Methods

High rooting frequency and functional analysis of GUS and GFP expression in transgenic Medicago truncatula A17

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Summary

• An effective transformation method is described for Medicago truncatula A17, verifying its suitability as a model legume for functional genomics.
• Media and culture methods are detailed that yielded an average frequency of 35% for recovery of transgenic shoots from cotyledonary node explants and 39% for root induction and regeneration of entire plants from 419 phosphinothricin-resistant shoots.
• Fertile plants transgenic for both 35S-GFP and phas-GUS were obtained in five of eight independent experiments. The presence and stable inheritance of transgenes was confirmed by GFP or GUS expression and by genomic DNA blots. GFP expression driven by the normally constitutive CaMV 35S promoter diminished as the leaves matured. Although GUS was very strongly and uniformly expressed in seed cotyledons of most lines, one line exhibited an aberrant, patchy pattern. Additionally, weak GUS expression was evident in leaf veins from the normally stringently spatially regulated phas promoter.
• Stably transformed, fertile, M. truncatula A17 plants were generated. The unconventional expression patterns for 35S-GFP and phas-GUS expression obtained in some transformants suggest the occurrence of novel epigenetic events.

Key words: Medicago, legume, transformation, rooting, 35S, phaseolin.

Introduction

With over 650 genera and 18 000 species, the family Leguminoseae is the third largest family of higher plants and is second only to grasses in agricultural importance (Young et al., 2003). Legumes are major sources of protein and oil for humans and livestock. Some legumes have beneficial medicinal qualities; others can be highly allergenic. While Arabidopsis is proving to be an excellent organism for gene discovery and functional genomics, it does not have the ability to establish symbiotic interactions with rhizobia and mycorrhizae exhibited by legumes such as Lotus japonicus and Medicago truncatula. The lack of symbiosis in Arabidopsis justifies the development of a legume model. In Europe, Lotus japonicus has been established as a valuable legume system for gene discovery. Effective systems for Agrobacterium-mediated transformation have been established (Handberg & Stougaard, 1992; Stiller et al., 1997), enabling the use of transposon (Thykjaer et al., 1995) and T-DNA (Martirani et al., 1999) tagging as tools to identify novel plant genes.

Medicago truncatula is also emerging as a model legume system for future gene discovery. Attractive features are its small, diploid genome, self-fertility and short generation time. Rapid progress with sequencing its ca. 500 Mbp genome and in comparative genomics is underway (Lamblin et al., 2003). However, a facile system for molecular transformation is of great importance for functional genomics. Tissue-culture-based transformation methods have been reported for four...
genotypes of M. truncatula: R108-1 (Hoffmann et al., 1997; Trinh et al., 1998; Scholte et al., 2002), Jemalong 2HA (Thomas et al., 1992; Chabaud et al., 2003), Jemalong J5 (Kamaté et al., 2000) and Jemalong A17 (Chabaud et al., 1996; Trieu & Harrison, 1996). Among these, Jemalong A17 is of special interest because the genome of this single-seed descent line was selected for sequencing. Considerable excitement was engendered by the development of two very efficient in planta transformation methods, one utilizing the inflorescence and the other seedling infiltration by a medium containing Agrobacterium (Trieu et al., 2000). These approaches were of special interest as they did not require tissue culture and could potentially be used for T-DNA insertion analysis.

Unfortunately, the in planta methods have not been reproduced (Somers et al., 2003) and difficulty has been experienced with M. truncatula A17 in effectively establishing previously described tissue culture-based transformation systems (Chabaud et al., 1996; Trieu & Harrison, 1996). Therefore, great need exists for a reproducible and convenient transformation method for this line. Here, we describe in detail an effective method for transformation and regeneration of M. truncatula A17 based on shoot organogenesis from the cotyledonary node. It has thus far been successful in producing transgenic shoots from each of eight completely independent experiments, five of which resulted in stable fertile transformants that were of special interest as they did not require tissue culture (T

Preparation of the Agrobacterium inoculation medium
pCB302-phas-GUS (Fig. 1a) was electrophoretically transformed into Agrobacterium tumefaciens strain EHA105 or AGL1; the bacterial cells were selected on 100 mg l

Preparation of cotyledonal explants and infection with Agrobacterium

Materials and Methods

Plant material

Medicago truncatula cv. Jemalong (line A17) was used for all experiments. Individual wild-type plants were grown in Redi-Earth (Scotts) in 4” pots under glasshouse conditions of 14 h day length (23°C day, 20°C night). Plants were watered daily and once weekly with Miracle-Gro® nutrient at the concentration recommended by the manufacturer. Primary transgenic plants were grown under similar conditions in a growth chamber.

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Preparation of cotyledonal explants and infection with Agrobacterium

Procedures for permeabilization and surface-sterilization of M. truncatula seeds were modified from Trieu et al. (2000) and the preparation of cotyledonal node explants was altered from Trieu & Harrison (1996). Mature seeds were exposed to conc. H

Plant regeneration and selection

Co-cultivated explants were washed twice by shaking in sterile distilled water at 100 °C (10 min each time), blotted dry on sterile filter paper and placed on 25 × 100 mm Petri dishes (c. 10 explants per dish) containing a regeneration medium consisting of SH salts and vitamins, 20 g l

Park, NC) in 0.8% agar, pH 5.8. The explants were incubated for 15 d under the same conditions as those used for cocultivation and then transferred to selection medium (regeneration medium supplemented with 1.6 mg l⁻¹ phosphinothricin (PPT) from Duchefa Biochemie, Netherlands) and transferred to fresh medium every other week. Untransformed shoots started to die after 1 wk and were removed at the time of transfer.

Induction of adventitious roots

Well developed resistant shoots (ca. 1.5 cm) were separated from explants with a surgical blade and laid with the cut end exposed on the surface of a plant development and rooting medium consisting of SH salts and vitamins, 10 g l⁻¹ sucrose, 0.5 mg l⁻¹ IBA (Sigma), 100 mg l⁻¹ Cloran, 300 mg l⁻¹ Timentin and 0.25% Phytagel (Sigma), pH 5.8, in Phytatrays (Sigma), with 3–4 shoots per Phytatray. If no roots were induced within 1 month, the ends of the shoots were cut with a surgical blade and transferred to fresh medium. The plantlets with well-developed roots were transplanted into soil and cultured (25°C, 16/8 h photoperiod) in a growth chamber, initially with high humidity by covering with plastic bags. The plants usually started to flower after one month.
Genomic DNA blot analysis of transgenic plants

Total genomic DNA from transgenic plants was extracted using cetyltrimethylammonium bromide (CTAB) (Woodhead et al., 1998), digested with HindIII, loaded (10 µg per lane) onto an agarose gel (1%) and, after electrophoretic separation, blotted onto Hybond-N + membrane (Amersham, Piscataway, NJ, USA). The EcoRV-XbaI fragment of the T-DNA construct containing the GUS coding sequence was used as a GUS probe (Fig. 1a). A DNA fragment PCR amplified from the binary vector pCB302-phas-GUS with the primer pair 5′-CACTGGAG-TTGTCCCAATTCTTG-3′ and 5′-GTCTGGTAAAAGGACAGGGC-3′ was used as a GFP probe (Fig. 1a). Hybridization was carried out using ULTRAhyb ultrasensitive hybridization solution (Ambion, Austin, TX, USA) for 14 h at 42°C. The hybridization results were recorded using a Fuji BAS 2000 phosphorimager system (Fuji, Stamford, CT, USA).

PCR analysis of GUS transgenic plant

Total genomic DNA was used as the template for PCR analysis with the primer pair 5′-GGTGGGAAACCGGCTTACAAG-3′ and 5′-GTCTGGTAAAAGGACAGGGC-3′ and 30 cycles of amplification (94°C for 1 min, 60°C for 1 min and 72°C for 2 min).

Detection of GFP expression in transgenic plants

The expression profile for intact transgenic plants was observed using a LT9700 Little Luma excitation light (Lighttools, Encinitas, CA, USA) with 470 nm excitation and 500 nm emission wavelengths. Precise localization of GFP expression in transgenic plants was undertaken using a Zeiss Stemi SV11 microscope with a Zeiss AttoArc 2 light source (Zeiss, Thornwood, NY, USA). Excitation and emission wavelengths were 470 nm and 500 nm, respectively. Expression patterns were recorded using a Zeiss AxioCam HRc.

Detection of GUS transcript using RT-PCR

Total RNA was extracted from young and mature leaves of M. truncatula plants transgenic for 3SS-GFP using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase (Invitrogen, 1 unit µl−1 at 25°C for 15 min to remove DNA and then heated at 65°C for 10 min in the presence of 2.5 mM EDTA to inactivate DNase. RT-PCR reactions were performed using a OneStep-RT-PCR kit (Qiagen) according to the manufacturer’s recommendations. Reverse transcription was performed at 50°C for 30 min, followed by PCR activation at 90°C for 15 min and then 25 cycles of PCR amplification (94°C for 1 min, 52°C for 1 min and 72°C for 1 min) using primer pair 5′-CACTGGAGTTGTCCCAATTCTTG-3′ and 5′-GTCTGGTAAAAGGACAGGGC-3′ for GFP transgene, and primer pair 5′-CTCTCCGCCACCGATTAC-3′ and 5′-CCATCTCTCTCAGCTGC-ATAC-3′ for M. truncatula EF-1α.

Histochemical localization of GUS activity

Embryos and leaves from transgenic plants were stained for GUS activity as described (Jefferson et al., 1987). Tissue samples were immersed in the GUS staining solution, vacuum infiltrated for 5 min to facilitate penetration, and then incubated at 37°C in the dark. The stained samples were then rinsed with distilled water and immersed in 95% ethanol to remove chlorophyll.

Results

Agrobacterium-mediated transformation of M. truncatula

Eight independent transformation experiments were performed using CaMV 35S-GFP and phas-GUS constructs (Fig. 1a) inserted into binary vector pCB302 (Xiang et al., 1999) in Agrobacterium strain EHA105 (Hood et al., 1993) for experiments 1–3, and in strain AGL1 (Lazo et al., 1991) for experiments 4–8 (Table 1). In these experiments, 100–150 explants were inoculated with Agrobacterium suspension. Phosphinothricin (PPT)-resistant shoots were induced in 3% to 15% of the explants, with an average of 6% of the explants treated. More than one resistant shoot was often induced from a single explant; the ratios of resistant shoots to inoculated explants ranged from 3% to 174%, with an average of 35%. The clear distinction between transgenic, PPT-resistant explants with green leaves and nontransformed with browning leaves on selection medium is shown in Fig. 2(a). The difficulty in inducing roots in initial experiments using the protocol of Trieu & Harrison (1996) is evident from the data shown for experiments 1, 2, 4, 7 and 8 (Table 1) in which no rooted plants were recovered. However, using the improved rooting procedure (see experimental procedures for details), considerably higher proportions of the regenerating resistant shoots form roots, as seen for experiments 1 (27%) and 8 (45%). Efficient induction of roots was very dependent on both the composition of the medium and the positioning of the cut shoot, as shown in Fig. 2(b).

In total, on the basis of GFP expression, more than 30 transgenic plants have been obtained from five independent experiments. To confirm the integration of T-DNA into the genomes of these plants, genomic DNA was extracted from 10 of the T0 plants derived from five separate experiments (experiments 1, 2, 4, 7 and 8) and subjected to hybridization analysis using the GUS and GFP probes indicated in Fig. 1(a). The results showed that, except for line 7, the T0 plants regenerated from PPT-resistant shoots carry at least one copy of each reporter (Fig. 1b,c). Of the 10 lines tested, eight were shown to be independent. Lines 3a, 3b and 4a, 4b were siblings, reflecting the recovery of multiple PPT-resistant shoots.
from a single transformation event. The hybridization fragments for GUS are all larger than 6 kb, as expected from the 5.8 kb distance from the HindIII site between the nos terminator and nos promoter and the right T-DNA border (Fig. 1a). The 2.0 kb GFP hybridization fragments reflect the distance between the two HindIII sites flanking the 35S-GFP gene. The hybridization patterns are consistent with the presence of one copy of the transgene insert in lines 4 and 8, two copies in lines 5 and 6, three copies in line 1 and four copies in line 3. Line 2 appears to have one intact copy and a second, partial copy.

The inheritance of GUS in the T1 generation of line 2 was confirmed by PCR amplification. A fragment with the predicted length of 1.2 kb was amplified from nine out of 10 T1 plants (Fig. 1d). For lines 1, 2, 3 and 4, the presence of transgenes in T1 and T2 progeny was confirmed by the expression of GFP and GUS.

Diminution of 35S-driven GFP expression in mature tissues

Expression of GFP has proven a useful visual screenable marker for plant transformation (Haseloff et al., 1997). To assess its value in Agrobacterium-mediated transformation of Medicago truncatula A17, a 35S-GFP fusion construct (Fig. 1a) was used. Strong green fluorescence was detected at some cut surfaces of inoculated explants 2 d after cocultivation and also in newly regenerating shoots from the cotyledonary node regions of the explants (Fig. 3a,b), permitting early identification of transformed tissues. Strong expression was also seen in young (ca. 1 d old) leaves of transgenic lines 1 (Fig. 3c) and 2; weaker expression was evident for lines 3–6 and 8. However, GFP expression diminished in all lines as the leaves aged, as seen for the 5-d-old trifoliate leaf of line 1 in Fig. 3(d) (although fluorescence at the leaf margin sometimes remained, as shown in the inset). Interestingly, as the shoots elongated, newly emerging leaves showed high GFP expression; again, this diminished as the leaves aged. The possibility that decreased expression resulted from gene silencing was evaluated by analysis of RNA transcript level using RT-PCR. In fact, similar transcript levels were detected in extracts of young and old leaves (Fig. 4), indicating that silencing was not the primary cause of decreased GFP expression.

Petals showed high levels of expression, but expression in the calyx was low (Fig. 3e). Evaluation of GFP expression in seed tissues was difficult because high levels of fluorescence were present in untransformed plants (not shown). The eight lines examined in detail were fertile although line 1 produced only a few pods. The germination rate for line 1 (c. 30%) was also lower than that of the other transgenic lines, which was similar to wild type.

GFP expression was evident in all tissues of the emerging T1 progeny seedling of line 1 shown in Fig. 3(f). Seedlings of other lines had lower overall levels of expression than line 1, but, in each case, the radicle region showed higher fluorescence than the other regions. An entire 20-d-old plant of line 1 is shown in Fig. 3(g); bright green fluorescence was seen throughout the root tissues but, in the shoot, GFP expression was limited to nodes and young leaves. As shown in Fig. 3(h,i), no green fluorescence was detected for 2-d- or 10-d-old wild-type seedlings.

| Table 1 | Efficacy of transgenic rooted shoot recovery from cotyledonary node explants of Medicago truncatula |
|---|---|---|---|---|
| Expt | Inoculated | Resistant<br>1 | Resistant<br> (%) | Regenerated<br>2 | Per inoculated explant<br> (%) | Rooted | Rooted<br> (%) |
| 1 | 140 | 11 | 8 | 41 | 29 | 11 | 27 |
| 2 | 150 | 23 | 15 | 261 | 174 | 103 | 39 |
| 3 | 150 | 5 | 3 | 12 | 8 | 0 | 0 |
| 4 | 150 | 9 | 6 | 53 | 35 | 21 | 40 |
| 5 | 150 | 4 | 3 | 15 | 10 | 0 | 0 |
| 6 | 100 | 3 | 3 | 3 | 3 | 0 | 0 |
| 7 | 140 | 6 | 4 | 24 | 17 | 10 | 42 |
| 8 | 100 | 7 | 7 | 40 | 7 | 18 | 45 |

1No. of inoculated explants from which shoots resistant to 1.6 mg l⁻¹ phosphinothricin (PPT) were recovered. 2No. of PPT-resistant shoots recovered from the resistant explants.

Histochemical staining was performed to detect GUS expression driven by the phaseolin promoter in transgenic Medicago truncatula

Histochemical staining was performed to detect GUS expression driven by the phas promoter in transgenic plants. For lines 1, 2 and 4 (Fig. 1b), blue staining was evident for embryos within a few minutes of incubation, and overnight staining resulted in intense blue staining throughout the embryo, indicating a high level of GUS expression (Fig. 5a). For line 3, discrete, dark blue spots were evident throughout the embryo (Fig. 5b), suggesting the occurrence of epigenetic silencing in this multicopy line.

Although expression from the phas promoter is known to be strictly confined to embryogenesis and microsporogenesis
in beans, transgenic tobacco (Sengupta-Gopalan et al., 1985; van der Geest et al., 1995) and Arabidopsis (Chandrasekharan et al., 2003), histochemical staining revealed GUS expression in the leaf veins of all the transformants (Fig. 5c); as for the embryo, line 3 exhibited spotty expression in the leaves (Fig. 5d).

GUS expression was assessed histochemically for 7-d seedlings of lines 1, 2 and 4; a representative seedling is shown in Fig. 5(e). GUS has a half-life of ca. 50 h (Jefferson et al., 1987) and residual expression was seen for tobacco seedlings 5 d after germination (Frisch et al., 1995). Similarly, residual GUS expression was detected (Fig. 5e) for 7-d-old M. truncatula

**Fig. 2** Regeneration of transgenic plants. (a) Selection of regenerating Medicago truncatula A17 shoots on medium containing 1.6 mg l⁻¹ phosphinothricin (PPT). Green, resistant shoots are indicated by arrows; brown, nontransformed shoots are indicated by starred arrows. (b) Optimal positioning of cut shoots for root induction; note that roots emerge from the upper edge of the cut surface. Both panels are for Expt 8 in Table 1. Scale bars: 1 cm in both (a) and (b).
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Fig. 3 GFP expression in transgenic Medicago truncatula A17. (a) Transient GFP expression (arrowhead) in explants 2 d after cocultivation. (b) Transgenic shoot 1 month after initiating regeneration. (c) Emerging trifoliate leaf after transfer of plant to soil. (d) Diminished GFP fluorescence in the mature trifoliate leaf, except at the margin of some leaves (inset); bright green fluorescence remains at the petiole nodes (arrowhead). (e) Strong fluorescence in the corolla but weak fluorescence in the calyx. (f) Ubiquitous fluorescence in germinating (2 d) T1 seedling. (g) Entire T1 plant (ca. 20 d) showing strong fluorescence in roots and nodal meristem (arrowhead), but no or low expression in mature leaves or stem. (h) A 2-d-old wild-type seedling. (i) A 10-d-old wild-type seedling. Scale bars: 5 mm in (a) (b) (d) (f) and (h); 1 mm in (c) and (e); 1 cm in (g) and (i).

Fig. 4 RT-PCR analysis of GFP transcription in young and old leaves of transgenic Medicago truncatula A17. Bottom panel shows GFP transcript levels in young and old leaf tissues of transgenic plants normalized relative to that of the internal control, EF-1α (top panel). WT, wild-type.

Discussion

Optimization of transformation, selection and regeneration condition

The direct induction of shoots from cotyledonary nodes has been used successfully for regeneration of transgenic plants following Agrobacterium-mediated infection for several legume species (Somers et al., 2003). Although an early report exists of M. truncatula A17 transformation by this approach (Trieu & Harrison, 1996), we are not aware of any subsequent articles that have utilized this system. While the work reported here is based on this earlier work, many small but important modifications were made to obtain reproducible, high frequencies for recovery of transgenic plants.

Compared with other tissue culture-based transformation methods, the cotyledonary-node approach is relatively fast and does not involve the induction of embryogenic calli and somatic embryos, which can be difficult. On the other hand, common problems with this approach are the low rates of transformation and regeneration, and recovery of transgenic plants can be less than 1% (Zhang et al., 1999; Donaldson & Simmonds, 2000). In this work, up to 15% inoculated explants gave rise to PPT-resistant shoots and up to 45% PPT-resistant shoots were rooted (Table 1). Excluding experiments 3, 5 and 6, for which no rooted transgenic shoots were obtained, the average recovery of rooted shoots was 39% from a total of 419 explants. Use of the conditions detailed in the Experimental Procedures section should greatly facilitate research in gene discovery and gene function in the model plant M. truncatula.

In our initial attempts to establish M. truncatula transformation, Agrobacterium strain LBA4404 was used, as in the original protocol (Trieu & Harrison, 1996), but without success. Agrobacterium strains EHA105 (Hood et al., 1993) and AGL1 (Lazo et al., 1991) were then tested because of their reported virulence; both proved to be effective in transforming M. truncatula.

An important component of the transformation procedure is inclusion of DTT and l-cysteine in both the inoculation
and the cocultivation media. The presence of these compounds in inoculation and cocultivation media substantially reduces tissue browning resulting from the release of phenolics (McCown et al., 1968) and greatly improved transformation efficiency in soybean (Olhoft et al., 2001; Olhoft et al., 2003). The inclusion of these compounds improved transient GFP expression immediately after cocultivation and yielded a higher percentage of explants from which PPT-resistant shoots were regenerated.

For successful transformation of *M. truncatula*, the selection stringency must be carefully determined, and should be coupled with the strength of the promoter used to drive the resistance gene to get effective selection of transformants, that is if a weak promoter is used, a lower concentration of selection agent should be employed. The presence of 1.6 mg l\(^{-1}\) PPT in the selection medium was effective in killing untransformed shoots within 2 wk while enabling the transgenic shoots to survive and develop. To avoid untransformed shoots from escaping, selection pressure was maintained for 2 months.

In summary, the parameters we found to be of major importance in successful recovery of transgenic *M. truncatula* A17 shoots include the use of hypervirulent *Agrobacterium* strains for inoculation, the incorporation of l-cysteine and DTT in inoculation and cocultivation media to suppress tissue browning, and careful determination of antibiotic selection stringency.

**Induction of adventitious roots from transgenic shoots**

Rooting of the regenerating shoots has been a bottleneck for transformation of *M. truncatula* A17. In the present work, a large array of modifications were tested towards improving rooting efficiency. Changes from the conditions used by Trieu & Harrison (1996) found to be effective were: supplementation of full strength Schenk and Hildebrandt salts and vitamins (Schenk & Hildebrandt, 1972) with 0.5 mg l\(^{-1}\) indole-3-butyric acid (IBA) instead of 0.2 mg l\(^{-1}\) and reduction of the sucrose concentration from 20 g l\(^{-1}\) to 10 g l\(^{-1}\); the use of...
0.25% Phytagel as the gelling agent instead of 0.8% agar, and the exclusion of the PPT selection agent. Various combinations of IAA and NAA were tested, but they were much less effective than IBA. A procedure found to be of vital importance is that the shoots must be laid on the surface of the medium with freshly cut ends exposed in the air, as illustrated in Fig. 2(b). For shoots that did not root within 1 month, the ends were cut again and the shoot transferred to fresh medium. Recent experiments revealed that a pH of 6.5 is more effective for root induction than is pH 5.8, yielding rooting frequencies approaching 50%. Even shoots that had failed to root after a year of culture responded favourably to this procedure and the numbers of rooted shoots, and hence their percentage of resistant shoots (Table 1) is increasing.

Intriguing expression patterns of 35S-GFP and phas-GUS transgenes in M. truncatula

The progressive loss of GFP expression in aerial parts as the plant aged was dramatic and occurred in each of the four transgenic plants studied. Consequently, transgene copy number does not appear to be a major factor. The possibility that decreased expression resulted from gene silencing was evaluated by analysis of RNA transcript level using RT-PCR, but the similar GFP transcript levels found in young (1–2 d) and old leaves (> 7 d) (Fig. 4) detracts from this notion. In detailed studies of ageing leaves of Brassica napus, Halfhill et al. (2003) found that the decrease in GFP expression was positively correlated with soluble protein content. However, this does not satisfactorily explain the rapid disappearance of GFP seen as the M. truncatula leaves age. Possibilities to be examined in the future include the synthesis of a quenching agent in the maturing leaves or the accumulation of a degrading agent such as a protease. The present analysis of transcript levels was not exhaustive and it also remains formally possible that gene such as a protease. The present analysis of transcript levels was maturing leaves or the accumulation of a degrading agent above and below the cotyledonary node evident in Fig. 3(g) (and seen in three additional lines) is dramatic. Further resolution of the situation will require evaluation of expression of GFP from alternative promoters and, conversely, to examine expression of GUS from the 35S promoter.

Evidence that aberrant transgene expression in M. truncatula is not confined to the 35S promoter is provided by the patchy pattern of GUS expression from the phas promoter in T1 embryos of lines 3a and 3b. The nonuniform expression suggests silencing, a phenomenon not previously encountered for the phas promoter. This may reflect the fact that the transgene construct is present in relatively high copy number in lines 3a and 3b (Fig. 1b) since intense uniform staining, and hence GUS expression, was seen for all other lines tested (1, 2 and 4). Lack of the usual spatial stringency of expression from the phas promoter in transgenic M. truncatula was also evidenced by the weak expression of GUS in mature leaf veins (Fig. 5c,d). While this may reflect the genomic environment of the transgene, the observation of expression in several independent lines detracts from this explanation. These findings are especially interesting since it might be assumed that the phas promoter would behave more, rather than less, faithfully in a legume background than in evolutionarily distant species such as tobacco and Arabidopsis. As evidenced in Table 1, many additional transformants will be available, and their analysis will permit a more statistically sound evaluation of transgene expression patterns in M. truncatula.

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References


