Review

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Cytosolic glutamine synthetase: a target for improvement of crop nitrogen use efficiency?

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Overexpression of the cytosolic enzyme glutamine synthetase 1 (GS1) has been investigated in numerous cases with the goal of improving crop nitrogen use efficiency. However, the outcome has generally been inconsistent. Here, we review possible reasons underlying the lack of success and conclude that GS1 activity may be downregulated via a chain of processes elicited by metabolic imbalances and environmental constraints. We suggest that a pivotal role of GS1 may be related to the maintenance of essential nitrogen (N) flows and internal N sensing during critical stages of plant development. A number of more refined overexpression strategies exploiting gene stacking combined with tissue and cell specific targeting to overcome metabolic bottlenecks are considered along with their potential in relation to new N management strategies.

Plant nitrogen use efficiency and the role of glutamine synthetase

Nitrogen (N) is one of the major plant nutrients limiting crop production worldwide. In many parts of the world the availability of N fertilizers is limited, whereas in other parts of the world too much N fertilizer is applied, leading to serious negative environmental consequences [1]. Hence, there is a demand to optimize the use of N fertilizers to make agriculture more sustainable. One such optimization is the improvement of plant N use efficiency (NUE), which in this context is defined as yield of plant biomass per unit N available for uptake [2,3].

For decades, glutamine synthetase (GS; EC 6.3.1.2) has been a major focus area in plant research owing to its essential role in the assimilation of inorganic N [4–6]. Plant GS occurs in most species as a single isoform in plastids (GS2) and as three to five isoforms localized in the cytosol (GS1) [7]. Cytosolic GS1 is important for primary NH_4^+ assimilation in roots and for reassimilation of NH_4^+ generated during protein turnover in leaves, whereas the dominating role of GS2 is in reassimilation of photorespiratory NH_4^+ in the chloroplasts and assimilation of NH_4^+ deriving from NO_3^- reduction in plastids [8] (Figure 1A). In leaves of C3 plants, GS2 activity is normally higher than that of GS1, although the ratio decreases with progressing senescence as chloroplasts are degraded [4,9].

In *Arabidopsis* (*Arabidopsis thaliana*), GS1 is encoded by five individual isogenes with distinct tissue localization and expression patterns as well as distinct affinities to NH_4^+ and glutamate [10–12]. Phylogenetically, the nucleotide and amino acid sequences of the *Arabidopsis GS1* isogenes do not cluster with the *GS1* sequences from cereals (Figure 1B) and, hence, the function of *Arabidopsis* and cereal *GS1* genes and their corresponding proteins may not be directly comparable, highlighting the importance of studying both model and crop species [13].

Quantitative trait locus (QTL) analyses performed in several cereal species have suggested that genotypic differences in NUE can partly be explained by *GS* loci. In maize, QTLs for grain yield and thousand grain weight colocalize with the *GS1* isogene *ZmGln4* (i.e., *ZmGln1;3*) at both high and low levels of N supply [14]. In rice, *OsGS1* colocalizes with a QTL region for yield of single spikelets [15]. A QTL for flag leaf GS activity in wheat (*Triticum aestivum*) has been mapped to the *TaGSr* locus (a *GS1* isogene) and colocalizes with a QTL for grain N concentration [16]. Another wheat *GS1* gene *TaGSe* (*GS1.3*) is associated with QTLs for grain protein content [17]. In senescing leaves, the cytosolic isoform *TaGS1.1* in wheat [9,18] as well as *BnaGLN1.1* and *BnaGLN1.4* in oilseed rape (*Brassica napus*) [19] are upregulated. GS1 polypeptides and NH_4^+ assimilation furthermore shift from phloem companion cells to mesophyll cells during senescence, highlighting the important role of GS1 for N remobilization to generative plant parts [9,20–22].

Expression analyses and knockout studies of individual *GS* isogenes have demonstrated that they have cell and tissue specific localizations and play essential roles in plant development and yield structure in cereal species. For example, in maize (*Zea mays*), the leaf-localized *GS1.1* isogenes *ZmGln1;3* and *ZmGln1;4* are of specific importance for the development of the cob with respect to kernel number and kernel size, respectively [23,24]. These specific functions seem to reflect differences in the cellular localization and temporal expression pattern of the two genes. *ZmGln1;3* is constitutively expressed in the mesophyll cells until plant maturity, suggesting a primary role in

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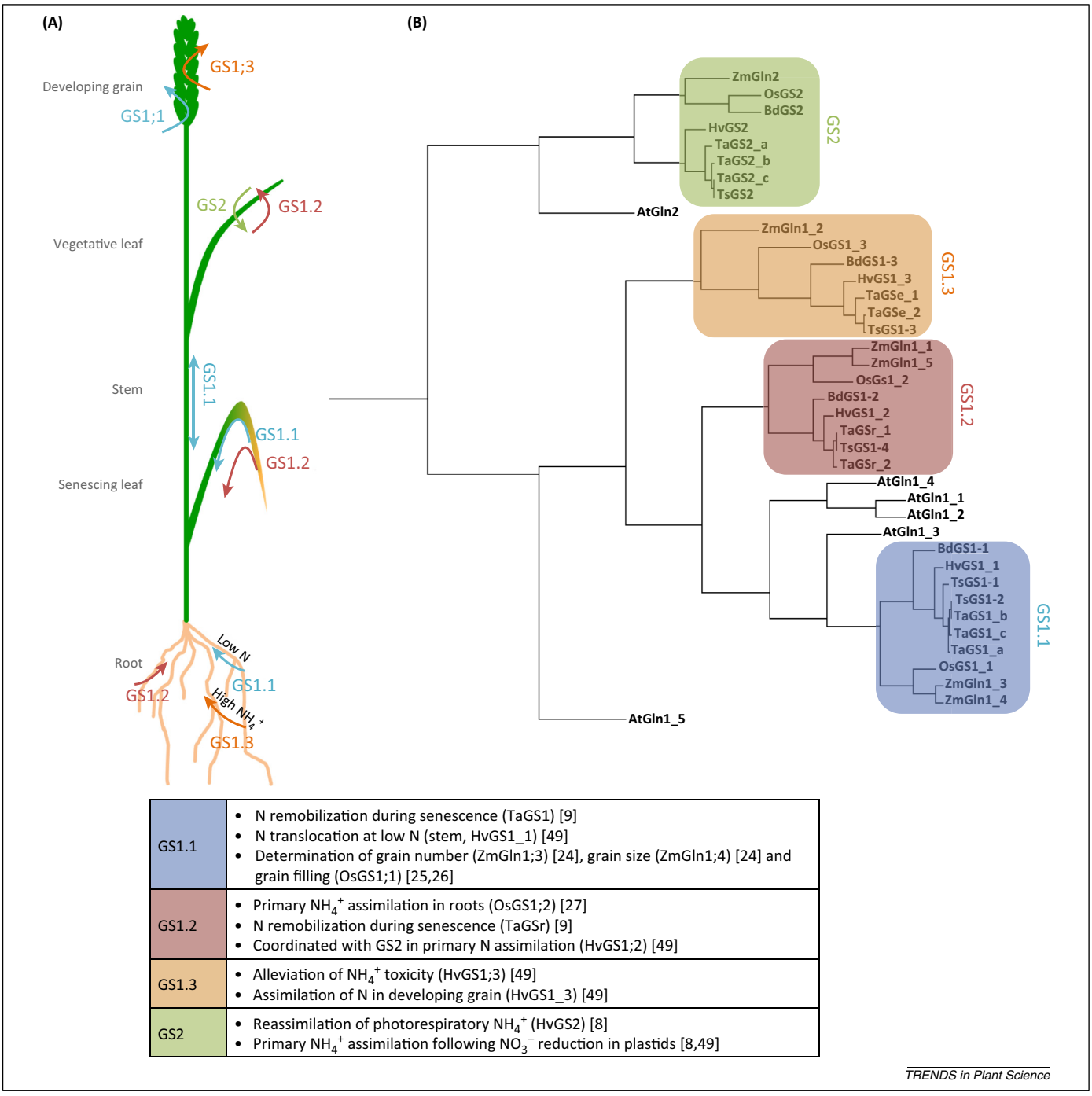

Figure 1. **(A)** Overview of glutamine synthetase (*GS*) protein isoforms. Tissue localization of *GS* isogenes and their proposed involvement in fundamental plant processes. **(B)** Phylogenetic tree showing clustering of individual GS protein isoforms of cereals and *Arabidopsis*. Sequences were obtained by searching the National Center for Biotechnology Information (NCBI) database using various search terms (e.g., glutamine synthetase, species, and gene name). Multiple alignment was performed using Clustal Omega and a subsequent phylogenetic tree was generated in ClustalW2 phylogeny. AtGln1;1 (AED94209.1); AtGln1;2 (AEE34476.1); AtGln1;3 (AEE76011.1); AtGln1;4 (AED92312.1); AtGln1;5 (AEE32297.1); AtGln2 (Q43127.1); BdGS1-1 (XP_003570690.1); BdGS1-2 (XP_003558466.1); BdGS1-3 (XP_003559528.1); BdGS2 (XP_003580719.1); HvGS1_1 (AFX60875.1); HvGS1_2 (AFX60876.1); HvGS1_3 (AFX60877.1); HvGS2 (P13564.2); OsGS1;1 (BAA95678.1); OsGS1;2 (BAD77931.1); OsGS1;3 (BAD99301.1); OsGS2 (P14655.1); SxTGS1_1 (AEM42903.1); SxTGS1_2 (AEM42900.1); SxTGS1_3 (AEM42901.1); SxTGS2 (AEM42904.1); TaGS1_a (AAZ30057.1); TaGS1_b (AAZ30058.1); TaGS1_c (AAZ30059.1); TaGSr_1 (AAR84347.1); TaGSr_2 (AAR84348.1); TaGSe_1 (AAR84349.1); TaGSe_2 (AAR84350.1); TaGS2_a (AAZ30060.1); TaGS2_b (AAZ30061.1); TaGS2_c (AAZ30062.1); TsGS1-1 (JN188397.2); TsGS1-2 (JN188396.1); TsGS1-3 (JN188395.1); TsGS1-4 (JN188394.1); TsGS2 (JN188398.2); ZmGln1;1 (P38559.1); ZmGln1;2 (CAA46720.1); ZmGln1;3 (CAA46721.1); ZmGln1;4 (CAA46722.1); ZmGln1;5 (P38563.2); ZmGln2 (CAA46724.1).

the synthesis of glutamine based on NH_4^+ from NO_3^- reduction [24]. By contrast, *ZmGln1;4* expression is induced during senescence and confined to the bundle sheath cells, indicating a function in the reassimilation of NH_4^+ released during Rubisco degradation [24]. The importance of the spatial distribution and expression of individual *GS* isogenes is also evident from studies in rice (*Oryza sativa*). Knockout of *OsGS1;1*, which localizes to vascular tissues of mature leaves, results in reduced growth and grain filling [25,26]. *OsGS1;2* is expressed in root surface cells and has recently been identified as being important for primary root NH_4^+ assimilation [27]. In the *OsGS1;1* knockout mutant, the isogenes *OsGS1;2* and *OsGS1;3* are not able to compensate for the loss of *OsGS1;1*, which suggests that