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Immunolocalization of glutamine synthetase in senescing tobacco (*Nicotiana tabacum* L.) leaves suggests that ammonia assimilation is progressively shifted to the mesophyll cytosol

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Abstract. Glutamine synthetase (GS) catalyses the formation of glutamine (a major form of nitrogen transport in plants) in an ATP-dependent reaction using ammonium and glutamate. This enzyme is present in the plastids and/or in the cytosol depending on the plant or the organ examined. In order to understand the role of GS isoforms in the remobilization of leaf nitrogen, we studied the localization of GS isoenzymes during natural senescence of tobacco (*Nicotiana tabacum* L.) leaves. Parallel to the progression of leaf senescence, an increase in cytosolic GS polypeptides was detected in the mesophyll cytosol of senescing leaves while a significant decrease in GS protein content was observed in the phloem companion cells. The presence of GS polypeptides in the leaf cytosol of senescing leaves appears to be the result of an induction of the *Gln1-3* gene, the transcripts of which are not detected in mature leaves but are abundant in senescing leaves. Alltogether, our results suggest that during senescence, ammonia assimilation is progressively shifted from the chloroplasts to the cytosol of leaf mesophyll cells.

Key words: Cytosol (GS, leaf) – Glutamine synthetase – *Nicotiana* (N-metabolism) – Nitrogen remobilization – Senescence

Introduction

Leaf senescence is a controlled process initiated by some unknown combination of both genetically determined internal and environmental triggers (Smart 1994; Thomas 1994). Drastic modifications in terms of leaf cell structure (Woolhouse 1984; Thompson 1988) and metabolism (Feller and Fisher 1994) are generally observed in senescing leaves of most plant species. In the early steps of leaf senescence, membrane integrity and cell walls are maintained during catabolism of leaf cell constituents (Woolhouse 1982; Matile 1992). The degradation products of these constituents must be efficiently remobilized by the plant in order to minimize the loss of nutrients available for growing organs such as young leaves or fruits (Boyd and Walker 1972). For some nutrients, such as potassium, no metabolization is necessary before phloem loading and export. Other nutrients, such as nitrogen, sulfur and phosphorus, are essentially present in the cell as constituents of macromolecules that cannot be directly transported (Peoples and Daling 1988; Feller 1990). Remobilization of carbon and nitrogen during senescence is of major importance for annual plant species. Leaves generally represent the most important organ for protein reserves to be remobilized during senescence, mainly because they contain a very large amount of Rubisco. During senescence, plants undergo a switch from a "nitrogen assimilation" status to a "nitrogen remobilization" status when senescence takes place within the leaf (Feller and Fischer 1994; Masclaux et al. 2000). During this highly controlled stage of development, amino acids and ammonia are produced as a result of protein catabolism. It is, therefore, of particular importance to characterize more fully the biochemical and molecular events associated with carbon and nitrogen remobilization.

It is well known that during plant senescence, several changes occur in the pathways for nitrate reduction, ammonium assimilation and amino-acid biosynthesis. For example, leaf nitrate reductase (NR: EC 1.6.6.1) activity decreases quickly during the early phase of leaf senescence and becomes undetectable in late stages (Feller and Fischer 1994). A concomitant decrease in ferredoxin-dependent glutamate synthase (Fd-GOGAT: EC 1.4.7.1), aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) is also generally

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Abbreviations: GS = glutamine synthetase; GS1, GS2 = cytosolic and plastidic glutamine synthetase; PAS-NBB = Periodic Acid Schiff's-Naphthol Blue-Black

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observed (Streit and Feller 1982, 1983; Peters and Van Laere 1992). The maximum activity of NADH-dependent glutamate dehydrogenase (EC 1.4.1.2), whose participation in the assimilation or production of ammonium is still not clearly defined (Lea and Ireland 1999), is reached during senescence in wheat and tobacco leaves (Peters and Van Laere 1992; Masclaux et al. 2000). Chloroplastic glutamine synthetase (GS2) gene expression and/or protein content have been shown to decrease during natural or dark-induced senescence, whereas cytosolic glutamine synthetase (GS1) gene expression and protein have been found to increase or remain stable during the same period (Kawakami and Watanabe 1988; Kamachi et al. 1991; Bernhard and Matile 1994; Pérez-Rodríguez and Valpuesta 1996; Masclaux et al. 1999). However, no attempt has been made to localize the different GS proteins in situ during leaf senescence. During this study, immunocytochemical experiments were performed to analyse in parallel the histological and the cellular localization of GS in senescing tobacco leaves. In addition, RNase protection experiments were performed to identify which of the two genes encoding GS1 (*Gln1-3* or *Gln1-5*) was induced in senescing leaves.

The physiological role of GS1 during senescence or related status is discussed according to polypeptide localization and the pattern of gene expression in leaf tissues.

Materials and methods

Plant material. Tobacco (*Nicotiana tabacum* L. cv. Xanthi; Institut National de la Recherche Agronomique, Versailles, France) was grown on a clay loam soil. From the base of the seedlings each emerging leaf was numbered and tagged. From a batch of 8-week-old plants, eight plants of uniform development and numbering 10 leaves were selected. These were transferred to a controlled-environment growth chamber (16 h light, 350–400 mmol photons m^{-2} s^{-1}, 26 °C; 8 h dark, 18 °C) and watered with N12 (10 mM NO^{3-} and 2 mM NH_4^+) solution (Coïc and Lesaint 1971). Plants were automatically watered for 1 min (flow rate for each plant: 50 ml min^{-1}) every 2 h. We selected four of the 11-week-old plants in the vegetative state of development, and harvested leaves numbered 10 (senescing yellow leaves), 15 (senescing green leaves) and 30 (mature green leaves) among the 35 leaves which had emerged (Masclaux et al. 2000).

The main midrids were removed and 1-cm^2 sections of mesophyll tissue were randomly collected and pooled for total RNA extraction. The pooled mesophyll tissue was frozen in liquid nitrogen and immediately reduced to a homogenous powder which was stored at −80 °C until further use.

Immunolocalization. Mature green leaves and senescing leaves (green and yellow) were fixed in freshly prepared 1.5% paraformaldehyde dissolved in sodium phosphate buffer for 4 h at 4 °C. The black squares in Fig. 1a,b indicate the position of the leaf areas used for the experiment. For immunolocalization, the material was dehydrated in an ethanol series (final concentration 90% ethanol) then embedded in LR White resin (Polysciences, Warrington, Penn., USA). Polymerization was carried out in gelatin capsules at 50 °C.

For structural investigations, thin sections (1 μm) were stained by the Periodic Acid Schiff's-Naphthol Blue-Black (PAS-NBB) method as described by Sangwan et al. (1992).

For immuno-transmission electron microscopy, ultrathin sections were mounted on 400-mesh nickel grids and allowed to dry at 37 °C. Sections were first incubated with 5% normal goat serum in Tl buffer (0.05 M Tris-HCI buffer containing 2.5% NaCl, 0.1% BSA and 0.05% Tween 20, pH 7.4) for 1 h at room temperature then with anti-GS or anti-Rubisco rabbit serum diluted 1:70 in Tl buffer for 6 h at room temperature. Section were then washed five times with Tl buffer, two times with T2 buffer (0.02 M Tris-HCI buffer containing 2% NaCl, 0.1% BSA and 0.05% Tween 20, pH 8) and incubated with 10-nm colloidal gold-goat anti-rabbit immunoglobulin complex (Sigma) diluted 1:50 in T2 buffer for 2 h at room temperature. After several washes, grids were treated, with 5% uranyl acetate in water and observed with a CMI2 electron microscope (Philips, Eindoven, The Netherlands) at 80 kV.

For immuno-light microscopy, thin sections of 2 μm were floated on drops of sterile water on slides coated with Biobond (British Biocell, Cardiff, UK), and the same procedure of labelling as described above was used, except that the primary serums were applied at a 1:200 dilution for 2 h and that 1-nm, instead of 10-nm, colloidal gold-goat anti-rabbit immunoglobulin complex (British Biocell) was used at a 1:600 dilution. Immuno-gold labelling was revealed by silver enhancement as described by the supplier (British Biocell) and sections were back-stained with 1% fuchsin before microscopical observations under bright-field and/or epipolarised light on a Nikon Eclips 800 epifluorescent photomicroscope.

For both techniques, controls were run either by omitting the primary antibody or by its substitution with preimmune serum.

Protection assay for RNase. Total RNA was isolated from 1 g of frozen mature green leaves or senescing leaves using a hot-phenol extraction procedure and a selective precipitation with 4 M LiCl to remove traces of DNA and small RNA species (Verwoerd et al. 1989). The RNase protection assays were carried out according to Dubois et al. (1996) and Brugière et al. (1999) with ^{32}P-labelled single-stranded RNA probes synthesised in vitro using SP6 polymerase (SP6/T7 Transcription Kit; Boehringer Mannheim, Mannheim, Germany) and [^{32}P]UTP (Dupont-NEN, Boston, Mass., USA) as described in Dubois et al. (1996).

Results

Leaf senescence and GS isoenzyme composition. Study of the GS isoenzyme composition in the various leaf stages of tobacco showed that GS1 is progressively induced during leaf ageing (Masclaux et al. 2000). Quantification of GS1 protein content following protein gel blot analysis revealed that GS1 was lacking in mature green leaf 30 whereas maximal amounts of GS1 protein were detected in senescing yellow leaf 10. Senescing green leaf 15 represented a stage of tobacco leaf development in which GS1 started to accumulate. These three stages of leaf development were therefore selected for further ultrastructural and immunocytochemical studies to investigate the localization of the GS1 in senescing leaves.

Figure 1a,b shows typical mature green and senescing yellow leaves. The phenotype of senescing green leaves (picture not shown), which did not exhibit any visible symptoms of chlorosis, was similar to that of mature green leaves. Transverse sections of both mature green and senescing yellow leaves were stained with PAS-NBB in order to study cell organization. The PAS-NBB dye has the property of staining proteins in blue and polysaccharides in red. Therefore, cell walls stain red whereas chloroplasts and nuclei appear dark-blue and the cytosol light blue. A comparison of the cell-wall

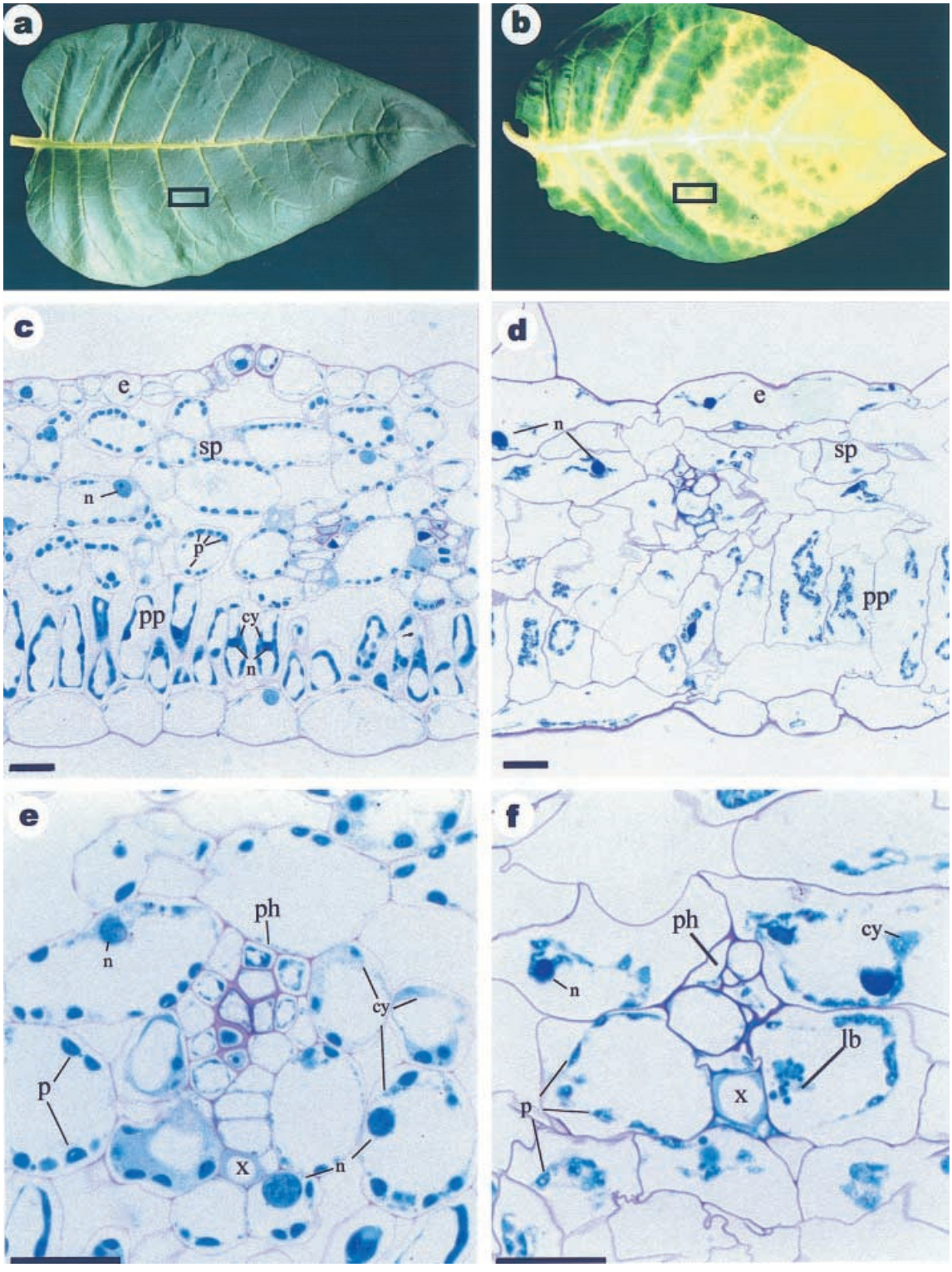


Fig. 1a–f. Morphological and structural observations of mature green leaves and senescing yellow tobacco leaves. **a**,**b** General view of mature green leaves (**a**) and senescing yellow leaves (**b**) used for our study. The *black rectangles* indicate the position of the leaf areas used for immunolocalization. **c–f** Comparative histological study of mature green leaves (**c**,**e**) and senescing green leaves (**d**,**f**) after staining with PAS-NBB. *p*, chloroplasts; *cy*, cytoplasm; *e*, epidermis; *lb*, lipoidal blebs; *n*, nucleus; *pp*, palisade parenchyma; *ph*, phloem pole; *sp*, spongy parenchyma; *x*, xylem pole. Bars = 50 μm

architecture of mature green and senescing yellow leaves is presented in Fig. 1c,d, respectively. In both types of leaf the palisade parenchyma, the spongy parenchyma, and the epidermal cells could still be easily distinguished; however, in senescing yellow leaves, where cell integrity had been preserved, the cell size had increased in

comparison to mature green leaves (Fig. 1d,f; see also Fig. 2e,f). In mature green leaves the large and protein-rich cytoplasm of both palisade and spongy parenchyma cells surrounding the chloroplasts and nuclei (dark blue color) stained light blue (Fig. 1c,e). The same cell types in senescing yellow leaves had a smaller and less-stained cytoplasm (Fig. 1d,f) as well as lipoidal blebs that were not present in the cells of mature green leaves (Fig. 1e,f). Nuclei and chloroplasts were detected on sections of both mature green and senescing yellow leaves (Fig. 1e,f). However, chloroplasts of senescing yellow leaves (Fig. 1f) appeared smaller and less abundant than those of mature green leaves (Fig. 1e). A higher magnification of the vascular tissue area of senescing yellow leaves (Fig. 1e,f) showed that cells in the phloem pole are less abundant and not as well organised as in mature green leaves. In particular, a decrease in the cytoplasmic content of phloem parenchyma cells and phloem companion cells can clearly be observed (Fig. 1e). The histological structure of senescing green leaves was intermediary between the mature green and senescing yellow stages and characterised by the persistence of practically intact chloroplastic and cytosolic structures similar to those of mature green leaves (data not shown; see also Fig. 2d).

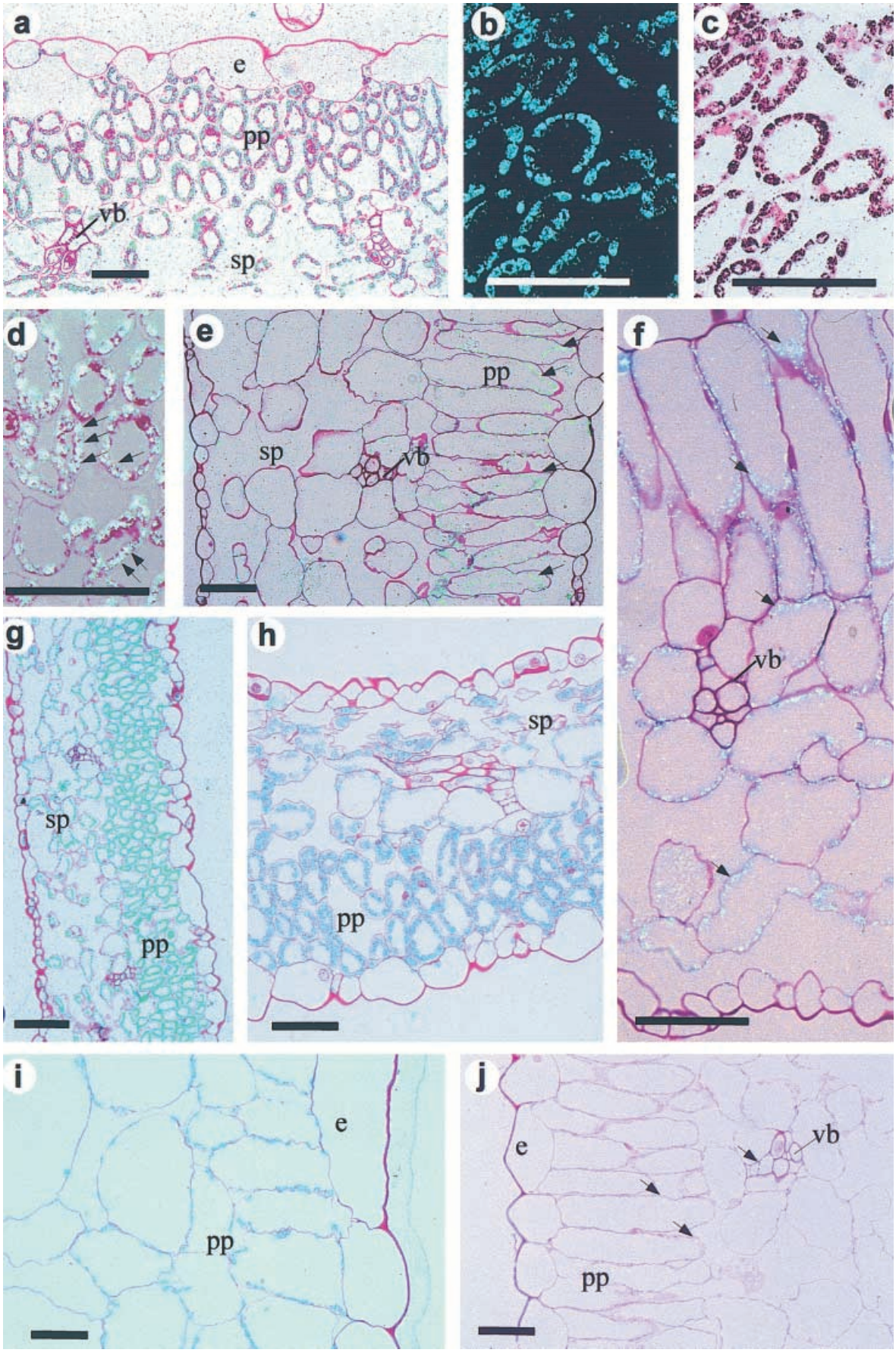
Intracellular and subcellular localization of GS in green and senescing leaves. Localization of GS in mature and senescing leaves was investigated using both immuno-light microscopy and immuno-gold transmission electron microscopy techniques which allowed visualization of the protein within tissue and subcellular structures. The polyclonal antiserum raised against purified tobacco GS2 recognizes plastidic or cytosolic GS with similar efficiency. Therefore, GS1 and GS2 can be unambiguously localised on plant tissue sections using either immuno-light microscopy or immuno-gold transmission electron microscopy techniques. These two techniques allow a good quantitative estimation of the amount of each isoenzyme both in the chloroplasts and in the cytosol (Dubois et al. 1996).

For histological localization of GS (Fig. 2) we used immuno-gold labelling followed by silver enhancement (Dubois et al. 1996). A bright-field light microscope emitting epipolarised light was used for these experiments and the silver-enhanced gold particles appeared as a bright blue-green colour. Sections of a mature green leaf treated with gold-labelled anti-GS antibodies (Fig. 2a) allowed the detection of GS in the chloroplasts of both palisade parenchyma cells and spongy parenchyma cells. Higher magnifications of the same parenchyma area observed under epipolarised light only (Fig. 2b) or bright field only (Fig. 2c) unambiguously confirmed that GS was only present in the chloroplasts. Unlabelled small areas inside the chloroplasts correspond to small starch granules. In contrast, there was a different pattern of GS localization in senescing green tobacco leaves (Fig. 2d). In addition to the strong labelling in the chloroplasts, a weak and diffuse labelling was visible in the cytosol. The presence of GS in the cytosol was confirmed when senescing yellow leaves were treated with the antiserum (Fig. 2e,f). Although the chloroplasts were smaller (as already observed in Fig. 1c,e) a diffuse labelling was still visible inside the organelles. Interestingly, cytosolic GS seemed to be still detectable in the vascular bundle cells of senescing leaves (Fig. 2f). However, due to the lack of resolution of the technique, it was difficult to detect if changes in the relative amount of protein occurred during leaf ageing. Therefore, additional experiments using immuno-gold electron microscopy were perfomed.

Fig. 2a–j. Histological immunolocalization of GS and Rubisco in thin sections of mature and senescing tobacco leaves using immuno-gold labelling followed by silver enhancement. **a–c** Immunolocalization of GS in mature green leaves. **a** Overview of a leaf transverse section under bright field and epipolarised light. **b**,**c** Transverse sections of the same parenchyma area observed under epipolarized light only (**b**) or bright field only (**c**), showing a specific localization of GS inside plastids. Bars = 50 μm. **d–f** Immunolocalization of GS in senescing tobacco leaves. **d** Immunolocalization of GS in senescing green leaves showing the localization of the protein both in the chloroplasts and in the cytoplasmic zone (*arrows*). **e**,**f** Overview (**e**) and detail (**f**) of a section of a senescing yellow leaf. Plastidial localization of GS is not observed but there is a diffuse labelling both in the cytosol (*arrows*) and in degenerating chloroplasts. Bars = 50 μm. **g**,**h** Immunolocalization of Rubisco in mature green leaves. **g** Overview showing a very high concentration of Rubisco in the palisade parenchyma. Bar = 100 μm. **h** Detail of the transverse section showing the specific location of Rubisco in the plastids. Bar = 50 μm. **i** Immunolocalization of Rubisco in senescing yellow leaves. Intensity of the labelling is strongly decreased but remains restricted to small plastids. Bar = 50 μm. **j** A control section treated with preimmune serum showing the specificity of labelling. Only a weak background labelling was observed (*arrows*). *e*, epidermis; *pp*, palisade parenchyma; *sp*, spongy parenchyma; *vb*, vascular bundle. Bar = 50 μm

A reduced content of most chloroplastic proteins, including Rubisco, is characteristic of leaf senescence as the result of chloroplast degradation associated with protein breakdown (Feller and Fisher 1994). Therefore, Rubisco was used as a marker to follow, at the intracellular level, the fate of a plastidic protein in senescing leaves. Immuno-light localization of Rubisco in mature green leaves (Fig. 2g) showed that the enzyme is mostly present in the palisade parenchyma cells. Higher magnifications confirmed its chloroplastic location (Fig. 2h). In senescing yellow leaves, Rubisco was also found in the plastids of parenchyma cells despite a disorganization of the cytoplasm and a reduction in chloroplast size (Fig. 2i). This latter observation excludes the possibility that the presence of GS in the cytosol of senescing leaves arose from chloroplast degradation leading to GS2 protein leaching into the cytosol. Control sections treated with preimmune serum confirmed the specificity of the labelling with either the GS or Rubisco antiserum (Fig. 2j), as only a very weak background signal was observed.

Immuno-gold transmission microscopy experiments were performed to confirm the apparent induction of GS in the cytosol and to investigate more accurately the possible modifications in the GS protein content within the vascular tissues of senescing leaves. In mature green leaves, GS was exclusively located in the mesophyll chloroplasts (Fig. 3a,b), whereas all the other cell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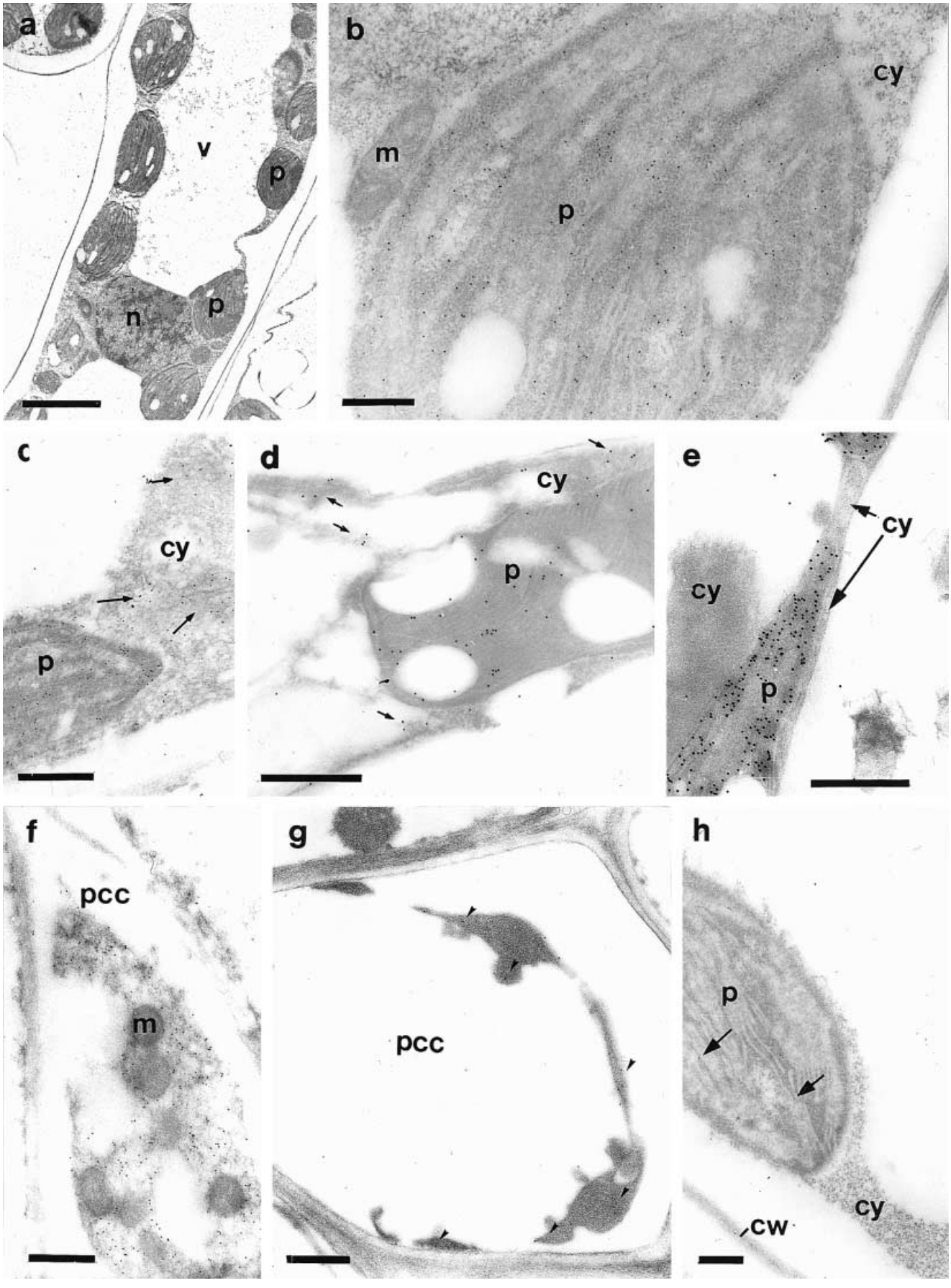
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Fig. 3a–h. Transmission electron microscopic immunolocalization of GS and Rubisco on thin sections of mature and senescing tobacco leaves (immuno-gold labelling). **a**,**b** Localization of GS in a mesophyll cell of mature green leaves. **a** Overview of a mesophyll parenchyma cell. Bar = 5 μm. **b** High magnification of a very densely labelled chloroplast. Note that the cytoplasm, mitochondria and observable cell wall are free of labelling. Bar = 0.5 μm. **c**,**d** Localization of GS in a mesophyll cell of senescing tobacco leaves. **c** Overview indicating clearly the localization of GS both inside the chloroplast and in the cytoplasm (*arrows*) of senescing green leaves. **d** View of a highly disorganised senescent plastid in senescing yellow leaves, confirming the presence of GS in the surrounding cytoplasm (*arrows*). Bars = 0.5 μm. **e** Localization of Rubisco in senescing yellow leaves cells. Note that the labelling is exclusively restricted to the senescent plastid and that the thin strand of cytoplasm surrounding the plastid (*cy and arrows*) is absolutely free of label (compare with **c** and **d**) **f**,**g** Localization of GS in the cytoplasm of phloem companion cells of mature green leaves (**f**) and senescing yellow leaves (**g**) of tobacco. Note that the density of cytosolic GS in this cell type is considerably decreased during senescence (*arrows* point out the few gold particles observed in the senescing companion cell). Bars = 0.5 μm. **h** Control section incubated with preimmune serum. Note that only a few gold particles (*arrows*) are visible, indicating insignificant labelling. Bar = 0.5 μm. *cy*, cytoplasm; *cw*, cell wall; *m*, mitochondrion; *n*, nucleus; *p*, plastid; *pcc*, phloem companion cell; *v*, vacuole

components were unlabelled (Fig. 3b). In contrast, immunolocalization of GS in parenchyma cells of senescing green leaves showed that the protein was present in both the chloroplasts and the cytosol (Fig. 3c). In senescing yellow leaves, the dual compartmentation of GS was still clearly visible, although most of the chloroplasts were highly disorganised (Fig. 3d). This series of observations also confirmed that chloroplast size was largely reduced in senescing leaves (see Fig. 3b,c and d for comparison) and that in senescing yellow leaves the cytosol was highly disorganised (Fig. 3d). The GS immuno-gold labelling detected in the cytosol was not the result of GS2 leaching from degenerated chloroplasts since Rubisco protein was only detected in the plastids of senescing yellow leaves (Fig. 3e). As already described in a number of previous reports (Carvalho et al. 1992; Dubois et al. 1996), in mature green leaves there was a high concentration of GS within the cytoplasm of phloem companion cells (Fig. 3f). Interestingly, in the phloem cells of senescing yellow leaves the amount of cytosolic GS protein was visibly reduced, probably due to the shrinking of a disorganised cytosol (Fig. 3g). The absence of almost any signal in the leaf sections treated with preimune serum indicated that GS and Rubisco labelling was highly specific (Fig. 3h).

Molecular characterization of GS1 gene expression in mature and senescing leaves. In a previous experiment (Masclaux et al. 2000) a northern blot using the full-length ^{32}P-labelled *Gln1-5* cDNA clone as a probe, allowed the demonstration that GS1 transcripts were progressively induced during leaf ageing and that the highest level of expression was found in senescing yellow leaves. In tobacco, the expression of two genes encoding GS1 (*Gln1-3* and *Gln1-5*) has previously been studied. The gene *Gln1-3* is expressed in roots and flowers

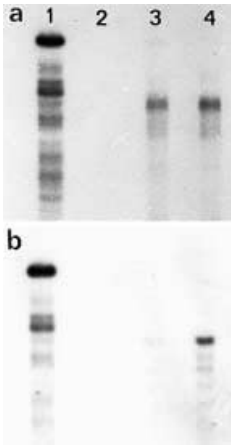

Fig. 4a,b. Molecular analysis of GS1 gene expression in senescing leaves. The RNase protection experiment was performed using 10 μg of total RNA isolated from mature green leaves (*lanes 2*), senescing yellow leaves (*lanes 3*) or roots (*lanes 4*) following hybridization with an antisense RNA ^{32}P-labelled probe corresponding to the 3′ non-coding region of the *Gln1-3* cDNA (**a**) or *Gln1-5* cDNA (**b**) (Dubois et al. 1996). *Lanes 1* show the position of the generated *Gln1-3* (298 b; **a**) and *Gln1-5* (243 b; **b**) antisense probe, respectively. The length of the probe protected from S1 nuclease digestion in RNA-RNA hybrids is 289 bp for *Gln1-3* and 218 bp for *Gln1-5*

whereas *Gln1-5* is transcribed in the vascular tissue (Dubois et al. 1996). To identify if GS1 found in the cytosol of senescing leaves could be the result of induced expression of either one or both genes, we performed an RNase protection experiment using radioactive antisense RNA probes corresponding to the 3′ non-coding regions of the two GS1 genes (Dubois et al. 1996). In this experiment, the amount of antisense probe protected from digestion by its hybridization with the specific transcript allowed the quantification of the mRNA corresponding to a specific gene (*Gln1-3* in Fig. 4a and *Gln1-5* in Fig. 4b). The transcripts of the *Gln1-3* gene (Dubois et al. 1996) were undetectable in mature leaves (Fig. 4a, lane 2) but were present in senescing leaves (Fig. 4a, lane 3), demonstrating that *Gln1-3* gene expression is induced during senescence. As shown by the absence of signal, the transcripts of the phloem-specific *Gln1-5* gene (Dubois et al. 1996) were undetectable in both mature green (Fig. 4b, lane 2) and senescing yellow (Fig. 4b, lane 3) leaves. A positive control showing that both genes are expressed in roots (Dubois et al. 1996) is depicted in Fig. 4a,b (lanes 4).

Discussion

The role of GS1 in photosynthetically active or senescing leaves is still a matter of debate mainly because, in most plant species studied so far, the physiological role of the enzyme within a particular tissue or cellular structure has yet not been definitively assessed. It has already been proposed that GS1, by virtue of its location in the vascular tissue of C_3 plants (Carvalho et al. 1992; Pereira et al. 1992; Dubois et al. 1996), plays a major role during glutamine transport via the phloem vessels

whether the plant is vegetative (Lam et al. 1996) or senescent (Sakurai et al. 1996). However, at least in the former case, recent results obtained using transgenic plants with impaired GS activity in the phloem did not confirm this hypothesis. The enzyme was shown to be involved in controlling proline production rather than the synthesis of glutamine for nitrogen export (Brugière et al. 1999). In parallel, it has clearly been shown that, in a large number of plant species GS1 is induced whether senescence is natural (Perez-Rodriguez and Valpuesta 1996), dark-induced (Kawakami and Watanabe 1988) or provoked by a pathogen infection (Pérez-Garcia et al. 1995). Immunocytochemical experiments showed that only the vascular GS1 was detected in senescing rice leaf tissue (Sakurai et al. 1996). However, in tomato leaves infected by *Pseudomonas* the GS enzyme was induced in the mesophyll (Pérez-Garcia et al. 1998) although GS in the phloem did not seem to be affected by the bacterial infection because the amount of protein in the vascular tissue was comparable to that of uninfected control plants. However, it remains difficult to compare these experiments performed on detached green mature leaflets with senescence and transport existing naturally.

In the present study we show that the structural and progressive changes occurring during tobacco leaf development from a fully photosynthetically active stage to an advanced senescent stage is tightly correlated to the induction of GS1 in the mesophyll. An enlargement of most cellular components, likely to be the result of water influx to compensate for the increase in solutes such as nitrate (Masclaux et al. 2000), accompanied by a reduction in chloroplast size, number and integrity (Martinoia et al. 1983; Matile 1992) were the most characteristic events observed either by transmission or electron microscopy. In addition, lipoidal globules, appearing as small dark spots in the cytoplasm of senescing cells could contain chloroplast components in transit for degradation in the cytoplasm or the vacuole where a number of proteolytic enzymes are present (Noodén et al. 1997). Despite the decrease of cytoplasm integrity in senescing cells, the presence of an apparently intact nucleus suggests that the transcription of different genes encoding enzymes (GS1 and glutamate dehydrogenase for example) involved in reassimilation of leaf carbon and nitrogen constituents, is still able to occur (Buchanan-Wollaston 1997). A parallel induction of GS protein in the mesophyll cell cytosol together with the progression of leaf senescence was clearly observed along the main plant axis. This result strongly suggests that part of the ammonia assimilation is shifted to the cytosol although a lower, but significant, amount of GS2 is still present in the degenerating chloroplasts. The disorganization of the vascular tissue in senescing leaves accompanied by a reduction in the GS1 protein content tends once again to argue against the role of the enzyme in the synthesis of glutamine for export to sink organs (Brugière et al. 1999). However, it cannot be completely ruled out that species-specific characteristics in terms of GS1 function during senescence may exist whether we are dealing with cereals (Sakurai et al. 1996), Solanaceae (Pérez-Garcia et al. 1998; Masclaux et al. 2000) or trees (Garcia-Gutierez et al. 1998).

Using an RNase protection experiment we showed that *Gln1-3* transcripts are present in senescing leaves but not in green leaves, whereas *Gln1-5*, a gene encoding GS1 specifically expressed in the phloem of green leaves, is not induced. This result strongly suggests that in senescing leaves, the newly synthesised GS1 protein is a *Gln1-3* translation product. The *Gln1-3* transcripts were also shown to be expressed in the cytosol of root and flower cells (Dubois et al. 1996), which indicates that senescence-associated signals trigger *Gln1-3* gene expression specifically in the leaf tissues where it is not normally transcribed. Whether senescing leaf cells and assimilating root cells share a common signal responsible for the expression of the *Gln1-3* gene still remains to be investigated. A similar situation has been shown to occur in senescing tomato leaves (Pérez-Rodríguez and Valpuesta 1996) in which the *Leu147* gene encoding a root GS1 is also induced. Moreover, the high similarity of the 3′ non-coding region of the tobacco *Gln1-3* cDNA to the same region of the *Leu147* GS cDNA (data not shown) is a striking feature, suggesting that within the Solanaceae conserved regulatory mechanisms are involved in the regulation of nitrogen remobilization.

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