Improved growth in a field trial of transgenic hybrid poplar overexpressing glutamine synthetase

Zhong Ping Jing[1], Fernando Gallardo[1], María Belén Pascual[1], Rafael Sampalo[1], José Romero[2], Augusto Torres de Navarra[2] and Francisco M. Cánovas[1]

[1]Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias e Instituto Andaluz de Biotecnología, Unidad asociada UMA-CSIC, Universidad de Málaga. Campus de Teatinos, 29071 Málaga, Spain; [2]Apliforesta Internacional S.L., Parque Tecnológico de Andalucía, Edificio Bic Euronova, Campanillas, 29590 Málaga, Spain

Author for correspondence:
Francisco M. Cánovas
Tel: +34 952131942
Fax: +34 952132000
Email: canovas@uma.es

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Summary

- In the present work the performance of transgenic poplars expressing a pine glutamine synthetase (GS) transgene was studied in natural conditions.
- A field study of eight independent transgenic lines and control plants was carried out for 3 yr in the province of Granada (Spain).
- Transgenic poplars reached average heights that were 21, 36 and 41% greater than control plants after the first, second and third year of growth, respectively. Transgene expression affected plant features with time resulting in increased protein, total GS and ferredoxin-dependent glutamate synthase (Fd-GOGAT) in leaves. However, neither differences in the large subunit of Rubisco (LSU) abundance nor water content were detected between lines. Furthermore, no significant differences were found in total polysaccharide and lignin content in tree trunks.
- The analyses of stem diameter, and protein contents in the bark suggest that higher levels of nitrogen reserves accumulated in the stem of transgenics. Our results suggest that modification of GS1 expression may be a useful strategy to complement traditional tree breeding in short rotation plantations.

Key words: field trial, glutamine biosynthesis, nitrogen metabolism, *Populus*, transgenic trees.

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Introduction

Plant development depends on the availability of inorganic nitrogen (N) in the soil. However, the recycling of N released in metabolism is also a relevant issue for the N economy of the plant. N recycling involves reassimilation of ammonium released in photorespiration and in other metabolic processes such as phenylpropanoid biosynthesis and mobilization of stored N in reserve proteins. The metabolic reaction catalysed by glutamine synthetase (GS, EC 6.3.1.2) is considered a key point of control in the synthesis and mobilization of N compounds, since the enzyme catalyses the incorporation of ammonium into glutamine, an amino acid precursor of glutamate and of all N compounds in plants (Miflin & Lea, 1980). Plant GS is a holoenzyme composed of eight subunits and exists as two major isoenzymes located in different subcellular compartments. GS in chloroplasts (GS2) acts in coordination with ferredoxin-dependent glutamate synthase (Fd-GOGAT, EC 1.4.7.1) to assimilate ammonium derived from nitrate reduction and photorespiration. By contrast, cytosolic GS (GS1) has still not got a well-defined role in plants, although it has been proposed to be involved in primary assimilation in roots and in the reassimilation of ammonium released in other metabolic processes (i.e. phenylpropanoid biosynthesis). In the photosynthetic organs of angiosperms, GS1 expression is restricted to vascular bundles, indicating a relevant role for this isoenzyme in the transport of glutamine from the leaf to other organs (Lea, 1997; Cren & Hirel, 1999). In addition, GS1 is expressed in photosynthetic cells of conifers, where GS2 is absent

(García-Gutiérrez *et al.*, 1998), and also in angiosperms during physiological differentiation of plastids (Gallardo *et al.*, 1988; Kawakami & Watanabe, 1988; Gálvez *et al.*, 1990) or under stressful situations such as water stress (Bauer *et al.*, 1997) or pathogen attack (Pérez-García *et al.*, 1995). GS1 has been found to co-localize with quantitative trait loci (QTL) for yield components in the plant genome (Hirel *et al.*, 2001; Obara *et al.*, 2001). All these recent reports suggest that cytosolic GS (GS1) plays a central and pivotal role in N metabolism that is essential for nitrogen-use-efficiency in higher plants. These data lead us and other research groups to generate transgenic plants with GS1 transgenes in order to obtain additional information about the biological role of cytosolic GS (revised in Miflin & Habash, 2002).

Populus has been considered a model in angiosperm tree research because of its small genome, easy vegetative propagation, and transformation with *Agrobacterium* (Bradshaw *et al.*, 2000; Taylor, 2002). We have introduced a pine GS gene in the hybrid *Populus tremula* × *P. alba* 7171 B4 clone (Gallardo *et al.*, 1999) to study its function in transgenic trees. Our results indicated that transgenic poplar produced a new GS holoenzyme in the cytosol of photosynthetic cells that was composed of pine GS subunits (Fu *et al.*, 2003). The ectopic expression of GS1 in young leaves was associated with increased GS activity, protein and chlorophyll content in the leaf, and with a higher number of leaves, leaf length and vegetative growth with respect to control plants (Gallardo *et al.*, 1999; Fu *et al.*, 2003). These studies were carried out with poplar plants cultivated in growth chambers and in the greenhouse, and the observed differences in growth between transgenics and control plants could be of interest in breeding. However, environmental conditions and stress factors in nature are difficult to reproduce in controlled growth conditions. These conditions may greatly influence the performance of plants (and consequently plant growth) and the plant vigour of transgenics selected *in vitro* may differ from field-grown plants (Confalonieri *et al.*, 2003). Moreover, the growth of trees over several years is also influenced by C and N reserves accumulated during each growing period. Therefore, field tests are essential to confirm the performance of transgenic trees for commercial applications, and also to improve our knowledge about the biological role of transgenes and their encoded protein products.

Field tests of transgenic poplars with altered lignin metabolism, flowering, resistance to herbicides and insect attacks have recently been reported (Pilate *et al.*, 2002; Confalonieri *et al.*, 2003). In this work we present the results obtained from a 3-yr field test of transgenic poplars expressing cytosolic pine GS. The field trial was carried out in the Province of Granada (Andalusia, Spain), although some of the results included in this paper correspond to 5-yr-old plants that had been maintained in soil at the Experimental Research Station of the University of Málaga, Málaga, Spain. In both cases, and as described in other field tests of transgenic poplars (revised in Confalonieri *et al.*, 2003), the expression of the pine transgene was stable along the study. To our knowledge, this is the first report describing the behaviour of transgenic plants overexpressing a gene involved in N assimilation under field conditions.

Materials and Methods

Plant material

Transformed poplar lines with a pine GS1 transgene (*Populus tremula* × *P. alba*, INRA clone 7171-B4; Gallardo *et al.* 1999) were micropropagated *in vitro* and cultivated in the greenhouse before they were transferred to the field. The trial was approved by the Spanish Comisión Nacional de Bioseguridad, Ministerio de Medio Ambiente (reference B/ES/98/27). A total of 80 rooted plants (average height 44–50 cm) corresponding to control and eight independent lines were planted in an experimental plot in the province of Granada (Andalusia, Spain) inside a regular poplar plantation (Fig. 1). Samples of young and expanding leaves were taken each month during the period of study and frozen in liquid N for chemical and biochemical analyses. The analyses of 5-yr-old plants corresponded to control and transgenics

Fig. 1 Field trial of transgenic poplar overexpressing cytosolic glutamine synthetase.

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cultivated in soil at the Experimental Research Station, University of Málaga.

Statistical analysis

Plant lines in the field test were located randomly, with 3 m between plants. The control group corresponded to eight plants that was divided into two sets and randomly distributed with transgenics. This design was carefully considered as the experimental plot was small (120 × 10 m). Growth studies of controls were conducted with eight plants ($n = 8$) and the results from transgenics corresponded to the mean of each transgenic line ($n = 8$). When indicated, significant differences in growth between control and transgenics were contrasted by a statistical test at a significance level $\alpha \leq 0.05$. In molecular and biochemical studies the means were obtained from at least four control plants ($n = 4$) and three transgenics from five independent lines ($n = 5$) selected at random. Differences between control and transgenics were tested at a significance level $\alpha = 0.05$ or $\alpha = 0.1$. Significant differences were also considered at $\alpha = 0.1$ because of the small number of replicates. In all plots, the data are presented as mean ± SD. Analysis of variance (ANOVA) with $\alpha = 0.05$ indicated significant differences of growth between plants with different GS1 content.

Protein analysis

Total soluble proteins were extracted as described earlier (Gallardo *et al.*, 1999). Bradford's method was used for the quantification of proteins using bovine serum albumin as standard (Bradford, 1976). Protein profiles were analysed by fractionation of samples by SDS-PAGE according to the procedure of Laemmli (1970) followed by Coomassie blue staining. Proteins from leaf and stem samples were fractionated in 10% polyacrylamide gels, and 12.5% polyacrylamide gels were used in the analysis of bark proteins. Specific detection of proteins was conducted by SDS-PAGE followed by Western blot using antisera raised against pine GS (Cantón *et al.*, 1996) and Fd-GOGAT (García-Gutiérrez *et al.*, 1995). Protein quantifications in gels and blots were conducted using Mac Bass image analysis software.

Determination of nitrate, water, polysaccharide and lignin

The determination of nitrate in soil samples was carried out colorimetrically after reduction to nitrite with a Bran & Luebbe Technicon TRACCS 800 Autoanalyzer (Norderstedt, Germany) following the manufacturer's instructions (Industrial method nos 818–871). Water content was determined in 1–2 cm leaf disks after desiccation at 80°C for 2 d. Total polysaccharides were determined in stem samples by the phenol-sulfuric method of Dubois *et al.* (1956). Lignin determination was performed according to the Klason method described in Kirk & Obst (1988).

Results

Growth of field-grown transgenic poplars overexpressing a pine glutamine synthetase gene

In previous studies we reported that poplars expressing cytosolic pine GS showed higher vegetative growth than control plants grown in the greenhouse and growth chambers (Gallardo *et al.*, 1999; Fu *et al.*, 2003). In order to establish whether differences in growth between transgenic and control plants are also maintained in open fields, we started a field trial test in the Province of Granada (Spain) in 2000 (Fig. 1). Rooted plants with an average height of 52.1 and 44.4 cm and corresponding to control and eight independent transgenic lines, respectively, were sown in December 2000, and their growth was monitored during a 3-yr period. The growth curve of the plants is shown in Fig. 2. The period of active growth corresponded to the April–October period, since apparent signals for bud break after dormancy was observed in April in the 3 yr, and symptoms of senescence in leaves were observed in October. During the first year, the growth rate of transgenic and control plants was very similar in April–May, but from June to October 2001 transgenic lines exhibited a higher vegetative growth, resulting in an increase in height of 21% with respect to the control plants. Similar results were observed in the stem diameter since transgenics were on average 19% thicker than control plants (data not shown). A similar behaviour in the growth of the plants was observed in the second and third year, but differences in the growth rate between transgenic and control lines were immediately visible

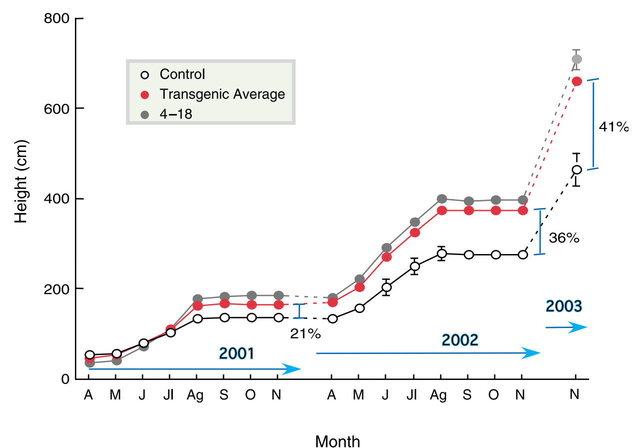

Fig. 2 Growth curve of control and transgenic poplar lines in an experimental plot in the province of Granada (Spain). A, April; M, May; J, June; Jl, July; Ag, August; S, September; O, October; N, November Vertical bars represent SD from mean. There were significant differences in height between control and transgenic plants from August 2001 ($\alpha \leq 0.05$).

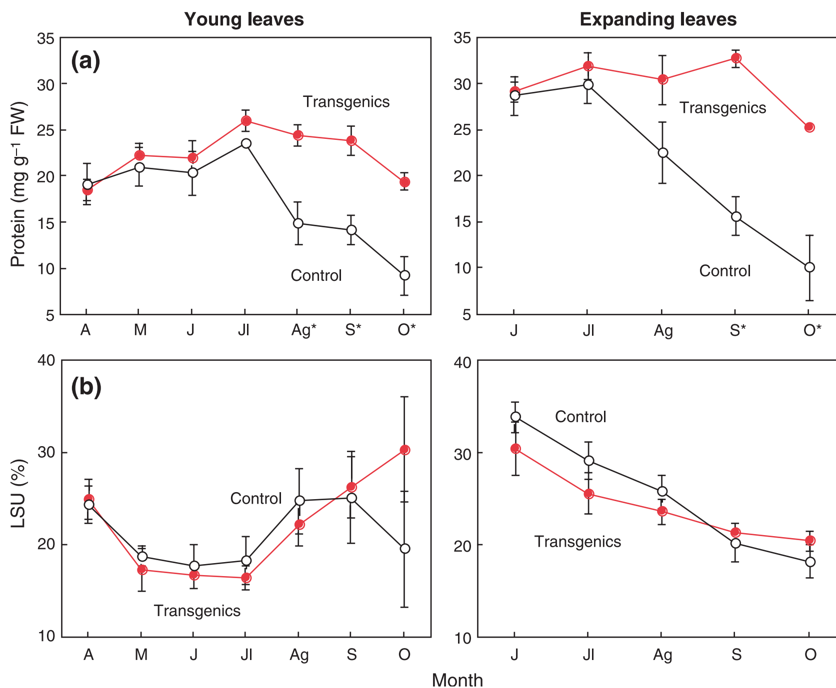

Fig. 3 Protein and LSU contents in young and expanding leaves of control and transgenic poplar during the first year of growth. Results are the mean ± SD of at least three independent determinations from different plants. Significant differences in protein content ($\alpha = 0.05$) are indicated by asterisks in the *x*-axis.

after bud break (not shown). At the end of the second and third period, the transgenics reached the average height of 3.7 and 6.6 m, respectively, representing an increase in height of 36 and 42% with regard to the height of control plants (Fig. 2). An average increase in stem diameter of 36% was also observed in transgenic trees with respect to control plants at the end of this study (data not shown). The 4–18 line was the best performance transgenic clone during the first two periods of tree growth, and the recorded values of increasing height growth are also included in Fig. 2 for comparison. This line reached an average height of 4.0 and 7.1 m after the second and third year, respectively, and the stem diameter increased by 44% with respect to control plants after the third year. Besides the differences in their vegetative growth no flowering was observed in either transgenic or control plants during the 3-yr study.

Characteristics of the leaf in transgenic and control plants

Water and protein contents were examined in young and expanding leaves. No difference in water content was observed between lines (data not shown). However, significant differences in protein contents were observed for both young and expanding leaves in the samples taken between July and October (Fig. 3, upper panels). Protein levels declined from July to October in young leaves of control plants; by contrast, the protein content in the young leaves of transgenic trees remained at high levels during the same period, with a slight decrease in October. These differences resulted in average protein content 2-fold higher in transgenic than in control leaves at the end of the studied period of growth (Fig. 3, upper panels). A related behaviour was observed for the protein content in expanding leaves, with significantly higher values in transgenic than in control leaves in the September–October period.

In order to investigate whether or not the observed changes in protein content were associated with changes in gene expression, the profile of total proteins and the level of LSU were monitored by SDS-PAGE followed by Coomassie blue staining. Neither difference in the protein profiles (not shown) nor in the relative LSU content (Fig. 3, lower panels) was observed between lines. In young leaves the content of LSU accounted for 17–23% of total proteins during the growing season, whereas in expanding leaves the LSU content was 30–34% of total protein in June, declining to 19% in October. The differences in the LSU plots indicate that young and expanding leaves correspond to different physiological states, and also suggest that the genetic modification does not affect the normal development of the leaf in the stages considered in this study. However, the differences in the protein content indicated above suggested that the GS/GOGAT cycle could be altered by the expression of cytosolic pine GS.

Expression analysis of GS and Fd-GOGAT

In order to determine the GS/GOGAT capacities of plants in the field, we conducted immunoblot experiments to quantify GS and GOGAT polypeptides. The analyses confirmed the presence of the pine GS polypeptide in the transgenic trees. As previously reported in greenhouse and growth-chamber studies, the presence of pine GS polypeptide was identified

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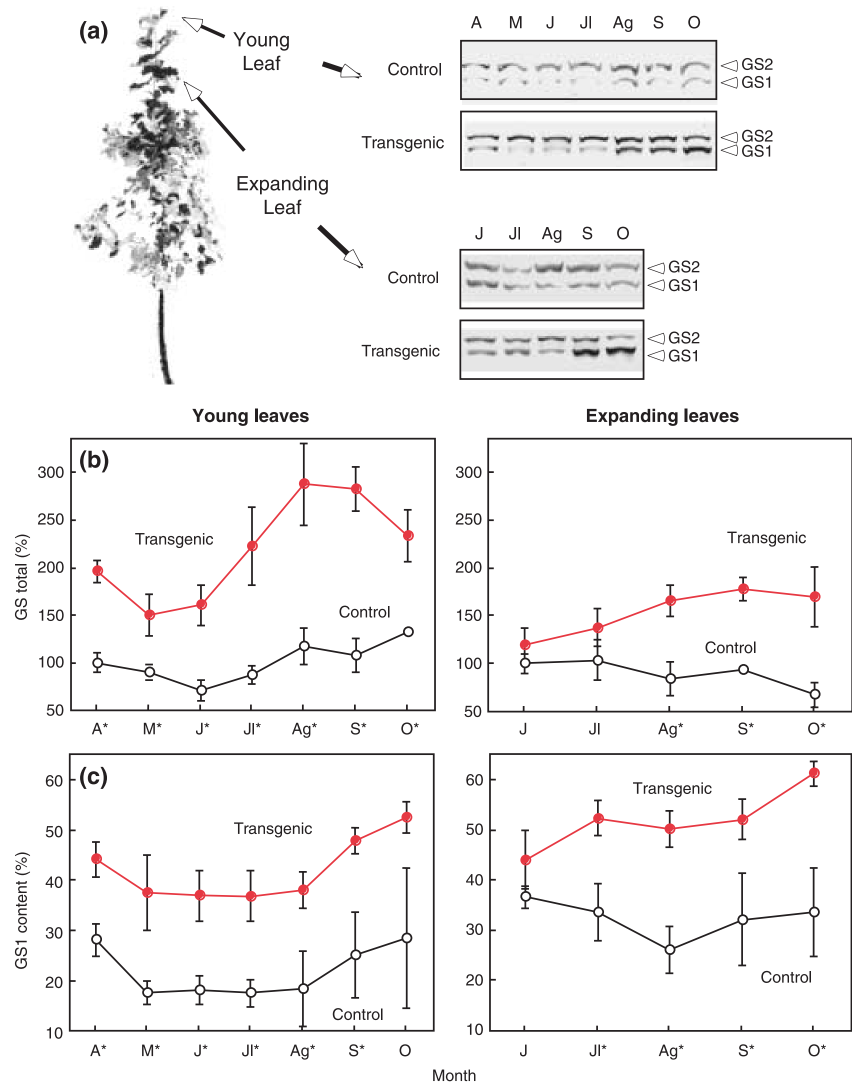

Fig. 4 GS content in control and transgenic poplar leaves during the first year of growth. GS polypeptides were separated by SDS-PAGE and detected by Western blot analysis. (a) Representative westerns of control and transgenic plants. (b) Total GS content in control and transgenic leaves. Total GS is expressed as a percentage of the polypeptide levels detected in control plants in April (young leaves) and June (expanding leaves). (c) GS1 content in control and transgenic leaves. GS1 content is represented as its relative content with respect to total GS content. Asterisks in the horizontal axis indicate significant differences ($\alpha \leq 0.1$).

as a band of 41 kDa, which substitutes the endogenous GS1 polypeptide of 40 kDa in the samples from transgenic plants (Gallardo *et al.*, 1999; Fu *et al.*, 2003; Fig. 4a). Total GS polypeptide content, comprising the bands for GS1 and GS2, was determined in the blots from young and expanding leaves during the first year of growth (Fig. 4b). In young leaves total GS was higher in transgenics than in controls. However, in expanding leaves, GS content was only higher in transgenics from August to October. Related results were observed when the GS1 content was recorded (Fig. 4c). GS1 content represented 18–28% and 26–36% of total GS in young and expanding leaves of control plants, respectively, whereas in the transgenics the content of GS1 was 37–52% and 44–61% in young and expanding leaves, respectively. Therefore, a main difference between transgenic and control poplar trees was their relative content of GS1.

When Fd-GOGAT level was analysed, a unique band of 160 kDa was detected in samples from young and expanding leaves, in both transgenic and control plants (Fig. 5a). The relative abundance of the Fd-GOGAT polypeptide was quantified and the results are shown in Fig. 5b. The corresponding protein profiles for Fd-GOGAT were related to the evolution of protein levels plotted in Fig. 3; thus, similar levels of Fd-GOGAT were observed in samples taken in spring, from April to July, but higher levels in transgenic leaves were detected in the leaf samples taken between August and October (Fig. 5b). Together, these results suggest that transgenic leaves had a higher GS/GOGAT cycle capacity than control ones.

Tree growth and GS1 content in leaf

The relationship between growth of poplar plants and GS1 content in leaves was analysed during the main growing months. A total of 148 leaf samples from control and transgenic plants were assayed to determine their GS1 content.

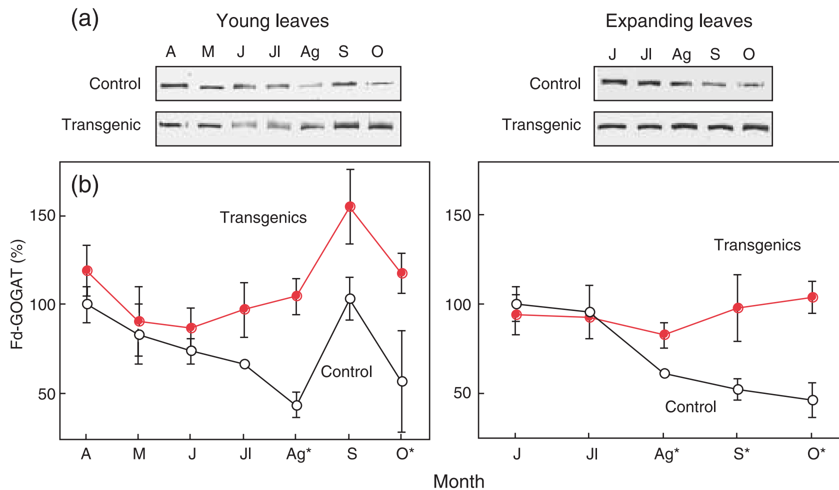

Fig. 5 Fd-GOGAT content in poplar leaves during the first year of growth. (a) Representative Western blots of control and transgenic leaves. (b) Fd-GOGAT content is represented as percentage of the polypeptide levels detected in control plants in April (young leaves) and June (expanding leaves). Asterisks in the horizontal axis indicate significant differences ($\alpha \leq 0.1$).

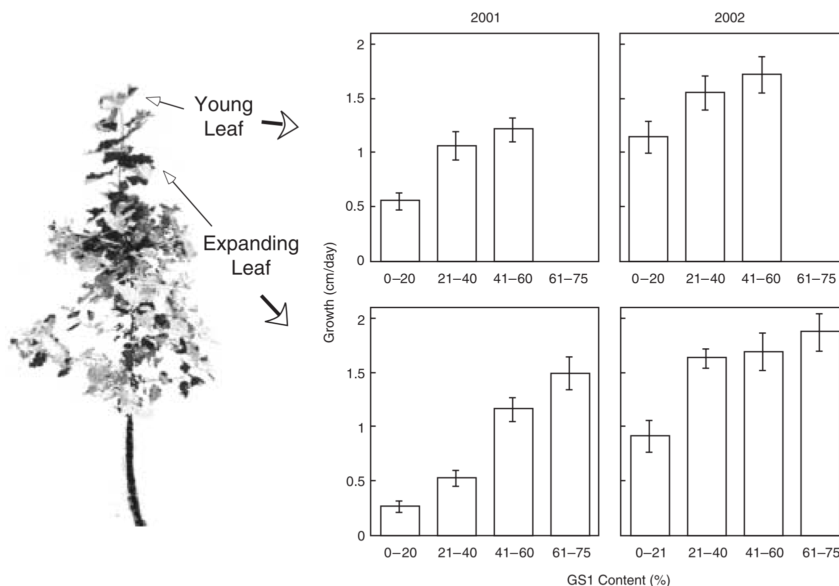

Fig. 6 Growth and GS1 content in poplar plants during the first (2001) and the second year (2002) of tree growth. Samples (148) from young and expanding leaves were grouped according to their GS1 content and plotted with the average growth of the plants. The contribution of GS1 content to the growth rate of the plants was significant ($\alpha = 0.05$, ANOVA) in young and expanding leaves in 2001 and in expanding leaves in 2002.

The samples, corresponding to young and expanding leaves, were taken in 2001 and 2002 during the period of higher growth rate for all lines (June–August). The samples were grouped according to their GS1 content as percentage of total GS (0–20, 21–40, 41–60 and 61–75% of GS1). Plots of plant growth vs the GS1 content in young and expanding leaves are shown in Fig. 6. The samples from young leaves were grouped in three categories (0–20, 21–40 and 41–60% of GS1), while the samples from expanding leaves were distributed in the four GS1-content groups. Surprizingly, distribution of control samples was different from the total of samples because most control samples (97%) contained a GS1 content lower than 40% of total GS, while 45% of transgenic samples contained GS1 levels higher than 41% of total GS. Increased growth of plants was significantly associated with their GS1 content in young and expanding leaves for the first year (2001) (Fig. 6, left panels). However, in 2002 there were no significant differences in young leaves, and only significant differences were observed between 0 and 20% of GS1 content and other GS1 groups in expanding leaves (Fig. 6, right panels). These results indicate that GS1 content in young and expanding leaves affects the growth of the tree, and also suggest the existence of other factor(s) that limit(s) the growth of plants with GS1 content higher than 20% of total GS.

Characteristics of poplar stem: vegetative storage protein, polysaccharide and lignin content

Once plants have reached the end of the growing season, the excess of N assimilated in the leaf is then stocked as vegetative storage proteins (VSP) in the stem. In trees, VSP are mainly accumulated in the bark, where they represent the most abundant proteins of the tissue. The analyses of proteins from stem sections of poplar plants cultivated in growth chambers

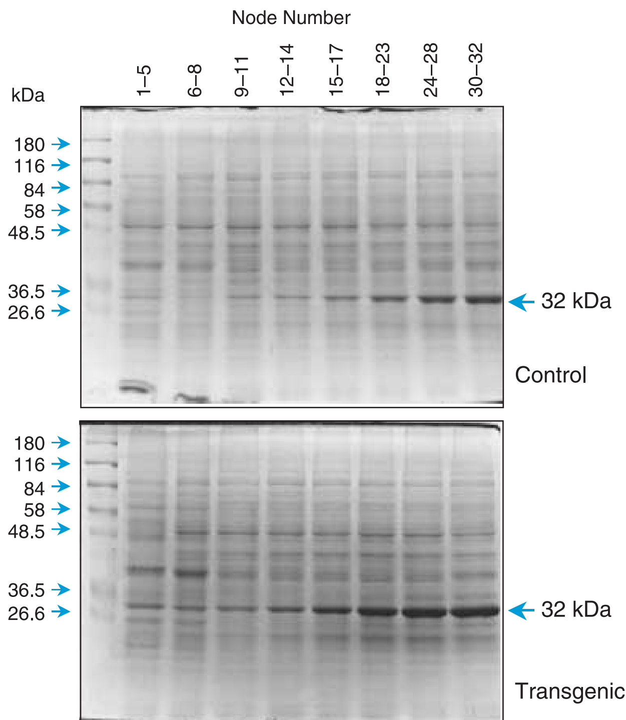

Fig. 7 Protein profiles from differentiating stems of control and transgenic poplar plants. A representative gel is depicted. Protein (20 µg) from stem extracts corresponding to different node intervals were fractionated by SDS-PAGE and stained by Coomassie blue. Node intervals are indicated from the apex. The position of the 32-kDa polypeptide is indicated on the right.

revealed that a 32-kDa polypeptide accumulated with the differentiation state of the stem (Fig. 7). This protein band corresponds in size to VSP previously characterized in poplar bark (Coleman *et al.*, 1991; Langheinrich & Tischner, 1991). The comparison between stained SDS-PAGE gels from control and transgenic plants showed that the 32-kDa polypeptide accumulated in transgenic stems more quickly than in control plants (Fig. 7). Thus, the level of the 32-kDa protein evolves from 5 to 7% of total protein in the apical part (nodes 1–5 in controls and transgenics) to 14 and 36% of total protein in nodes 30–32 of control and transgenic plants. The relative abundance of the 32-kDa protein was also monitored in the bark of plants in the field trial at the end of the dormancy period after the first and second year of growth. The protein profile revealed that the 32-kDa polypeptide was the most abundant protein in bark, representing 33–40% of the total protein content in all bark samples, regardless of plant line and year of growth. A representative gel of bark samples corresponding to the second year of growth is shown in Fig. 8a. Interestingly, the analysis of protein indicated that protein levels in transgenic bark were 21% higher than in control samples after the first year of growth. However, the protein abundance was similar in the samples taken after the second year (Fig. 8b). Since differences in the stem diameter were observed during this study, these data suggest that a limit for the accumulation of protein in the bark is reached in the second year in all the plants, regardless of their stem diameter.

In order to estimate if transgene expression affects other stem features, extracts from trunk slices were prepared to determine total polysaccharide and lignin content. The samples were taken from 1-yr-old plants from the field trial in Granada and from 5-yr-old plants cultivated in soil at the Experimental Research Station at the University de Málaga. No significant differences were observed between lines, with total polysaccharide content of 48 and 59% and lignin contents of 22 and 21% in 1- and 5-yr-old trees, respectively.

Discussion

Field trials of transgenic plants represent a relevant test to learn about the biological role *in planta* of introduced genes and proteins, and also to verify their potential use and interest for commercial applications. This type of study is a laborious but necessary task, since growth conditions in the field do not usually match experimental conditions maintained in greenhouse or growth chambers studies. In the particular case of trees, very limited information is available about seasonal variation in gene expression or how environmental conditions affect primary and secondary metabolism. In this work, transgenic hybrid poplars expressing cytosolic pine GS exhibited a higher vegetative growth than control plants during a 3-yr study. The data derived from this study are in agreement with our previous results obtained in growth chamber and greenhouse

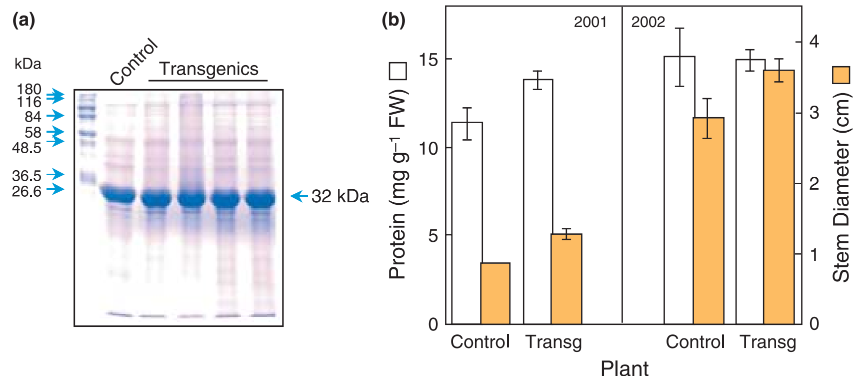

Fig. 8 Protein content in bark of control and transgenic poplar trees. (a) Representative gel of bark proteins (10 µg) during dormancy after the first (2001) and second (2002) years of growth in the field. The position of the 32-kDa VSP polypeptide is indicated on the right. (b) Comparison of protein content in bark and stem diameter of plants. Vertical bars represent SD from mean.

studies (Gallardo *et al.*, 1999; Fu *et al.*, 2003). A main difference between earlier studies and our field trial was the availability of nutrients in soil, since the effect of a GS1 transgene on plant growth was previously observed in tobacco and poplar in conditions of low N nutrition or without the addition of N fertilizers (Fuentes *et al.*, 2001; Gallardo *et al.*, 1999; Fu *et al.*, 2003; Gallardo *et al.*, 2003). By contrast, the analysis of N in soil indicated that our growth study was carried out with a nitrate concentration of 50 ± 6 ppm, which is probably due to the proximity of the experimental plot to a side brook. Therefore our results indicate that the effect of pine GS1 transgene expression was relevant even under medium-high nitrate availability in soil.

The analysis of GS polypeptides confirmed the presence of GS1 polypeptide in transgenic leaves as described in previous reports (Gallardo *et al.*, 1999; Fu *et al.*, 2003), and the existence of higher levels of total GS in the transgenic trees. These characteristics were observable during the first year, once young leaves appeared after bud break (April). However, their effects on growth rate were only apparent from July, and their effects on other parameters considered in this study, such as protein and Fd-GOGAT levels, were observed later. Therefore, it seems that the transgene expression affects plant features with time. Interestingly, water and LSU content were similar in control and transgenic leaves, suggesting that higher GS/Fd-GOGAT cycle capacities of transgenics were responsible for the changes detected, without altering the differentiation state of young and expanding leaves. Nevertheless we cannot exclude that GS1 transgene expression may alter the expression of other enzymes involved in N and C metabolism. Further characterization of the transgenic lines by transcriptomic and/or proteomic approaches (Bhalerao *et al.*, 2003; Cánovas *et al.*, 2004) are required to contrast this possibility.

During the growing period, N reserves such as VSP are accumulated in the bark. The synthesis of VSP is subjected to seasonal changes and they accumulate, mainly due to leaf senescence during autumn and winter (Wetzel *et al.*, 1989). After dormancy, bud break and leaf development depends, in the first instance, on the abundance of VSP accumulated in bark. When protein profiles of stem sections were investigated, the accumulation of a 32-kDa VSP was observed concomitantly with the differentiation of the stem in poplar trees. This polypeptide may represent up to 40% of the total protein detected in bark during dormancy. According to its pattern of accumulation and to its abundance in bark, differences between plants in their VSP content were only observed during the first months of the development of the tree. Interestingly, it has been reported that expression of VSP in poplar is regulated by glutamine (Zhu & Coleman, 2001) and this amino acid is the main form of N transported in poplar (Sauter & van Cleve, 1992). Therefore faster accumulation of the 32-kDa VSP in transgenics could be a consequence of the differences in the GS content detected in transgenic and control plants, and increased availability of organic N in the form of glutamine for tree growth and development. Although the relative abundance of VSP in bark of control and transgenic plants was similar, the total content of protein in bark differed between lines with higher levels in transgenics after their first year of growth. However, these differences were not observed after the second year. These data could indicate that direct and non-direct effects of transgene expression were affecting the growth rate of the tree. Thus, during the first year, and in agreement with our previous studies (Fu *et al.*, 2003), the higher GS content in leaf contributes to the higher growth of transgenics, and their higher content in protein and Fd-GOGAT detected from July could also affect their growth rate during the first year. However, the faster accumulation of VSP and, mainly, the higher content of protein in bark at the end of the first year could influence plant growth on the next period, since tree development after dormancy depends on the reserves accumulated in the stem. Although protein and VSP content in bark is similar in the plants after the second year, the stem of transgenics was thicker and therefore their higher reserves could explain the differences observed in growth at the end of this study. Nevertheless, it cannot be ruled out that transgene expression could also be a relevant factor on the growth of plants during the second period and beyond. Cytosolic GS (GS1) has been recently proposed as a key component of N use efficiency in plants (Miflin & Habash, 2002) and its metabolic role is particularly important for N remobilization and recycling in woody plants (Suárez *et al.*, 2002; Gallardo *et al.*, 2003). The findings reported here support a higher capacity of transgenic trees, not only in primary N assimilation but also in the reassimilation of ammonium released in different metabolic processes. This enhanced efficiency for N recycling could result in better exploitation of nutrient resources, improved efficiency of photosynthetic cells, and faster plant growth.

Besides the differences in growth and stem diameter, the content of total polysaccharide and lignin were similar in transgenic and control stem samples taken from 1- and 5-yr-old plants cultivated in soil. We did, however, notice, but did not quantify, a tendency for transgenic plants to have a higher rate of rhizome production (i.e. new shoots developed from spreading roots, away from the main stem). This capacity, if real, could contribute to improved uptake of nutrients and therefore may be an important cause of the higher growth seen in transgenic plants. In addition this could result in a higher rate of vegetative spread from plantations, a relevant issue to be considered for biosafety of transgenic forest exploitations. However, we do not expect that the modest increase observed is likely to exceed the great variation in vegetative spread that occurs among wild and cultivated poplars.

In conclusion, most of the characteristics of the transgenic GS poplar trees we studied seem to be within the normal range, with the exception of a higher rate of growth than control plants. Therefore modification of GS1 expression could be considered as a reasonable approach to improve the

growth of poplars in commercial settings. Further research, especially longer term field trials that include a range of genotypes, GS transgenes, and environments, will be needed to verify its commercial potential.

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