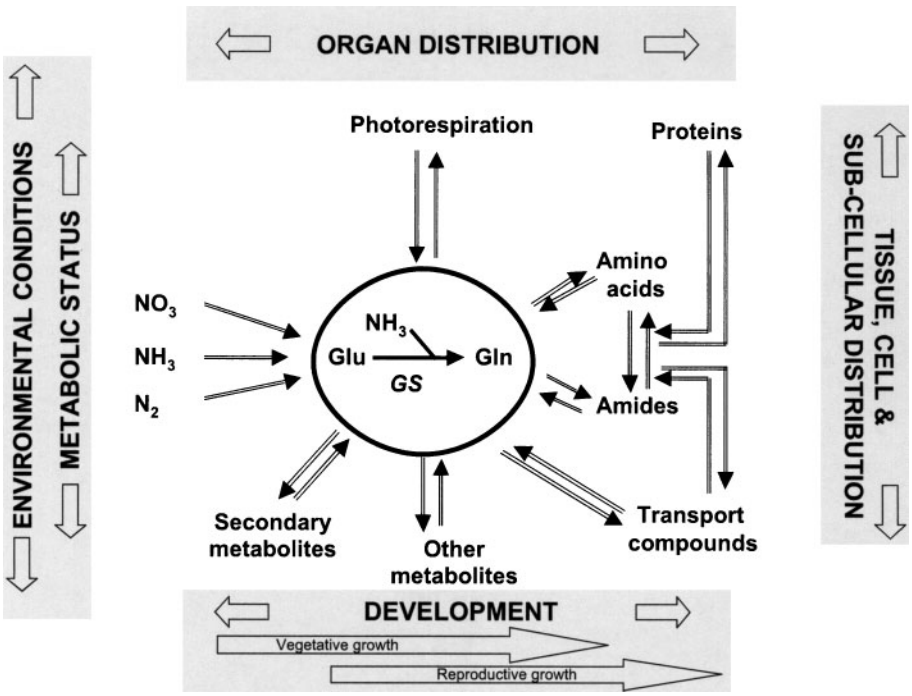

Fig. 1. The central role of GS in the complex matrix of plant N metabolism. The central scheme encompasses the total role of GS. The boxes around the outside indicate the matrix of various locations and environments in which GS may be operating. The direction of the flow of N (and thus the arrows) will depend on which part of the matrix is under consideration. Thus in the developing seed the flux will be from incoming transport compounds towards proteins whilst in the germinating seed the flow will be in the reverse direction.

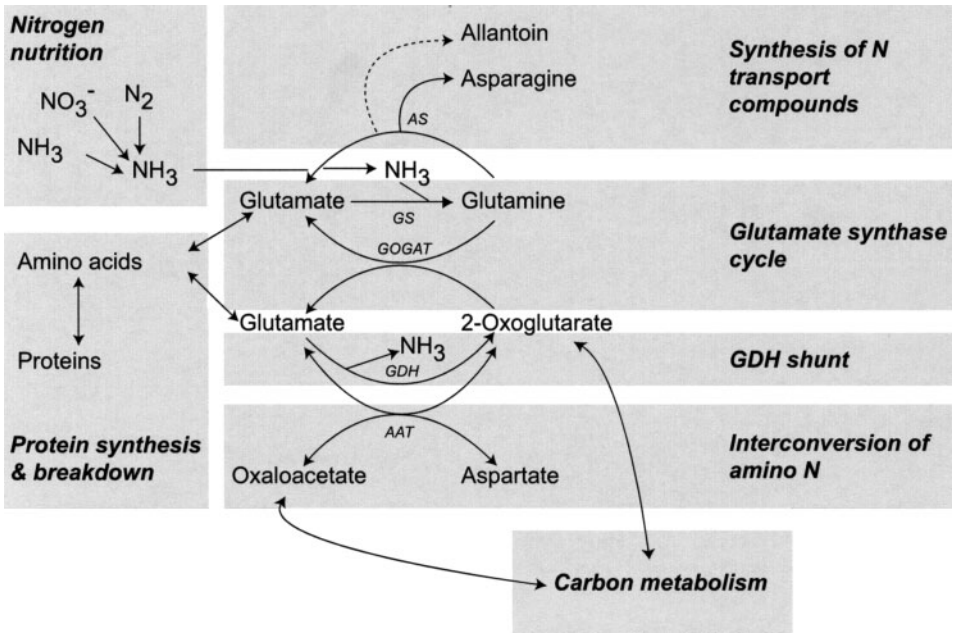

Fig. 2. Enzyme pathways important in the balance of C and N metabolism. AAT, aspartate amino transferase; AS, asparagine synthetase; GS, glutamine synthetase; GOGAT, glutamate synthase.

subject to continued controversy (Fox *et al*., 1995; Oaks, 1995; Srivastava and Singh, 1987). GDH is one of the few enzymes capable of releasing amino nitrogen from amino acids to give a keto-acid and NH_3 that can be separately recycled to be used in respiration and amide formation, respectively. GDH may be expected to function in the deaminating direction in tissues that are converting amino acids into transport compounds with a low C/N ratio, for example, germinating seeds and senescing leaves. Aubert *et al*., using NMR and labelling studies and coupling glutamate oxidation to glutamine synthesis, have recently produced strong experimental evidence that GDH does function in this direction in isolated mitochondria (Aubert *et al*., 2001).

The addition of a GDH-shunt to the glutamate synthase cycle (Fig. 2) would provide a mechanism that could respond to the differing needs of cells for carbon and nitrogen compounds. It would also provide a means for regulating the internal glutamate concentration, which Stitt *et al*. have shown to remain remarkably constant in leaves (Stitt *et al*., 2002). At first glance the GDH-shunt might appear to generate a futile cycle. However, the reactions in Fig. 2 are situated in the same conceptual matrix as depicted for Fig. 1. Thus they occur in different subcellular compartments, and may also occur in discrete cells and are separated by time over the course of the daily cycle. This gives plenty of possibilities for regulation of the system to prevent a useless cycling of ammonia. It also suggests that there is an important role for the transporters of amino and keto acids and of NH_3 across the membranes separating the different compartments, particularly across the mitochondria and chloroplasts. The way in which they function and their regulation would enable the plant to balance the operation of the glutamate synthase cycle and the GDH-shunt. In general, but particularly under C shortage, the operation of transaminases (e.g. such as oxaloacetate-aspartate aminotransferase {AAT}, Fig. 2) will ensure that the different keto-acids needed by the plant will be available. Other key enzymes involved in the regulation of C/N balance are asparagine synthetase (AS) and the enzymes of ureide metabolism. How these might act to link nitrogen and the carbon metabolism is indicated in Fig. 2. The GDH-shunt may be particularly important when the plant is carbon limited. This is supported by the work of Aubert *et al*. (Aubert *et al*., 2001) and by studies on GDH genes in *A. thaliana* (Melo-Oliveira *et al*., 1996) that show the expression of one of the GDH genes, *GDH-1*, is at its highest in dark-adapted or sucrose-starved plants. The operation of the shunt is probably also important under conditions of abundant NH_3 nutrition. Such conditions enhance the expression of GDH genes and lead to high levels of GDH activity and, even though the plants may not be carbon-limited overall, NH_3 might be expected to produce local C/N imbalances that require the operation of the GDH-shunt. GDH levels also increase under various stress conditions (Srivastava and Singh, 1987) and again the plant may well need to give priority to carbon metabolism and keto-acid production over nitrogen metabolism, by means of the GDH-shunt, although further evidence is needed to support this hypothesis.

The operation of a GDH-shunt as proposed further predicts that: (1) plants fed glutamate will convert it to NH_3, glutamine and 2-oxoglutarate (this is borne out

by the results of Stewart *et al*., 1995, Stitt *et al*., 2002); (2) plants lacking GDH may have inhibited growth, particularly under stress (this has been observed for GDH-nulls of maize by Pryor, 1990, and Magalhaes *et al*., 1990, and of *A. thaliana* by Melo-Olivera *et al*., 1996); and (3) GDH null mutants would be unable to convert glutamate to NH_3, glutamine and oxoglutarate (the labelling studies of Stewart *et al*., 1995, support this).

The role of GS and the glutamate synthase cycle in NH_3 assimilation is now generally accepted (Oaks, 1994). However, there have been continued suggestions over the last 25 years, that GDH also plays a significant role in NH_3 assimilation. However, as Oaks has remarked 'science is not democratic; it requires experimental proof' (Oaks, 1995). The basis for these suggestions is the presence of the enzyme and the fact that it is easier to assay *in vitro* in the assimilatory direction. The presence of the enzyme suggests it has a role but not what that role is. The key question is which direction does it function *in vivo* within the matrix presented in Fig. 1? It is clear that GDH can catalyse the exchange of NH_3 into the amino group of glutamate (Aubert *et al*., 2001) and that limited synthesis of glutamate can occur in mitochondria (Yamaya and Oaks, 1987). Mitochondria in photorespiring leaves generate large amounts of ammonia in a situation where reductant should not be limiting. Under such circumstances, if GDH is an assimilatory enzyme, it might be expected to provide a major contribution to the reassimilation of ammonia. However, the evidence from biochemical (Keys *et al*., 1978) and genetic (Somerville and Ogren, 1982; Wallsgrove *et al*., 1987) studies indicates it does not. Recent work on conditions that limit the growth of GDH null mutants has also led to the suggestion that GDH functions in ammonia assimilation (Melo-Olivera *et al*., 1996). However, there are problems with this interpretation. Firstly, GS is present and able to function, so there is no clear reason why assimilation of NH_3 should limit growth; secondly, no labelling or inhibitor evidence was presented to support the assimilatory function of GDH. The labelling, and inhibitor studies done with GDH nulls of maize suggest that GDH is not acting in the assimilatory direction (Magalhaes *et al*., 1990). The observed limitations of growth of the GDH null mutants can be explained by the importance of the GDH-shunt (see above) and do not need the invocation of a GDH assimilatory pathway, for which there is a lack of evidence.

In conclusion, the maintenance of carbon and nitrogen balance at the levels required for different metabolic processes in the different parts of the cell and the plant can (at least in part) be achieved by the operation of the reactions outlined in Fig. 2. At the core of these reactions is the glutamate synthase cycle and GDH acting in the deaminating direction. Evidence for this important role of GDH in nitrogen metabolism is accumulating, whereas there is still a lack of convincing evidence for GDH having a significant role in NH_3 assimilation, however attractive this idea might seem. The challenge is now to describe how the GDH-shunt and its associated reactions might operate within the matrix shown in Fig. 1.

GS organization and distribution

Early studies showed that GS was widely distributed in the plant and occurred in two major forms, one in the chloroplast and one in the cytosol. Further work has shown that the pattern of distribution is highly sophisticated. Edwards *et al*. using promoter analysis of the *GS3A* gene of pea, suggested that cytosolic GS is preferentially expressed in the vascular tissue of leaves (Edwards *et al*., 1990). Many subsequent studies have confirmed the importance of the location of GS1 in the phloem and related vascular tissues (Tobin and Yamaya, 2001). However, expression of GS1 in the rest of the leaf may be dependent on the developmental age and species. Brugière *et al*. suggest that cytosolic GS is increasingly expressed in the mesophyll tissues during the ageing of tobacco leaves (Brugière *et al*., 2000). GS1 is also found in the mesophyll cells of barley (Tobin and Yamaya, 2001) and has been isolated from the mesophyll protoplasts of pea (Wallsgrove *et al*., 1979).

Besides its presence in roots and leaves, GS is also localized in a number of specialist tissues and organs involved in the generation and transport of reduced nitrogen. Thus a nodule-specific GS1 isoenzyme is formed during the onset of nitrogen fixation (Lara *et al*., 1983) and one of the maize GS1 genes is preferentially highly expressed in the pedicels of developing kernels (Rastogi *et al*., 1998).

The plastidic form of GS (GS2) is widely distributed in the chloroplast and generally regarded as universal. However, recent studies suggest it may be absent in pine chloroplasts (Avila *et al*., 2001), but this conclusion needs to be substantiated by further work. Its major role in leaves is thought to be reassimilating the NH_3 generated in photorespiration. GS2 is also present in plastids in roots and other non-green tissues; this distribution differs between species and differs with respect to plastid subtypes (Tobin and Yamaya, 2001).

GS is an octameric enzyme and may be either homomeric or heteromeric. This diversity may lead to more sophisticated changes in the nature of GS as the plant and its individual organs pass through different development stages. Thus, Brechlina *et al*. have shown that the GS1 subunit composition of sugar beet changes with respect to N nutrition and organ ontogeny (Brechlina *et al*., 2000). Changes in subunit composition in *Phaseolus vulgaris* leaves are due to the differential expression of the various GS1 genes during development

and ageing (Cock *et al*., 1991). Thus it is important to take the changes in GS1 subunit composition into account when studying the distribution of the enzyme in different tissues and developmental stages. Measurements of the bulk GS1 fraction may fail to reveal the sophistication of the regulatory mechanisms.

GS regulation

The direction and the flux of N through the scheme depicted in Fig. 1 varies in relation to a number of factors depicted in the boxes around the scheme. The nature of the metabolism occurring via GS will depend on the environment of the plant, which may act directly or through the metabolic status of the plant and its different tissues, and varies over the course of the day (Stitt *et al*., 2002). The reactions and the forms of GS involved will differ according to the plant organ under consideration. Within an organ, the role of GS and the metabolism in progress will differ according to the tissue, cell or subcellular compartment. Within any location, the metabolism will differ according to the developmental stage of that part of the plant. In this regard, it is important to realize that developmental stages such as vegetative and reproductive growth, are not linear but overlapping. Thus, the nature of GS and its regulation has to be approached by taking into account the multidimensional nature of nitrogen metabolism and appreciating the large differences that occur in glutamine metabolism between various locations in the matrix. The plant has evolved mechanisms to enable it to cope with this complexity and which enable it to survive in competition with other plants in its environment. In seed plants, this must place the greatest importance on the success of the seed, because mechanisms that do not support effective reproduction will not have been maintained during evolution.

Regulation of GS begins with the GS genes, for which there is now a good description in a number of plants (Forde and Cullimore, 1989; Tingey *et al*., 1988). GS1 is encoded for by a small subfamily of genes that varies in number from three in *A. thaliana* (Peterman and Goodman, 1991) to five in maize (Li *et al*., 1993). Some members of the gene family are expressed in an organ-specific manner; others appear to be less specific. GS2 is encoded by a smaller number of genes, often only one. A number of studies have been made of the promoters of these genes. These have helped to identify the organs in which specific genes are expressed. However, the situation may be more complex than is often revealed by analysing just one promoter fragment from a given gene. Marsolier *et al*. showed that a large fragment of the promoter of *Lotus GS15* gene drove the expression of a chimeric marker gene (GUS) in the nodule and in response to NH_3 nutrition (Marsolier *et al*., 1993). When the promoter fragment was resolved into smaller portions, further elements could be identified that conferred root, pulvinus and anther expression. The conclusion is that the complexity of genes and promoters can combine to give expression in the many and various cells, tissues and organs in which GS is required for plant nitrogen metabolism.

Transcription is clearly a necessary condition for the presence of GS within a cell, but it may not be determining the level of enzyme activity in the cell as other factors play an important part. Thus Cock *et al*. found that the levels of the GS-δ isoenzyme did not correlate with the level of *gln*-δ gene expression during leaf ageing (Cock *et al*., 1991). Ortega *et al*. have shown with transgenic alfalfa plants transformed with GS under the control of the 35S (CaMV) promoter accumulate transcripts without a corresponding increase in the level of enzyme activity (Ortega *et al*., 2001). Habash *et al*. have found that native GS1 is expressed late in the development of wheat flag leaves and that transgenic GS appears to follow the same pattern of expression when under the control of the *rbcS* promoter (Fig. 3) (Habash *et al*., 2001; DZ Habash, unpublished data). This would not be expected if the promoter was determining the expression, since its highest expression is early in leaf development. These results suggest that the post-translational controls that are important in controlling the level of GS in cells may be regulated by the turnover of GS.

Mechanisms that could control the stability and activity of GS have recently been discovered. Moorehead *et al*. demonstrated that GS interacts with 14-3-3 proteins (Moorehead *et al*., 1999) and Finnemann and Schjoerring

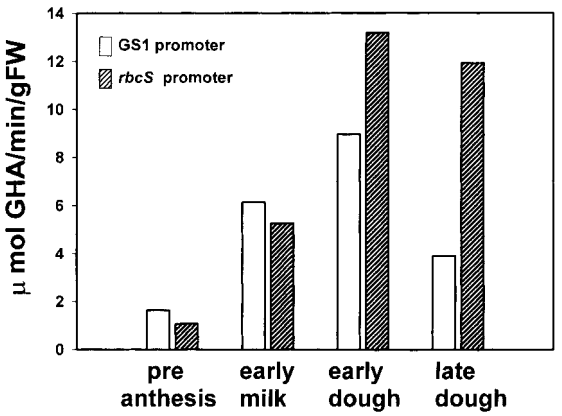

Fig. 3. Calculated GS1 activities of transformed wheat flag leaves at different developmental stages of the grain. GS activities, expressed as μmol γ-glutamyl hydroxamate min^{-1} g^{-1} FW, were determined by the transferase assay after the isoforms were separated by HPLC (according to Habash *et al*., 2001). GS1 activities ascribed to the native GS1 promoter of wheat are those of null segregant controls (open bars) and designated as GS1 promoter. GS1 activities ascribed to the introduced *rbcS* promoter (hatched bars) were calculated by taking the activities of *gln*-α plants and subtracting the activities of their corresponding null segregant controls. Representative results are shown for line 1 and were similar to trends found in two other independent transformed lines.

have recently presented a tentative model for the reversible control of GS1 by phosphorylation and dephosphorylation incorporating the roles of ATP, Mg^{2+} and 14-3-3 binding (Finnemann and Schjoerring, 2000). The model is based on the central role that the ATP/AMP ratio, under light and metabolic regulation, plays in controlling the activity and stability of GS1. It is proposed that in the dark, ATP/AMP levels are high and GS1 is phosphorylated and binds 14-3-3 proteins, which confers protection against degradation. Conversely, in the light, GS1 is unphosphorylated and may be susceptible to damage. New evidence has recently emerged demonstrating that GS2 is also phosphorylated in tobacco (Riedel *et al*., 2001). It is now necessary to characterize the elements of this model, kinases, phosphatases, 14-3-3 proteins, and diurnal changes to enable the precise physiological controls to be determined. This also necessitates care in leaf sampling and GS assay procedures. Such studies will undoubtedly reveal another complex regulation of a major N metabolism enzyme as sophisticated as that of nitrate reductase (Meyer and Stitt, 2001).

Important factors affecting the activity of GS and other enzymes in Fig. 2 are light, carbon status and nitrogen nutrition. Coruzzi and colleagues have described (see Oliveira *et al*., 2001, for a review) the way in which the expression of the genes for GS2 and AS are reciprocally regulated by light and carbon status. The level of GS1 is not significantly affected by light. The light repression of AS may be mediated via the action of phytochrome. Carbon compounds important in stimulating GS2 and GS1 synthesis include sucrose and 2-oxoglutarate. The latter may play a signalling role commensurate with its importance in C/N balance (Fig. 2). Looking for homologues of genes known to be important in the regulation of nitrogen metabolism in other organisms has led to the identification of PII like proteins in *A. thaliana* and *Ricinus communis* (Hsieh *et al*., 1998). PII in *E. coli* is a part of the nitrogen regulatory complex. The exact way in which these homologues work in plants remains to be described. There are also likely to be significant links between the regulation of nitrogen metabolism and regulatory mechanisms, such as the SNF1 protein kinases, involved in sucrose sensing in plants (Halford and Hardie, 1998).

Glutamine as a regulator of N metabolism

There are many studies that suggest that the plant can sense its reduced nitrogen status and regulate the uptake and reduction of nitrate. The question is, which are the metabolites that the plant uses to sense its reduced N status? A definitive answer is not available, however, several lines of evidence have suggested that glutamine may play an important role (Glass *et al*., 2002). However, there are arguments (Stitt *et al*., 2002) that suggest that it may be other amino acids which act as sensors of plant nitrogen status. Resolution of the control loops will require further experimental approaches, particularly identification of the metabolite pools in the multidimensional matrix of Fig. 1 that are effecting the control.

GS and plant development

Recent studies with transgenic plants suggests that altered or overexpression of GS may accelerate development. Thus Vincent *et al*. found with transgenic *Lotus corniculatus* that plants containing a transferred cytosolic GS gene from soybeans flowered prematurely (Vincent *et al*., 1997). Earlier flower and seed development were also observed in transgenic wheat lines containing a *Phaseolus vulgaris* GS1 gene under the control of the *rbcS* promoter (Habash *et al*., 2001; DZ Habash, unpublished data) and in transgenic oil seed rape overexpressing GS1 under the control of 35S (CaMV) promoter (JK Schjoerring, personal communication). Taken together these results suggest that the effects were not due to any chance event occurring during the transformation process, but due to the transformation with the GS gene. One explanation may lie in the link between GS1 and its role in senescent leaves. The extra expression in the later stages of flag leaf development (Fig. 3) may enhance the recovery of N during senescence and signal to the plant that the conditions have been reached for successful seed filling and maturation. There is likely to be a competitive advantage in evolving a mechanism that ensures rapid seed production once the requisite conditions warrant it. The results with wheat (Habash *et al*., 2001, and see below) suggest that there are no adverse side-effects in this early development since the seed weight and protein content are both enhanced in some of the early developing lines. The nature of the signalling mechanism and its transmission are as yet unknown, but presumably involve products of GS activity; this is now receiving further study.

Possible ways to improve the efficiency of nitrogen use in crops

Crop plants have been developed over the last 10 000 years and for most of this time they were not heavily fertilized. However, in the last 50 years the nitrogen fertilization of crop plants worldwide has increased more than 20-fold. The use of this fertilizer is generally inefficient with only about 50% being recovered in the harvested crop. Crop plants did not evolve under conditions of high nitrogen nutrition and many of the mechanisms discussed above are not necessarily suited to growth under such nutrition. The question therefore arises, can we, based on our knowledge and the experimental techniques now available to us, improve the efficiency of

nitrogen use by crop plants? Two ways appear possible, one to make best use of the available variation in nitrogen use characteristics within the gene pool and, the second, to try to introduce new genes which might increase that variation.

Marker-assisted breeding has opened up exciting possibilities for the more effective use of variation within crop gene pools and in searching for further useful variation in the wild relatives of crops (Tanksley and McCouch, 1997). Marker technologies are now being used to analyse crop traits and link them to regions in the genome. In maize, Hirel *et al.* and Masclaux *et al.* have analysed recombinant inbred lines, already assessed for several agronomic traits, for physiological traits such as nitrate content, and nitrate reductase and GS activity (Hirel *et al.*, 2001; Masclaux *et al.*, 2001). Significant variation occurred for all these traits. When the variation in physiological traits and yield components were compared it was found that there was a positive correlation between nitrate content, GS activity and yield. Loci that appeared to govern quantitative traits were determined on the map of the maize genome and the positions of the QTLs for yield components and the locations of the genes for cytosolic GS coincided. Obara *et al.* have followed a similar line of research in rice (Obara *et al.*, 2001). Again, coincidental locations were found for a QTL for a yield trait and a structural gene for GS1. These results suggest that it is possible that GS1 could represent a key component of nitrogen use efficiency and yield. The results of such mapping experiments have also indicated regions of the genome that are important in regulating the activity of GS, but which do not coincide with structural genes for GS. These regions could contain genes important in the control of GS activity. If these genes can be identified they might provide novel information on GS regulation.

The possibility that GS1 genes might affect yield provokes the question as to what might happen if an additional GS1 gene is introduced by transformation. A number of experiments have been done with different species including crop plants (Harrison *et al.*, 2000; Gallardo *et al.*, 1999; Habash *et al.*, 2001). Results with wheat suggest that the addition of an extra GS gene does not lead to an overall increase in the amount of GS. However, detailed analysis of the GS components of wheat leaves show that there is a change in the balance of GS1 and GS2 activity in the flag leaves (Habash *et al.*, 2001; DZ Habash, unpublished data). When some of the transgenic lines were grown in pots to maturity and their productivity analysed, definite phenotypic effects were found. These included changes in developmental pattern and in productivity. One line (line 3) had significantly more roots, more grain and the grain had a higher %N (Habash *et al.*, 2001). Increases were observed in the biomass of shoots and roots of *Lotus corniculatus* transformed with a soybean GS1 under the control of the 35S (CaMV) promoter. By contrast, expression of the same gene behind a *RolD* promoter, which led to more GS 1 in the roots of *Lotus corniculatus*, decreased biomass production (Harrison *et al.*, 2000). Increases in productivity of individual plants have also been described for poplar and oil-seed rape transformed with GS1 (Gallardo *et al.*, 1999; JK Schjoerring, personal communication). Transgenic tobacco plants containing a *gdhA* gene encoding a NADP-GDH from *E. coli* under the control of the 35S promoter have also been produced (Ameziane *et al.*, 2000). They contained high specific activities of NADP-GDH (non-transgenic plants only contained NAD-GDH) and glasshouse and field experiments show the *gdhA* plants produced more biomass at harvest. Whilst the authors suggest that such improvements may be due to the role that GDH may play in cellular homeostasis, more detailed work is necessary

Conclusions

GS plays a central role in nitrogen metabolism. This role is complex and varies according to the context in which the metabolism is taking place. There are multiple regulatory controls at the gene and protein level to modify its activity. It is important to take this complexity into account. GS functions in conjunction with a number of other key enzymes to enable the plant to balance its carbon and nitrogen metabolism in different parts of the cell, at different times of the day, in different organs and under a wide range of environments. There is strong evidence that NAD-GDH also plays an important role in N metabolism. In this role it acts as a shunt to the glutamate synthase cycle to release carbon from amino compounds in the form of keto-acids and to enable the synthesis of compounds with low C/N ratios. Despite many suggestions, convincing evidence that GDH can function *in vivo* in an assimilatory direction is still awaited.

Strategies to improve the nitrogen use efficiency of crop plants are being explored. QTLs have been identified that might lead to crop improvement through manipulation of nitrogen metabolism. A number of transgenic plants with different GS transgenes have been made. Most of these involve relatively unsophisticated control of the transgene expression because of the use of the 35S promoter. Given the complexity of the system, many different approaches may need to be tried to obtain robust results. So far studies have only been done on individual plants in glasshouses. Crop improvement is dependent of the behaviour of populations in fields and this might differ significantly from the behaviour of individual plants. Nevertheless, the initial results are sufficiently encouraging to suggest that the manipulation of N metabolism via transformation is worthwhile.

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