Plant Physiol. (1996) 111: 1109–1117

The Effect of Nitrogen Nutrition on the Cellular Localization of Glutamine Synthetase Isoforms in Barley Roots[1]

Lucy J. Peat and Alyson K. Tobin*

Plant Sciences Laboratory, Sir Harold Mitchell Building, School of Biological and Medical Sciences, University of St. Andrews, St. Andrews, United Kingdom KY16 9TH

Glutamine synthetase (GS) was detected by immunogold localization in the cytosol and plastids of roots of 7-d-old barley (*Hordeum vulgare* L. cv Klaxon) seedlings grown in the presence or absence of NO_3^- (15 mM) or NH_4^+ (30 mM). The number of GS polypeptides changed during root development, and this was affected by N nutrition. There was no evidence of a NO_3^--inducible root plastid GS. In apical 5- to 10-mm regions of the root the concentration of immunogold labeling of cytosolic GS was higher in the cortical parenchyma than in the vascular cells of the stele, irrespective of N nutrition. This labeling was at least 50% higher in both cell types in N-free compared with N-grown (either NO_3^- or NH_4^+) seedlings. In contrast, GS specific activity was highest in roots of NO_3^--grown seedlings. It is suggested that this indicates the presence of inactive GS in roots grown without N. This study has identified both cell- and development-specific responses of GS to N nutrition.

The role of GS (EC 6.3.1.2) in the GS/glutamate synthase cycle, which assimilates the majority of ammonia into Gln and glutamate, makes it an ubiquitous enzyme in higher plant cells (McNally et al., 1983; Oaks and Hirel, 1985). The enzyme occurs as several distinct isoforms: a plastidic form (GS2), a cytosolic form (GS1), a root isoform distinct from GS1 (Vézina and Langlois, 1989; Sakakibara et al., 1992; Marttila et al., 1993), and specific cytosolic root nodule forms (Cullimore and Miflin, 1984; Lea et al., 1991).

GS2 is probably a stromal protein of plastids (Brangeon et al., 1989; Pereira et al., 1992), and in leaves of C_3 plants, it plays a major role in the reassimilation of ammonia produced during photorespiration (Wallsgrove et al., 1987). The roles of leaf and root GS1 isoforms are still under investigation. Recently, the distinct and separate roles of GS1 and GS2 in leaves have been emphasized by evidence that GS1 is preferentially localized in phloem companion cells and other vascular tissues, suggesting a role in the synthesis of Gln for transport to sink tissues (Botella et al., 1988; Edwards et al., 1990; Carvalho et al., 1992; Kamachi et al., 1992; Pereira et al., 1992).

In barley (*Hordeum vulgare* L.), two isoforms of GS, GS1 and GS2, were initially identified in leaves and roots (Mann et al., 1979). However, Marttila et al. (1993) recently identified a second root GS isoform on western blots, and Mäck (1995) has isolated two cytosolic isoforms from leaves and roots. The ultrastructural localization of GS in barley has not been investigated to date, although Fentem et al. (1983) found that GS had higher activities in root tips, and Mann et al. (1980) found varying ratios of GS1:GS2 in different parts of mature barley plants.

The expression and synthesis of GS isoforms is a complex process that differs between species (McNally et al., 1983) and is influenced by plant development (Tobin et al., 1985; Walker and Coruzzi, 1989; Marsolier and Hirel, 1993), spatial (Cullimore et al., 1984) and temporal factors (Pearson and Ji, 1994), and external stimuli such as light (Hirel et al., 1982) and available N forms (Hirel et al., 1987; Miao et al., 1991; Stanford et al., 1993).

Given that N nutrition can influence whether N is assimilated in the root or shoot (Andrews, 1986) and also the form in which it is transported in the plant (Mann et al., 1979; Lewis et al., 1982; Imsande and Touraine, 1994), it may influence the relative distribution of GS isoforms. Although previous studies have identified NO_3^-- and NH_4^+-inducible GS isoforms (Emes and Fowler, 1983; Vézina and Langlois, 1989; Sakakibara et al., 1992), to our knowledge there have been no studies to date about the cellular localization of these responses. In this study we have used immunogold electron microscopy to investigate how N nutrition affects the cellular localization of GS isoforms in barley roots.

MATERIALS AND METHODS

Plant Growth

Barley (*Hordeum vulgare* L. cv Klaxon) seedlings were grown hydroponically using constantly aerated Long Ashton complete nutrient solution (Hewitt, 1966) modified to contain either no N, 15 mM KNO_3, or 15 mM $(NH_4)_2SO_4$. Where applicable, antibacterial and fungicidal reagents (0.002% [v/v] Myxazin and 0.002% [v/v] Protozin, respec-

[1] This work was supported by grant no. P02582 from the Biotechnology and Biological Sciences Research Council.

* Corresponding author; e-mail at6@st-andrews.ac.uk; fax 44–1–334–463375.

Abbreviations: GS, Gln synthetase; GS1, cytosolic Gln synthetase; GS2, chloroplastic Gln synthetase; NIS, nonimmune serum.

tively; Waterlife Research Ltd., West Drayton, UK) were added. Plants were grown in a controlled-environment cabinet (Sanyo Gallenkamp, Loughborough, Leicestershire, UK). Growth conditions were a 16-/8-h, 20/10°C light/dark period, RH of 80%, and a light intensity of 220 μmol m^{-2} s^{-1}. Nutrient solutions were changed every 24 h, and $CaCO_3$ was added to NH_4^+ solutions to avoid pH fluctuations. Seven-day-old seedlings were used for experiments, since we determined, by daily measurement of primary leaves and roots, that 7 d was the period of maximum growth rate. Mean root lengths were obtained from 30 individual seedlings from 3 separate experiments.

Electron Microscopy Preparation

Roots were harvested from three randomly selected seedlings and sampled at 5 to 10 mm distal to the tip. The tissue was fixed overnight at 4°C in 2.5% (v/v) glutaraldehyde, 0.05 M sodium cacodylate, pH 7.3. Tissue was dehydrated in ethanol series and infiltrated with LR White resin (London Resin Co, London, UK) at less than 0°C. Polymerization was at −20°C under UV light for 24 h, using 0.5% (w/v) Lowicryl K4M initiator (Chemische Werke Lowi, Waldkraiburg, Germany).

Silver-gold ultrathin sections were cut from three blocks per growth treatment using a Reichert (Vienna, Austria) ultramicrotome and placed onto Formvar (TAAB Laboratories, Aldermaston, UK) and carbon-coated nickel grids. After immunolabeling, grids were stained with 2% (w/v) uranyl acetate and 0.3% (w/v) lead citrate and examined on a Hitachi (Tokyo, Japan) 600 transmission electron microscope at 70 kV. Tissue that was fixed and stained with 1% (w/v) osmium tetroxide for structural observation showed good general preservation with little tissue shrinkage or membrane distortion.

Immunolabeling

The antiserum used in this study was raised against *Phaseolus* nodule GS, and it recognizes all GS isoforms tested from a wide range of higher-plant species (Cullimore and Miflin, 1984). On a western blot of a whole primary leaf extract of barley, the antiserum recognized two clear bands of approximately 42 and 44 kD, corresponding to GS1 and GS2, respectively (Fig. 1). Before incubation with antiserum, grids were floated onto 0.5 M NH_4Cl, followed by blocking buffer (10 mM sodium phosphate, 3% [w/v] NaCl, 1% [w/v] globulin-free bovine albumin, 0.1% [v/v] Tween 20, pH 7.3) for 1 h. GS antiserum was used at a dilution of 1:400 in blocking buffer for 4 h, followed by at least three 5-min washes in blocking buffer. Incubation with secondary antibody (goat anti-rabbit, 15-nm gold conjugate; Biocell, Cardiff, UK) occurred overnight at 4°C. Control sections were incubated with rabbit NIS (Sigma) also diluted at 1:400 with blocking buffer.

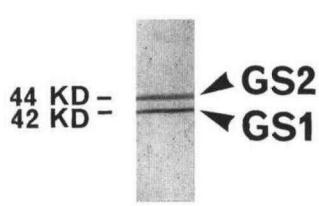

Figure 1. Barley leaf GS isoforms. Western blots of barley leaves were probed with GS antiserum, which revealed two bands. The lower band, at approximately 42 kD, corresponds to GS1 (cytosolic), and the upper band, at approximately 44 kD, corresponds to GS2 (chloroplastic). Twenty micrograms of protein was loaded per well.

Quantification of Immunogold Labeling

Random micrographs (10–30 for each cell type) were taken at a magnification of ×20,000. Immunogold label was quantified by counting gold particles, and areas of cell compartments were calculated by tracing, cutting, and weighing to give final mean values of gold label μm^{-2}. Data were analyzed using Minitab (State College, PA) software employing a one-way analysis of variance to each set of data. Data sets showing some variation from the group mean were then subject to a Student's t test with significant differences at $P \leq 0.05$.

GS Extraction and Assay

For each enzyme extract, 1-cm segments were taken from the roots of 20 individual seedlings, ground to a powder in liquid N_2 with a mortar and pestle, and then extracted in 0.5 mL of GS extraction buffer (25 mM Trizma base, 1 mM EDTA, 1 mM DTT, 1 mM 2-mercaptoethanol, 1 mM reduced glutathione, 10 mM $MgSO_4 \cdot 7H_2O$, 5 mM glutamate, pH 8.0). The extract was centrifuged at 12,500g for 20 min in a microfuge (Beckman model E). The supernatant was assayed using the GS synthetase and transferase assays (Rhodes et al., 1975) modified for microassay (200 μL of total reaction volume). A_{540} of γ-glutamyl hydroxamate was measured in a microplate reader (model MR5000, Dynatech Laboratories Ltd., Billingshurst, UK). Protein was assayed using the Bradford (1976) method (Bio-Rad) with thyroglobulin as standard.

Analysis of GS Polypeptides by Western Blotting

One-centimeter root segments from 20 seedlings were ground to a powder in liquid N_2 and extracted in 2.0 mL of 2.5% (w/v) SDS, 100 mM sodium phosphate, 0.5 mM EDTA, 1 mM 2-mercaptoethanol, 10 μM leupeptin, pH 7.7. Proteins (40 μg/well) were separated by SDS-PAGE (Laemmli, 1970) using 10% acrylamide gels and the Bio-Rad mini Protean II system. Protein transfer to nitrocellulose was done by the method of Towbin et al. (1979) using a Bio-Rad mini Trans-blot.

GS polypeptides were detected by incubation with GS antiserum (1:750 dilution) followed by goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma). The final color reaction used nitroblue tetrazolium/bromo-chloro-indolyl phosphate in sodium bicarbonate (pH 9.8) (Mierendorf et al., 1987).

RESULTS

General Plant Growth

Roots grown without N were consistently longer after d 5 (Fig. 2A) and appeared to develop lateral roots before those grown in other solutions. Roots grown with NH_4^+ tended to be straighter, whereas the NO_3^--grown roots appeared to have denser root hair growth at 7 d.

Soluble protein content (mg g^{-1} fresh weight) of 1-cm segments of roots differed between seedlings grown on different N-containing media (Fig. 2B). In all treatments the protein content increased toward the root tip. N-free-grown roots, however, showed a much larger increase in protein content at the root tip than the other roots.

Structural Observations

For the immunolocalization study, data were collected from two tissue types at 5 to 10 mm distal to the root tip: the vascular tissue, comprising xylem, phloem, vascular parenchyma, and pericycle, which is bound by the endodermis; and the surrounding cortex, which consists of several layers of cortical parenchyma cells (Fig. 3a). The single, outer layer of epidermal cells was not analyzed in the present study because of the difficulty in achieving good structural preservation.

Cells of the cortical parenchyma are highly vacuolate, with only a thin layer of cytoplasm, whereas vascular cells of the stele often appear to have a greater area of cytoplasm in the cross-section (Fig. 3, b and c).

Cellular Localization of GS: Quantification of Immunogold Labeling

Cortical parenchyma and vascular tissue showed significant levels of immunogold labeling with GS antiserum in both the cytosol and the plastid (Fig. 3, b and c). Background labeling of other cell compartments, e.g. nucleus and mitochondria, was extremely low, on the order of 0.3 gold particles μm^{-2}, which is similar to that found in control sections using NIS (Fig. 3d).

Within each growth treatment, mean cytosolic immunolabeling was significantly higher ($P \leq 0.05$) in the cortical parenchyma than in the vascular cells. In both cortical and vascular cells the mean density of cytosolic immunolabeling was significantly lower ($P \leq 0.05$) by at least 50% with growth on either 15 mM NO_3^- or 30 mM NH_4^+ (Fig. 4A). Given that the vascular tissue comprises a number of different cell types, it is possible that there may be differences in GS localization within this tissue. Our preliminary observations are that cytosolic immunolabeling is higher in the pericycle than in phloem companion cells, whereas immunolabeling is lowest in the metaxylem and xylem parenchyma (not shown).

The mean immunolabeling density of plastids from cortical parenchyma and vascular cells was significantly higher than that of NIS control sections in all N treatments, with the exception of vascular plastids of 15 mM NO_3^--grown plants (Fig. 4B). The level of immunolabeling of plastids was significantly higher ($P \leq 0.05$) in the cortical parenchyma than in the vascular tissue of both N-free and NO_3^--grown plants but not in NH_4^+-grown plants, where plastid labeling was the same in both cell types (Fig. 4B). Although growth on NO_3^- did not alter the labeling density of cortical plastids compared with those of N-free-grown plants, growth on NH_4^+ resulted in a large decrease in plastid GS immunolabeling.

The large SE values in some of these plastid samples, together with our own observations, lead us to suggest that there are different populations of plastids within the

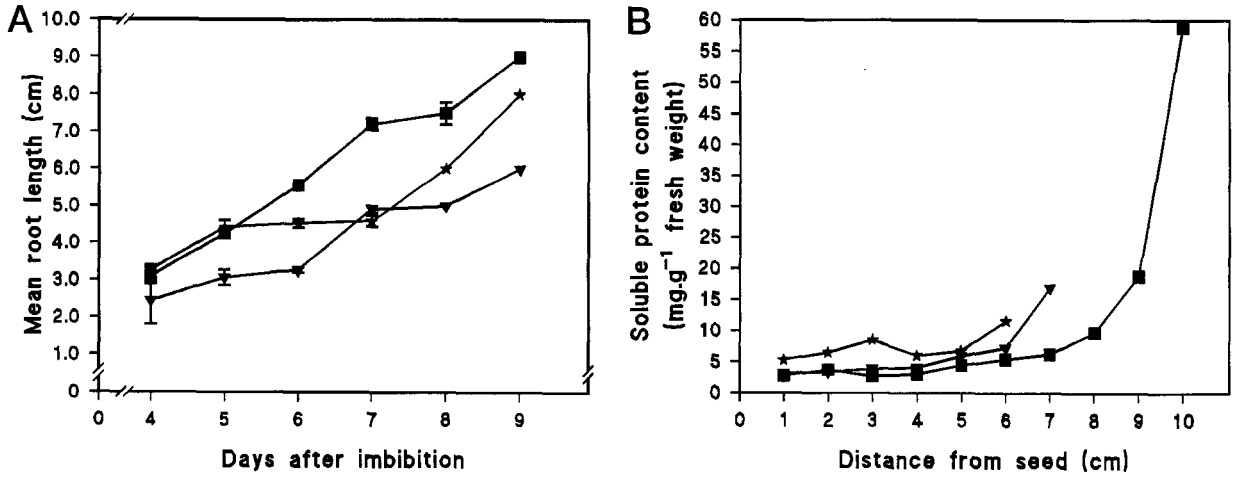

Figure 2. A, Barley root growth with different N media. Seedlings were grown hydroponically on modified Long Ashton solution containing no N (■), 15 mM KNO_3 (▼), or 15 mM $(NH_4)_2SO_4$ (★). Mean root measurements shown are from one experiment, $n = 30$; error bars represent ± SE where appropriate. Two replicate experiments revealed a similar pattern of growth under these conditions. B, Soluble protein content of roots grown with different N media. Protein was measured in extracts of 1-cm root segments along the entire length of roots of 7-d-old barley seedlings grown with no N (■), 15 mM KNO_3 (▼), or 15 mM $(NH_4)_2SO_4$ (★). The number of segments differs between treatments because of the difference in final root length.

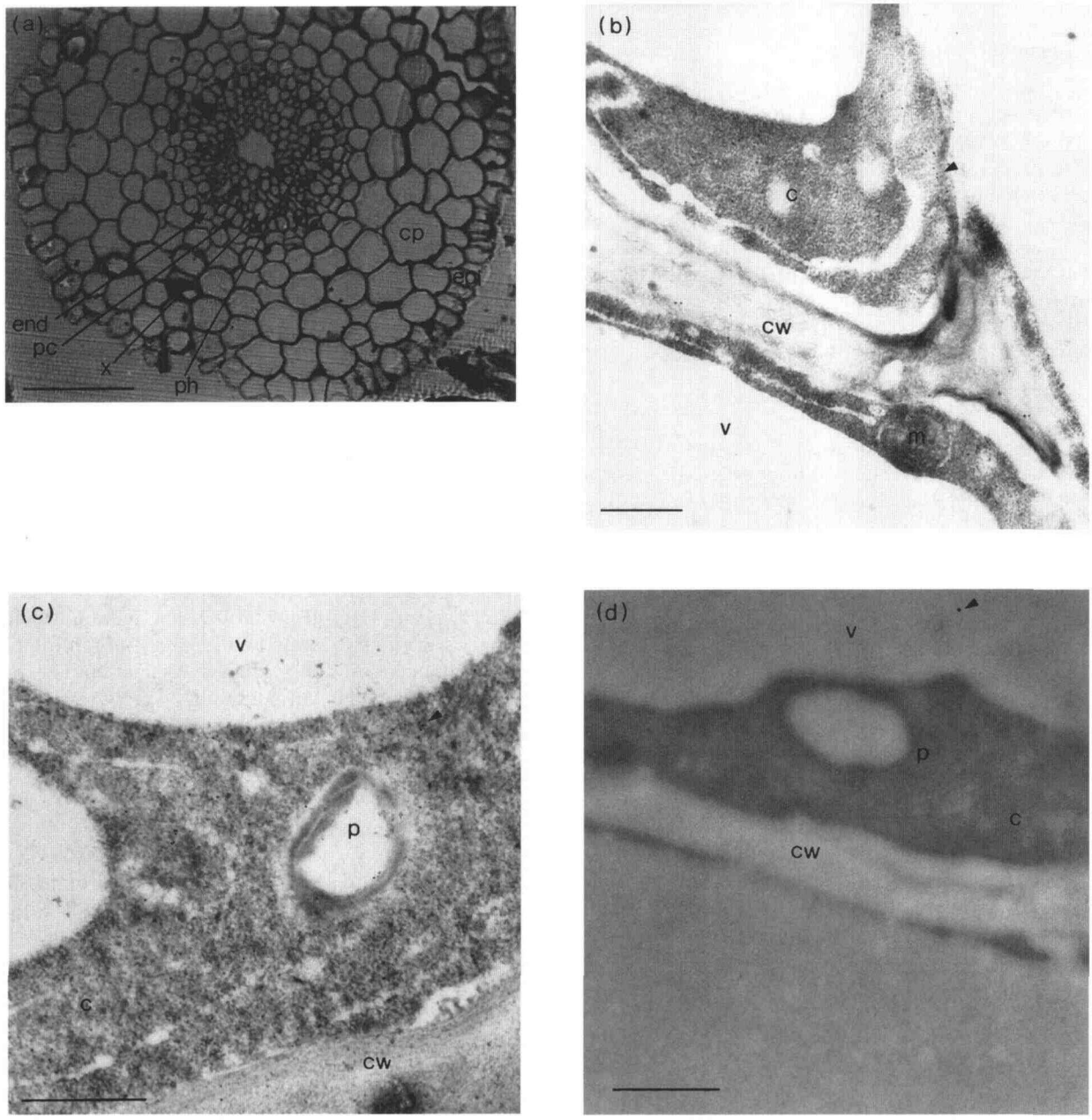

Figure 3. a, Barley root morphology in cross-section. Tissue is from a seedling grown with 15 mM KNO_3 and is representative of that used for immunogold studies, sampled from 5 to 10 mm distal to the tip. Contrast was obtained with 0.1% toluidine blue. epi, Epidermis; cp, cortical parenchyma; end, endodermis; pc, pericycle; ph, phloem; x, xylem. Bar = 0.1 mm. b to d, Immunogold localization of GS in barley roots. p, Plastid; c, cytosol; cw, cell wall; m, mitochondrion; v, vacuole. Arrowheads indicate gold label. Bars = 0.5 μm. b, Cortical parenchyma cell from a seedling grown with NO_3^-. GS is immunolabeled throughout the cytosol. Note the lack of immunolabeling on the mitochondrion and vacuole and low immunolabeling of the cell wall. c, Vascular pericycle cell from a seedling grown without N. Note the low immunolabeling of the starch-containing plastid and cell wall and high immunolabeling of the cytosol. d, Control section from a seedling grown with NH_4^+, incubated with rabbit NIS. Note the low amount of gold label.

root. Our preliminary observations are that these forms of plastids occur in a range of different cell types. Some of the plastids have well-defined starch grains (see Fig. 3c) and tend to be less densely immunolabeled; others appear to contain either globular membranous regions (Fig. 5a) or a single internal lamella (Fig. 5b), and these plastids are generally more intensely immunolabeled. Another group has a level of immunolabeling similar to that of the starch-containing plastids but has no discernible internal organization or starch grains (Fig. 5c). More work is necessary to establish these differences in plastid populations.

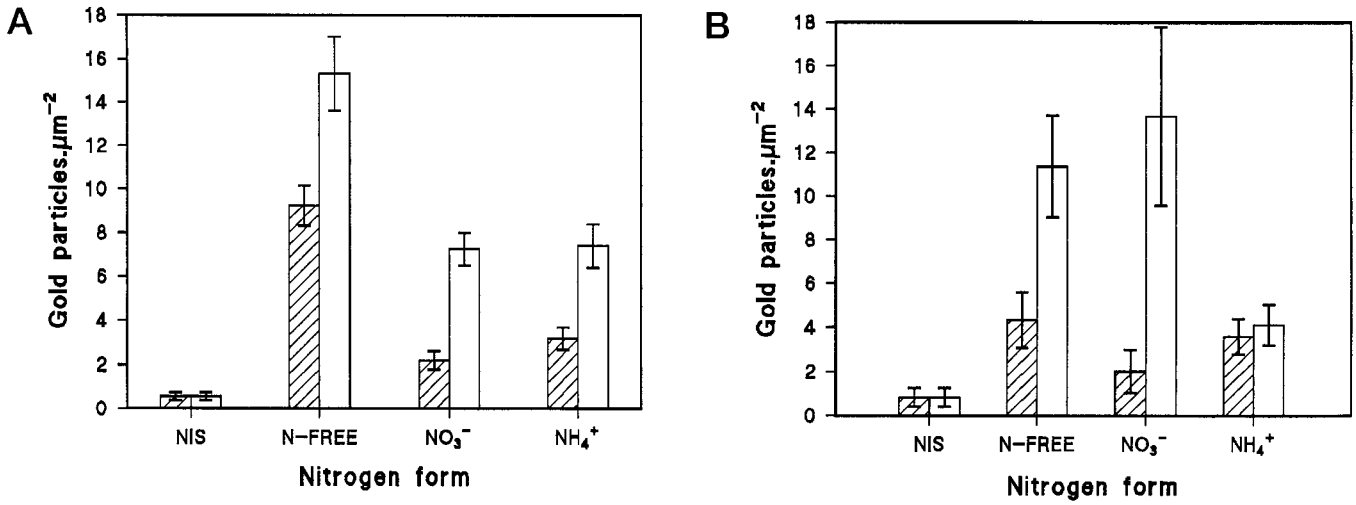

Figure 4. A, Quantification of cytosolic GS immunolabeling of root vascular and cortical parenchyma cells. Barley was grown for 7 d with no N, 15 mM KNO_3, or 15 mM $(NH_4)_2SO_4$. Ten to 30 micrographs of each cell type were taken of sections from 5 to 10 mm distal to the root tip and analyzed as described in "Materials and Methods." Values represent the mean immunolabeling density of the cytosol of vascular (▨) and cortical (□) cells ± SE. Control sections were stained with rabbit NIS. B, Quantification of plastid GS immunolabeling of root vascular and cortical parenchyma cells. Barley was grown for 7 d with no N, 15 mM KNO_3, or 15 mM $(NH_4)_2SO_4$. Ten to 30 micrographs of each cell type were taken of sections from 5 to 10 mm distal to the root tip and analyzed as described in "Materials and Methods." Values represent the mean immunolabeling density of the plastids of vascular (▨) and cortical (□) cells ± SE. Control sections were stained with rabbit NIS.

The Effects of N Nutrition on GS Polypeptides during Root Development

Western blot analysis of 1-cm root segments indicates a change in GS polypeptide content with root development (Fig. 6). Protein degradation during extraction was kept to a minimum by grinding the tissue directly in SDS (see "Materials and Methods"). In all extracts the upper band at approximately 42 kD (Fig. 6) corresponded to the GS1 protein from barley leaves (cf. Fig. 1). Up to three distinct bands were visible in sections of roots grown on NO_3^- or NH_4^+ (Fig. 6). NH_4^+-grown roots appeared to have three polypeptides in the five basal sections and only two at the tip (Fig. 6). NO_3^--grown roots showed two discernible bands in all five root sections, which showed a marked decrease in intensity at the root tip (Fig. 6). N-free-grown roots showed two bands in the basal sections, the lower-molecular-mass polypeptide of which gradually decreased toward the root tip (Fig. 6). In the root tip section, sampled for immunogold labeling, GS polypeptides were least abundant in NO_3^--treated plants. When plants were grown on media containing antibacterial and fungicidal agents (see "Materials and Methods"), there was no change in either the number or the intensity of the bands (not shown). This suggests that the proteins are all of plant origin.

The Effect of N Nutrition on GS Activity

Root segments were assayed for GS activity. Transferase and synthetase assays were performed but because of the very small amount of tissue used per assay (<0.2 g fresh weight), the sensitivity of the transferase assay was judged to be more representative of actual activities, and the synthetase assay was used only for comparative purposes.

N appeared to affect the GS transferase activity of root segments as expressed on a protein basis (Fig. 7A). NO_3^--grown roots showed the highest rates of activity, with a relatively constant rate along the root. N-free and NH_4^+-grown roots have similar activities with decreasing rates toward the root tip, the earlier decline in activity in NH_4^+-grown roots being due to their shorter final length (Fig. 7A). These activities gave a much different profile when expressed on a fresh weight basis (Fig. 7B). Whereas in NH_4^+-grown roots GS activity remained relatively constant along the root, in N-free and NO_3^--grown roots the activity increased markedly to a similar value toward the tip. The earlier rise in GS activity in NO_3^--grown roots is due to the shorter length of these compared with N-free-grown roots.

DISCUSSION

The small number of previous immunolocalization studies of GS in roots have been restricted to legumes, and no comparisons have been made between different types of root cells (Brangeon et al., 1989; Datta et al., 1991). To our knowledge, our study is the first to use quantitative immunogold electron microscopy to investigate the intercellular localization of GS in cereal roots and to examine how this is influenced by N.

Since the growth of barley seedlings was influenced by N, as reported previously (Dale et al., 1974), it was important to distinguish between a direct effect of N on root GS content and an indirect effect resulting from altered root

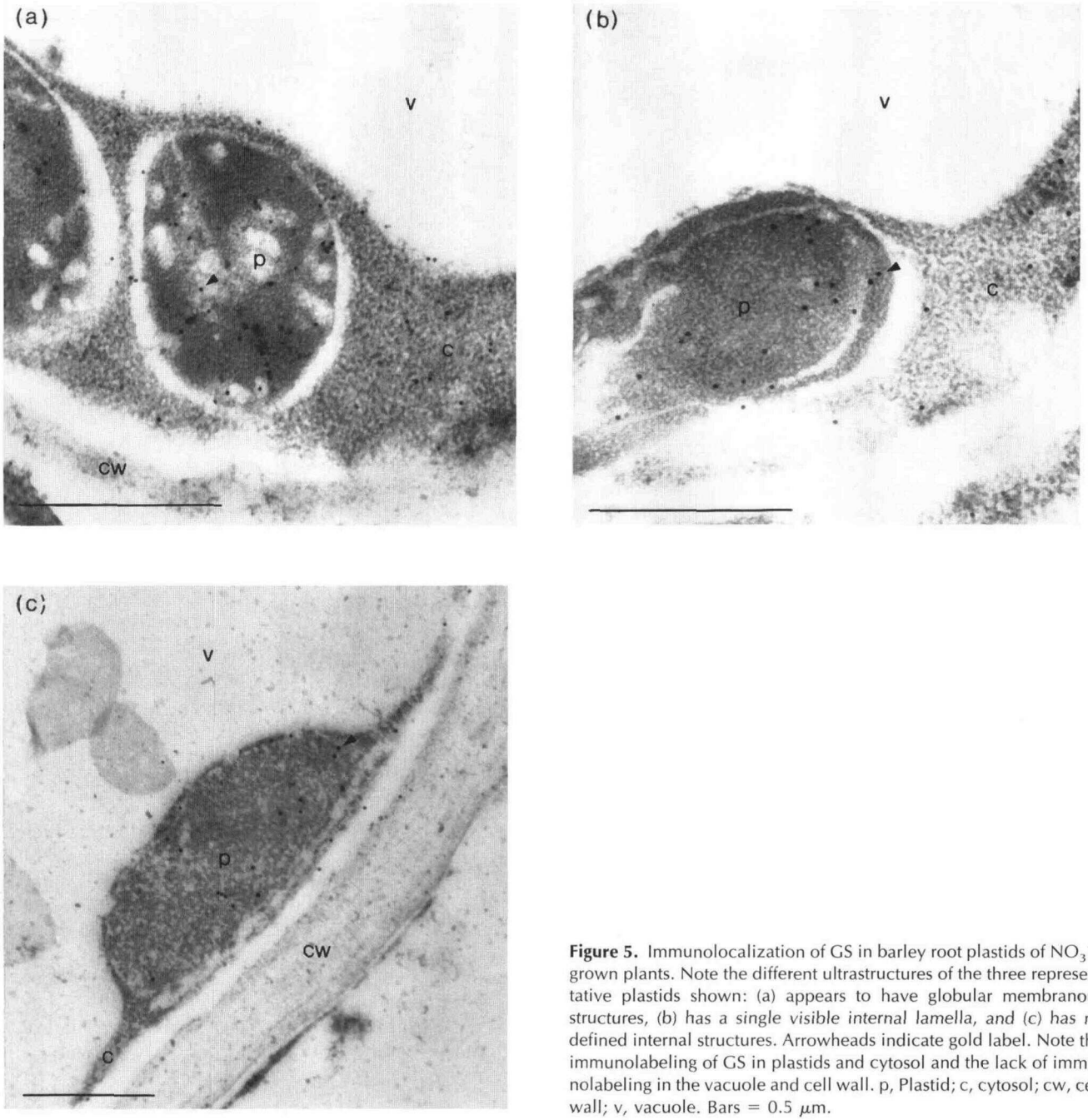

Figure 5. Immunolocalization of GS in barley root plastids of NO_3^--grown plants. Note the different ultrastructures of the three representative plastids shown: (a) appears to have globular membranous structures, (b) has a single visible internal lamella, and (c) has no defined internal structures. Arrowheads indicate gold label. Note the immunolabeling of GS in plastids and cytosol and the lack of immunolabeling in the vacuole and cell wall. p, Plastid; c, cytosol; cw, cell wall; v, vacuole. Bars = 0.5 μm.

development. Developmental influences are illustrated by the observed changes in GS activity and polypeptide composition along the length of the root. The number of GS polypeptides detected on western blots increased with root cell age in all N treatments, and this is analogous to the increase in GS2 activity with advancing cell age in wheat leaves (Tobin et al., 1985). Multiple isoforms of GS have been detected previously in barley roots (Marttila et al., 1993; Mäck, 1995). Although our immunolocalization detected GS in the plastids, none of the observed GS polypeptides corresponded to the molecular mass of barley chloroplast GS, which implies that either root plastid GS has a different molecular mass than leaf GS2, or it is present in amounts too small to be detected by this method, and the other polypeptides present on western blots are cytosolic in origin. This result requires further corroboration. In addition to these developmental effects, the presence of a third polypeptide only in the basal sections of the NH_4^+-grown roots suggests a specific induction by NH_4^+ in mature cells. N-inducible GS isoforms have been identified in maize roots (Sakakibara et al., 1992) and pea root plastids (Emes and Fowler, 1983; Vézina and Langlois, 1989). Previous studies have reported conflicting effects of NH_4^+ on GS activity of barley roots (Lewis et al., 1982; Mäck, 1995), but these discrepancies could be due to different developmental ages of the roots used in each study.

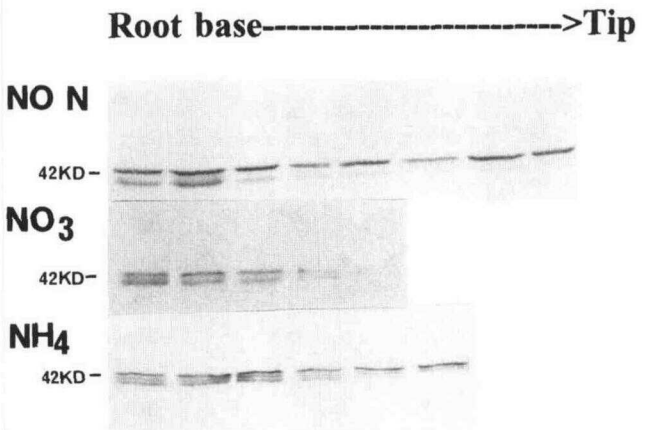

Figure 6. GS polypeptide composition along roots of barley grown with different N media. Western blots were made of extracts of 1-cm root segments along the entire length of roots of 7-d-old barley grown with no N, 15 mM KNO_3, or 15 mM $(NH_4)_2SO_4$. The number of segments differs between treatments because of the difference in final root length. GS polypeptides were visualized following incubation with the GS antiserum as described in "Materials and Methods." Forty micrograms of protein was loaded per well.

In view of these developmental influences on root GS content and the fact that differences in GS activity between N treatments were greater at the root tip than in basal regions, we sampled tissue for the immunolocalization study from just behind the tip, where cell age would be similar in all treatments. We found significant GS immunolabeling of root plastids from all N treatments, with the exception of vascular plastids from NO_3^--grown plants. We have also made preliminary observations of root plastids with distinct morphologies, similar to those previously reported by Lux (1986), and with apparent differences in GS immunolabeling. Additional work is necessary to determine whether these plastid forms differ in their capacity for N assimilation. There was no evidence of a NO_3^--inducible root plastid isoform, as has been reported in other species (Emes and Fowler, 1983; Vézina and Langlois, 1989), although plastid GS labeling was lowest in NH_4^+-grown seedlings. To our knowledge, ours is the first evidence of a root plastid GS isoform in barley; other studies analyzed whole root extracts and therefore may have failed to detect small amounts of plastidic GS (Mann et al., 1980; Marttila et al., 1993; Mäck, 1995).

In all N treatments we found a higher concentration of cytosolic GS in the cortical parenchyma rather than in the vascular stele cells, indicating that there is a greater capacity for NH_4^+ assimilation and Gln formation in the cortex. Our preliminary observation of lower GS immunolabeling in root phloem companion cells than in other root vascular cells also implies that in the region adjacent to the root tip Gln export is limited. The relatively small size of barley root companion cells reflects their function as sinks, i.e. importing rather than exporting metabolites (Warmbrodt, 1985). In contrast, leaf GS1 has been localized only in the vascular tissue of some species, particularly the phloem companion cells, suggesting a primary role in the synthesis of Gln for translocation (Kamachi et al., 1992; Pereira et al., 1992).

In both cortical parenchyma and vascular stele cells, immunolabeling of cytosolic GS is as much as 50% lower when seedlings are grown with either NO_3^- or NH_4^+, compared with that grown with N-free medium. Although there are no directly comparable studies, GS gene expression was extended from the vascular tissue and root apices to the entire root following NH_4^+ treatment (Miao et al., 1991). In maize roots, the cellular distribution of nitrate reductase activity was altered by increasing the NO_3^- supply (Rufty et al., 1986).

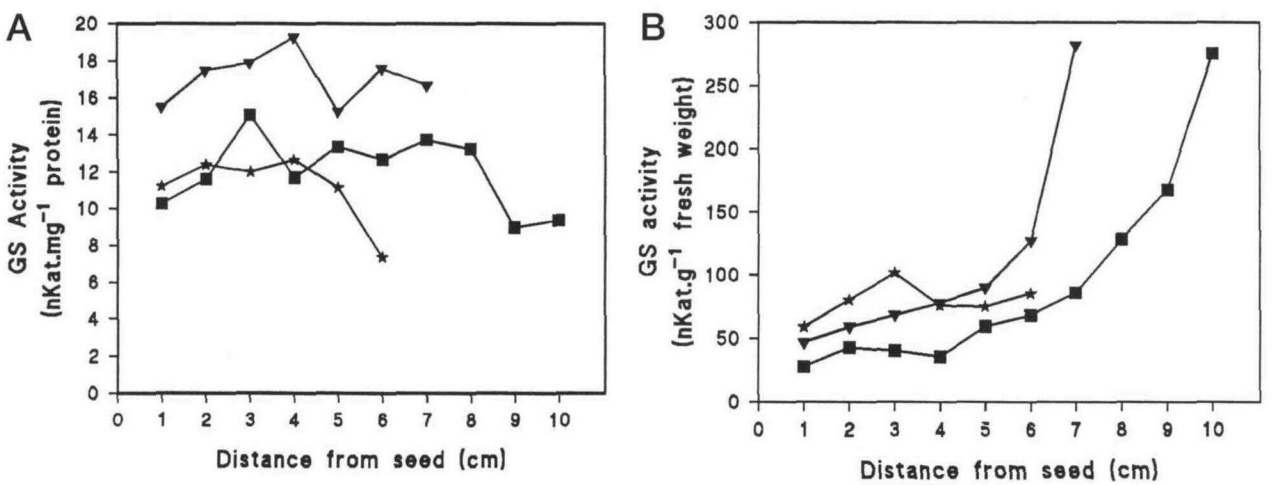

Figure 7. A, GS transferase activity (per mg protein) along roots of barley grown on different N media. GS transferase activity was determined in extracts of 1-cm root segments along the entire length of roots of 7-d-old barley grown with no N (■), 15 mM KNO_3 (▼), or 15 mM $(NH_4)_2SO_4$ (★). Results are the mean of three replicate assays of one representative experiment. B, GS transferase activity (per gram fresh weight) along roots of barley grown on different N media. GS transferase activity was determined in extracts of 1-cm root segments along the entire length of roots of 7-d-old barley grown with no N (■), 15 mM KNO_3 (▼), or 15 mM $(NH_4)_2SO_4$ (★). Results are the mean of three replicate assays of one representative experiment.

Overall, in the region behind the root tip, plants grown with NO_3^- had a higher GS specific activity but less immunolabeling, soluble protein, and GS polypeptides on western blots compared with those grown without N. NH_4^+-grown plants had slightly less GS specific activity and polypeptides but much less immunolabeling and soluble protein than those grown without N. These discrepancies between GS activity and immunolabeling can be explained in a number of ways. First, any change in either the number or the size of root cells, or in GS content of the epidermis, which was not analyzed in our study, would have to be very large to account for these differences.

A more likely explanation, however, is that in plants grown without N some GS is inactive. Inactive forms of GS protein have been reported in legume roots under N-limited conditions (Hoelzle et al., 1992). It may be that in N-free-grown roots, where there is little demand for NH_4^+ assimilation, GS is still synthesized but is largely inactive. This situation might serve to poise the roots for a rapid response, via GS activation, should NH_4^+ become available. In NO_3^--grown roots, a high demand for N assimilation would require GS protein to be fully active. In NH_4^+-grown plants the majority of assimilation would occur in the roots, and low amounts of NH_4^+ would translocate to the shoot (Lewis and Chadwick, 1983) and, therefore, active GS is present in the roots.

Our study highlights the need to support immunolocalization with measurement of enzyme activity wherever possible. In support of this, discrepancies between nitrate reductase activity (Rufty et al., 1986) and immunolabeling (Fedorova et al., 1994) have been previously reported.

In summary, we have found that there are both cell- and development-specific responses of GS to N in the growth medium. Developmental changes in NO_3^- uptake can occur within millimeter-sized regions of barley roots (Siebrecht et al., 1995). It is therefore important to understand how these localized changes in root N metabolism are regulated.

ACKNOWLEDGMENTS

We are grateful to Dr. J.V. Cullimore for the generous gift of the GS antiserum and to Prof. M.J. Emes for useful discussions.

Received January 3, 1996; accepted May 3, 1996.

LITERATURE CITED

Andrews M (1986) The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ **9:** 511–519

Botella JR, Verbelen JP, Valpuesta V (1988) Immunocytolocalization of glutamine synthetase in green leaves and cotyledons of *Lycopersicon esculentum*. Plant Physiol **88:** 943–946

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Brangeon J, Hirel B, Forchioni A (1989) Immunogold localization of glutamine synthetase in soybean leaves, roots and nodules. Protoplasma **151:** 88–97

Carvalho H, Pereira S, Sunkel C, Salema R (1992) Detection of a cytosolic glutamine synthetase in leaves of *Nicotiana tabacum* L. by immunocytochemical methods. Plant Physiol **100:** 1591–1594

Cullimore JV, Gebhardt C, Saarelainen R, Miflin BJ, Idler KB, Baker RF (1984) Glutamine synthetase of *Phaseolus vulgaris* L: organ specific expression of a multigene family. J Mol Appl Genet **2:** 589–599

Cullimore JV, Miflin BJ (1984) Immunological studies on glutamine synthetase using antisera raised to the two plant forms of the enzyme from *Phaseolus* root nodules. J Exp Bot **35:** 581–587

Dale JE, Felippe GM, Marriott C (1974) An analysis of the response of young barley seedlings to time of application of nitrogen. Ann Bot **38:** 575–588

Datta DB, Cai X, Wong PP, Triplett EW (1991) Immunocytochemical localization of glutamine synthetase in organs of *Phaseolus vulgaris* L. Plant Physiol **96:** 507–512

Edwards JW, Walker EL, Coruzzi GM (1990) Cell-specific expression in transgenic plants reveals nonoverlapping roles for chloroplast and cytosolic glutamine synthetase. Proc Natl Acad Sci USA **87:** 3459–3463

Emes MJ, Fowler MW (1983) The supply of reducing power for nitrite reduction in plastids of seedling pea roots (*Pisum sativum* L.). Planta **158:** 97–102

Fedorova E, Greenwood JS, Oaks A (1994) In-situ localization of nitrate reductase in maize roots. Planta **194:** 279–286

Fentem PA, Lea PJ, Stewart GR (1983) Ammonia assimilation in the roots of nitrate- and ammonia-grown *Hordeum vulgare* (cv Golden Promise). Plant Physiol **71:** 496–501

Hewitt EJ (1966) Sand and Water Culture Methods Used in the Study of Plant Nutrition. Commonwealth Bureau of Horticultural and Plantation Crops East Malling, Technical Communications no. 22, Ed 2. Commonwealth Agricultural Bureau, Farnham Royal, UK

Hirel B, Bouet C, King B, Layzell D, Jacobs F, Verma DPS (1987) Glutamine synthetase genes are regulated by ammonia provided externally or by symbiotic nitrogen fixation. EMBO J **6:** 1167–1171

Hirel B, Perrot-Rechenmann C, Suzuki A, Vidal J, Gadal P (1982) Glutamine synthetase in spinach leaves. Plant Physiol **69:** 983–987

Hoelzle I, Finer JJ, McMullen MD, Streeter JG (1992) Induction of glutamine synthetase activity in non-nodulated roots of *Glycine max, Phaseolus vulgaris,* and *Pisum sativum*. Plant Physiol **100:** 525–528

Imsande J, Touraine B (1994) N demand and the regulation of nitrate uptake. Plant Physiol **105:** 3–7

Kamachi K, Yamaya T, Hayakawa T, Mae T, Ojima K (1992) Vascular bundle-specific localization of cytosolic glutamine synthetase in rice leaves. Plant Physiol **99:** 1481–1486

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

Lea PJ, Blackwell RD, Joy KW (1991) Ammonia assimilation in higher plants. Proc Phytochem Soc Eur **33:** 153–186

Lewis OAM, Chadwick S (1983) An ^{15}N investigation into nitrogen assimilation in hydroponically-grown barley (*Hordeum vulgare* L. cv. Clipper) in response to nitrate, ammonium and mixed nitrate and ammonium nutrition. New Phytol **95:** 635–646

Lewis OAM, James DM, Hewitt EJ (1982) Nitrogen assimilation in barley (*Hordeum vulgare* L. cv. Mazurka) in response to nitrate and ammonium nutrition. Ann Bot **49:** 39–49

Lux A (1986) Changes of plastids during xylem differentiation in barley root. Ann Bot **58:** 547–550

Mäck G (1995) Organ-specific changes in the activity and subunit composition of glutamine-synthetase isoforms of barley (*Hordeum vulgare* L.) after growth on different levels of NH_4^+. Planta **196:** 231–238

Mann AF, Fentem PA, Stewart GR (1979) Identification of two forms of glutamine synthetase in barley (*Hordeum vulgare*). Biochem Biophys Res Commun **88:** 515–521

Mann AF, Fentem PA, Stewart GR (1980) Tissue localization of barley (*Hordeum vulgare*) glutamine synthetase isoenzymes. FEBS Lett **110:** 265–267

Marsolier MC, Hirel B (1993) Metabolic and developmental control of cytosolic glutamine synthetase genes in soybean. Physiol Plant **89:** 613–617

Marttila S, Saarelainen R, Porali I, Mikkonen A (1993) Glutamine synthetase isozymes in germinating barley seeds. Physiol Plant **88:** 612–618

McNally SF, Hirel B, Gadal P, Mann AF, Stewart GR (1983) Glutamine synthetase of higher plants. Plant Physiol **72:** 22–25

Miao G-H, Hirel B, Marsolier MC, Ridge RW, Verma DPS (1991) Ammonia-regulated expression of a soybean gene encoding cytosolic glutamine synthetase in transgenic *Lotus corniculatus*. Plant Cell **3:** 11–22

Mierendorf RC, Percy C, Young RA (1987) Gene isolation by screening lambda-gt11 libraries with antibodies. Methods Enzymol **152:** 458–469

Oaks A, Hirel B (1985) Nitrogen metabolism in roots. Annu Rev Plant Physiol **36:** 345–365

Pearson J, Ji Y-M (1994) Seasonal variation of leaf glutamine synthetase isoforms in temperate deciduous trees strongly suggests different functions for the enzymes. Plant Cell Environ **17:** 1331–1337

Pereira S, Carvalho H, Sunkel C, Salema R (1992) Immunocytolocalization of glutamine synthetase in mesophyll and phloem of leaves of *Solanum tuberosum* L. Protoplasma **167:** 66–73

Rhodes D, Rendon GA, Stewart GR (1975) The control of glutamine synthetase level in *Lemna minor* L. Planta **125:** 201–211

Rufty TW, Thomas JF, Remmler J, Campbell WH, Volk RJ (1986) Intercellular localization of nitrate reductase in roots. Plant Physiol **82:** 675–680

Sakakibara H, Kawabata S, Hase T, Sugiyama T (1992) Differential effects of nitrate and light on the expression of glutamine synthetases and ferredoxin-dependent glutamate synthase in maize. Plant Cell Physiol **33:** 1193–1198

Siebrecht S, Mäck G, Tischner R (1995) Function and contribution of the root tip in the induction of NO_3^- uptake along the barley root axis. J Exp Bot **46:** 1669–1676

Stanford AC, Larsen K, Barker DG, Cullimore JV (1993) Differential expression within the glutamine synthetase gene family of the model legume *Medicago truncatula*. Plant Physiol **103:** 73–81

Tobin AK, Ridley SM, Stewart GR (1985) Changes in the activities of chloroplast and cytosolic isoenzymes of glutamine synthetase during normal leaf growth and plastid development in wheat. Planta **163:** 544–548

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA **76:** 4350–4354

Vézina L-P, Langlois JR (1989) Tissue and cellular distribution of glutamine synthetase in roots of pea (*Pisum sativum*) seedlings. Plant Physiol **90:** 1129–1133

Walker EL, Coruzzi GM (1989) Developmentally regulated expression of the gene family for cytosolic glutamine synthetase in *Pisum sativum*. Plant Physiol **91:** 702–708

Wallsgrove RM, Turner JC, Hall NP, Kendall AC, Bright SWJ (1987) Barley mutants lacking chloroplast glutamine synthetase. Biochemical and genetic analysis. Plant Physiol **83:** 155–158

Warmbrodt RD (1985) Studies on the root of *Hordeum vulgare* L.: ultrastructure of the seminal root with special reference to the phloem. Am J Bot **72:** 414–432