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Transgene expression in strawberries driven by a heterologous phloem-specific promoter

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Abstract Strawberry is susceptible to diseases caused by phytoplasmas, mycoplasma-like prokaryotes restricted to sieve elements in the phloem tissue of infected plants. One strategy to improve strawberry resistance to phytoplasmas involves transgenic expression of anti-microbial peptide genes in phloem. For targeted phloem-specific expression, we constructed a binary vector with an expression cassette bearing the β-glucuronidase (GUS) reporter gene (*uidA*) under control of the *Arabidopsis* sucrose-H^+ symporter gene (AtSUC2) promoter. Transgenic strawberry lines were generated with high efficiencies by a modified trans-formation protocol, which combines the adoption of a 3-day pre-selection period following transformation, and the addition of 10-μM thidiazuron to the regeneration medium. Histological GUS activity indicated that the reporter gene was expressed specifically in phloem of leaves, petioles, and roots of transgenic plants. The results suggest that the transformation protocol and the *AtSUC2* promoter may be useful for engineering phytoplasma-resistant transgenic strawberries.

Keywords Strawberry · *AtSUC2*-promoter · Indirect regeneration · Phloem-specific expression

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Introduction

Strawberry (*Fragaria* ssp.) is susceptible to infection by phytoplasmas, which are cell-wall-less, non-culturable bacteria that cause diseases in more than 300 vegetable, ornamental, and perennial plant species representing over 100 families (Lee et al. 2000; McCoy et al. 1989). More than a dozen phytoplasmas are associated with strawberry diseases, causing deformation of fruit, leaf yellowing, stunting, and heavy fruit loss as well as plant death (Mass 1998). These phytopathogenic agents are restricted to sieve cells of phloem tissue and are transmitted from diseased plants to healthy ones by sap-sucking insect vectors, mainly leafhoppers and psyllids (Lee and Davis 1992; McCoy et al. 1989). Phytoplasmal infection affects phloem function, impairs amino acid and carbohydrate translocation, inhibits photosynthetic activities, and induces rapid senescence (Lepka et al. 1999; Bertamini et al. 2002a,b). Histological studies on a variety of phytoplasma-infected plants revealed anatomical aberration as a result of abnormal deposition of callose in sieve cells, followed by different degrees of necrosis and collapse of sieve cells as well as companion cells at late stage of phytoplasmal diseases (Batjer and Schneider 1960; Braun and Sinclair 1976; Kartte and Seemüller 1991).

Control of phytoplasmal diseases in strawberry has been very difficult because of the lack both of curative methods and plant varieties tolerant or resistant to phytoplasmas. Classic control measures rely on eradication of infected plants and use of insecticide treatment against the phloem-feeding vectors.

Over the past decade, artificially engineered resistance has emerged as a new strategy for plant disease control. Several different genetic approaches have been explored to generate disease-resistant plants. These include enhancing natural plant defense by promoting cell death at the site of infection via plant hypersensitive response (Belbahri et al. 2001), and inhibiting pathogen growth by transgenic expression of anti-microbial peptides (Osusky et al. 2000) or single-chain variable-fragment antibodies

(Le Gall et al. 1998). In addition, a few recent reports suggested that anti-apoptotic genes of human, nematode, and baculovirus, once introduced into plants, may provide broad-spectrum resistance to fungal, bacterial, and viral diseases by blocking programmed cell death and preventing tissue necrosis (Dickman et al. 2001; Lincoln et al. 2002).

To mitigate phytoplasmal diseases in strawberry, we wish to develop genetically modified strawberry plants that will produce and deliver anti-apoptotic and anti-microbial peptides specifically to phloem sieve elements where phytoplasmas reside and multiply. For such genetic engineering, a phloem-specific promoter is highly desirable. The *Arabidopsis* sucrose-H^+ symporter AtSUC2 is a main phloem-loading transporter (Sauer and Stolz 1994) and is essential for long-distance transport of sucrose in *Arabidopsis* (Gottwald et al. 2000). *AtSUC2* gene is specifically expressed in phloem companion cells of photosynthetic leaves (Stadler and Sauer 1996). The promoter activity of the *AtSUC2* gene was studied in transgenic *Arabidopsis* and tobacco plants using β-glucuronidase (GUS) or green florescent protein (GFP) as a reporter molecule (Truernit and Sauer 1995; Imlau et al. 1999). In both homologous and heterologous backgrounds, the *AtSUC2* promoter was able to direct phloem-specific expression, and the levels of the promoter activity were coupled to the source-strength of photosynthetic leaves. *AtSUC2* promoter-GFP fusion studies further revealed that the reporter protein produced in companion cells can traffic through plasmodesmata into adjacent sieve elements and migrate within the phloem (Imlau et al. 1999). In the present study, we established a modified leaf-segment culture method for efficient transformation and regeneration of strawberry plants and demonstrated that the promoter of the *AtSUC2* gene is active in transgenic strawberry and is capable of directing phloem-specific expression of the GUS reporter gene.

Materials and methods

Strawberry cultivars and micropropagation in vitro

Octoploid (2*n*=8×=56) *Fragaria* × *ananassa* ever-bearing cultivars 'Hecker' and 'La Sans Rivale' and diploid (2*n*=2×=14) *F. vesca* 'Alpine' accession FRA197 and FRA198 were obtained as in vitro shoots or seeds, respectively, from the National Clonal Germplasm Repository (Corvallis, Ore.). Both octoploid and diploid strawberry plants are day neutral in flowering habit. In vitro shoots were micropropagated and maintained on a multiplication medium consisting of MS salts (Murashige and Skoog 1962), B5 vitamins (Gamborg et al. 1968), 2.5 μM 6-benzylaminopurine (6-BA), 0.5 μM indole 3-butyric acid (IBA), 87.6 mM sucrose, and 6 g l^{-1} Phytagar (pH 5.6) at 25°C with a photoperiod of 16-h light (40 μmol m^{-2} s^{-1}, as provided by cool-white fluorescent light) and 8-h darkness. Seeds were germinated on a hormone-free medium with MS salts, B5 vitamins, 87.6 mM sucrose, and 6 g l^{-1} agar (pH 5.6). Leaf petiole and leaf-blade explants were excised from in vitro cultured shoots and used for transformation and regeneration experiments.

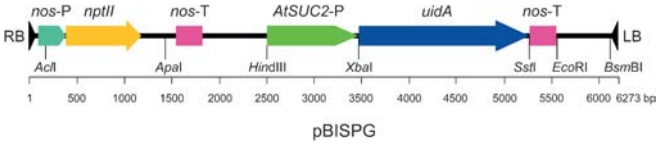

Fig. 1 Diagrammatic representation of the T-DNA region of the binary plasmid vector pBISPG. The plasmid vector was derived from pBI121, with the Cauliflower mosaic virus (CaMV) 35S promoter replaced by the promoter of the sucrose-H^+ symporter *AtSUC2* gene from *Arabidopsis*. *RB*, *LB* Right and left borders of T-DNA; *nos-P*, *nos-T* nopaline synthase gene promoter and terminator; *nptII* neomycin phosphotransferase II gene, *AtSUC2-P Arabidopsis* sucrose-H^+ symporter gene promoter, *uidA* β-glucuronidase (GUS) gene

Shoot organogenesis

Petiole section and leaf segment explants (5 mm in length) from 4-week-old micro-cultures were wounded by two transverse cuts and placed horizontally, abaxial side up, on a solid regeneration medium (MS-R, pH 5.6), which consisted of MS salts, B5 vitamins, 10 μM thidiazuron (TDZ), 1.5 μM IBA, 87.6 mM sucrose, and 6 g l^{-1} Phytagar. All explants were incubated in the dark for 14 days before being transferred to light conditions (40 μmol m^{-2} s^{-1}) under a 16/8-h (light/dark) photoperiod. Explants were subcultured onto fresh MS-R medium every 2 weeks. At the end of 8 weeks, all explants were transferred to the multiplication medium. Regeneration rate was calculated as the percentage of the explants that produced shoots.

Plasmid construction

The DNA sequence of the *AtSUC2* gene promoter (Truernit and Sauer 1995) was retrieved from GenBank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) under the accession number X79702. Primer pair SUC2PF (5′ gattataagcttgcaaaatagcacacc 3′) and SUC2PR (5′ tggaatcctctagatttgacaaaccaagaaagtaag 3′) was synthesized, with built-in recognition sites for restriction enzymes *Hin*dIII and *Xba*I, respectively, and used for PCR amplification of the *AtSUC2* promoter from *Arabidopsis* genomic DNA. The amplified *AtSUC2* promoter was cloned into the binary vector pBI121 (GenBank accession AF485783) at the *Hin*dIII/*Xba*I sites, replacing the Cauliflower mosaic virus (CaMV) 35S promoter to control the downstream GUS reporter gene *uidA*. The resultant plasmid was named pBISPG (Fig. 1).

Agrobacterium-mediated transformation and regeneration of transgenic plants

Agrobacterium tumefaciens strain EHA105 (Hood et al. 1993) was used as the host for the binary vector pBISPG. The bacterial cells were grown overnight (200 rpm, 28°C) in LBG liquid medium (LB with 0.5% glucose), pelleted, and resuspended to a density of 2×10^8 cfu ml^{-1} in MS-R liquid medium supplemented with 20 μM acetosyringone (pH 5.4). Leaf and petiole segments were dipped in the *A. tumefaciens* suspension for 30 min, transferred to sterile paper saturated with MS-R liquid medium, and incubated in the dark at 25°C for 72 h. Following co-cultivation, the explants were washed with water, blotted on sterile paper, and transferred to a solid pre-selection medium (MS-R agar medium supplemented with 100 mg l^{-1} cefoxitin and 100 mg l^{-1} carbenicillin) for a 3-day incubation in the dark at 25°C. The explants were, in turn, incubated in the dark for 10 days on a selection medium (the same composition as pre-selection medium but supplemented with 50 mg l^{-1} kanamycin) before resumption of the 16/8-h photoperiod. The explants were subcultured every 2 weeks for a total of 8 weeks. The green shoots were then transferred to the multiplication me-

dium with antibiotics for further selection. Transformation rate was calculated as the percentage of the explants that regenerated shoots on the selection medium. The shoots resistant to kanamycin were rooted on one-half strength MS medium supplemented with B5 vitamin, 0.5 μM IBA, and antibiotics.

Molecular confirmation of transgenic strawberry lines

Genomic DNA was isolated from putative transgenic and non-transgenic control plants using a DNeasy Plant Mini Kit (Qiagen, Valencia, Calif.). Integration of the transgene into the plant genome was screened by PCR using a GUS-specific primer pair (GUSF: 5′-caacgaactgaactggcaga-3′ and GUSR: 5′-tttttgtcacgcgctatcag-3′). A reaction mixture of 50 μl, containing 0.1 μg genomic DNA, 25 pmol each primer, and 1× AmpliTaq Gold PCR master mix (Applied Biosystems, Foster City, Calif.), was subjected to 35 rounds of the following cycle: 94°C for 30 s, 56°C for 45 s, and 72°C for 45 s. The PCR fragments were fractionated on a 0.8% agarose gel. For Southern blot analysis, 10 μg genomic DNA was digested with *Xba*I, or doubly digested with *Hin*dIII and *Sst*I, separated on a 1% agarose gel, transferred to a nylon membrane, and probed with a digoxigenin (DIG)-labeled *uidA* gene fragment. Pre-hybridization and hybridization with the probe were performed at 65°C in the presence 5× SSC, 1% blocking reagent (Roche, Indianapolis, Ind.), 0.1% *N*-lauroylsarcosine, and 0.02% SDS. After a 16-h hybridization period, a final high stringency wash of the membrane was carried out at 65°C for 20 min in a buffer containing 0.5× SSC and 0.1% SDS. The hybridization signal was detected by a chemiluminescent assay using anti-DIG alkaline phosphatase-conjugated Fab fragment and its substrate CDP-star (Roche).

Histochemical GUS assay

Histochemical assay of GUS activities in various parts of *uidA* gene transgenic strawberry plants was performed as described by Jefferson (1987) using the colorimetric substrate 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc).

Reverse transcriptase-PCR analysis of the reporter gene transcripts

Total RNA was isolated from leaf, flower bud, developing pseudocarp (green), flower petal (white), and ripe pseudocarp (red) of transgenic strawberry plants using a method previously described (Kiefer et al. 2000). Crude RNA preparations were purified with an RNeasy Plant Mini Kit (Qiagen). Trace amounts of DNA in the RNA preparations were removed by treatment with DNA-free reagent (Ambion, Austin, Tex.). Samples of 0.5 μg total RNA were subjected to a single-step reverse transcriptase (RT)-PCR with the primer pair GUSF/GUSR using MasterAmp RT-PCR Kit (Epicenter, Madison, Wis.).

Results and discussion

Transformation of diploid and octoploid strawberries

The key to successful genetic modification of cultivated strawberries, as with that of any other crop, is the establishment of an efficient regeneration system, which provides the means for the selection and recovery of genetically modified plants following gene transfer (James et al. 1988; Passey et al. 2003). Several regeneration protocols have been described to improve strawberry transformation (Haymes and Davis 1998; Alsheikh et al. 2002; Passey et al. 2003). However, strawberry cultivars with different genetic backgrounds may respond quite differently to a given regeneration culture regimen (Passey et al. 2003). In order to establish an optimal regeneration system for the diploid and octoploid strawberries being studied, we formulated a new regeneration medium (MS-R), in which B5 vitamins were added as supplements to the MS salts and 10 μM TDZ along with 1.5 μM IBA were included as growth regulators. Both diploid and octoploid strawberries responded to the MS-R medium very well. For example, during the 14-day dark incubation period, depending on the genotype of the cultivars, up to 100% of the leaf and petiole explants produced calli at their wounded edges. These calli subsequently differentiated into shoots over a course of 4–8 weeks. A shoot regeneration rate of 100% was achieved from leaf and petiole explants of diploid strawberries FRA197 and FRA198. Regeneration rates of 80% and 40% were obtained from the leaf explants of octoploid strawberry ‘Hecker’ and ‘La Sans Rivale’, respectively. These high regeneration rates enabled the transformation experiments that followed.

For *Agrobacterium*-mediated transformation, 73 and 53 leaf/petiole explants from diploid strawberry FRA197 and FRA198, respectively, were used. Following co-cultivation of explants with *Agrobacterium*, and prior to

Fig. 2a,b Regeneration of transgenic strawberry plants. **a** Shoot regeneration from calli derived from the transformed cells of parent explants. **b** Root formation at the base of regenerated shoots. For both **a** and **b**, the genotype of the parent explants was diploid *Fragaria vesca* ‘Alpine’

Table 1 Percentage of explants forming shoots on selection medium. Leaf and petiole explants from diploid and octoploid parent strawberry plants were co-cultivated with *Agrobacterium*. Following co-cultivation and pre-selection incubation, explants were cultured on a selection medium containing thidiazuron (TDZ), indole 3-butyric acid (IBA), and antibiotics. Transformation rate was calculated as percentage of the explants that regenerated shoots in 8 weeks. *NPE* number of total parent explants, *NSE* number of explants forming shoots on selection medium

Parent genotype	Explant type	NPE	NSE	Transformation rate (%)	
Diploid 'Alpine' FRA197	Leaf	53	33	62.3	Average 64.4
Diploid 'Alpine' FRA197	Petiole	20	14	70.0	
Diploid 'Alpine' FRA198	Leaf	38	29	76.3	Average 67.9
Diploid 'Alpine' FRA198	Petiole	15	7	46.7	
Octoploid 'Hecker'	Leaf	48	5	10.4	
Octoploid 'La Sans Rivale'	Leaf	54	4	7.4	

shoot organogenesis from transformed cells, a 3-day pre-selection phase was introduced, in which explants were held in the pre-selection medium without kanamycin (Alsheikh et al. 2002). This allowed any transformed cells to recover from the infection/transformation process and begin expression of the neomycin phosphotransferase II (NPTII) gene before being subjected to kanamycin selection. Forty-seven and 36 independent putative transgenic lines, capable of growth on 50 mg l^{-1} kanamycin, were generated from transformed cells derived from explants of FRA197 and FRA198, respectively (Fig. 2), representing a transformation efficiency of 64.4% for FRA197 and 67.9% for FRA198 (Table 1). Under the same conditions, only five and four such putative transgenic lines were obtained from 48 and 54 leaf explants of octoploid strawberry 'Hecker' and 'La Sans Rivale', respectively, representing a transformation frequency of 10.4% for 'Hecker' and 7.4% for 'La Sans Rivale' (Table 1).

The high transformation rates in strawberry varieties—especially those of diploid genotypes—achieved in the present study may be due to the favorable medium modifications, which fostered successful organogenesis from transformed cells through an indirect regeneration pathway. As, by nature, *Agrobacterium*-mediated genetic transformation is normally a single-cell event, the callus phase of the indirect regeneration pathway made it possible for single transformed cells to be selected and multiplied prior to regeneration. Inclusion in our selection/regeneration medium of TDZ, a synthetic growth regulator that exhibits the unique property of mimicking both auxin and cytokinin effects on growth and differentiation (Murthy et al. 1998), greatly enhanced both induction of callus formation from the transformed cells and subsequent shoot organogenesis from the calli (data not shown). Our medium composition and culture regimen is particularly effective for regeneration of strawberry plants from transformed cells with diploid genotypes.

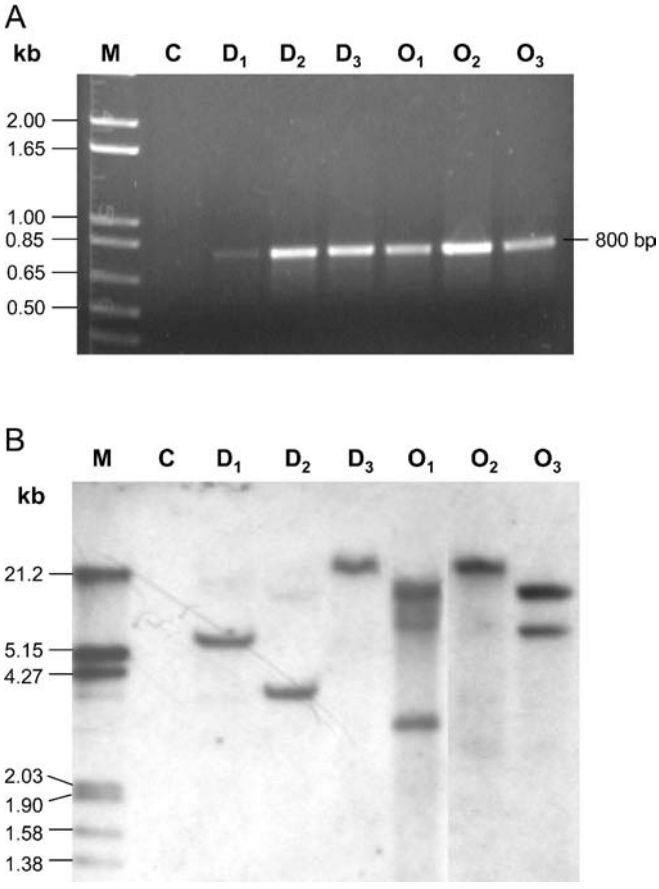

Fig. 3a,b Molecular confirmation of transformation. **a** PCR screening of transgenic lines. A replicon of 800 bp was amplified from three representative diploid and three representative octoploid strawberry plants transformed with the *AtSUC2–uidA* fusion construct. Lanes: *M* DNA molecular size marker (Invitrogen, #10787), *C* untransformed plant (control), D_1–D_3 diploid transformants, O_1–O_3 octoploid transformants. **b** Southern blot analysis with a DIG-labeled *uidA* gene probe. Lanes: *M* DIG-labeled DNA molecular size marker (Roche, #1218603), *C* untransformed plant (control), D_1–D_3 diploid transformants, O_1–O_3 octoploid transformants

Molecular confirmation of transformation

Integration of the GUS-encoding gene *uidA* in the genome of putative transgenic strawberry lines was initially verified by genomic PCR. With primer pair GUSF/GUSR, an expected fragment of 800 bp was amplified from genomic DNA of all putative transgenic plants that were recovered from the kanamycin-containing regeneration and rooting medium. Genomic PCR results from six representative transgenic lines are shown in Fig. 3a. Of the six putative transgenic lines, three are of diploid genotype (lanes D_1–D_3) and the other three are of octoploid genotype (lanes

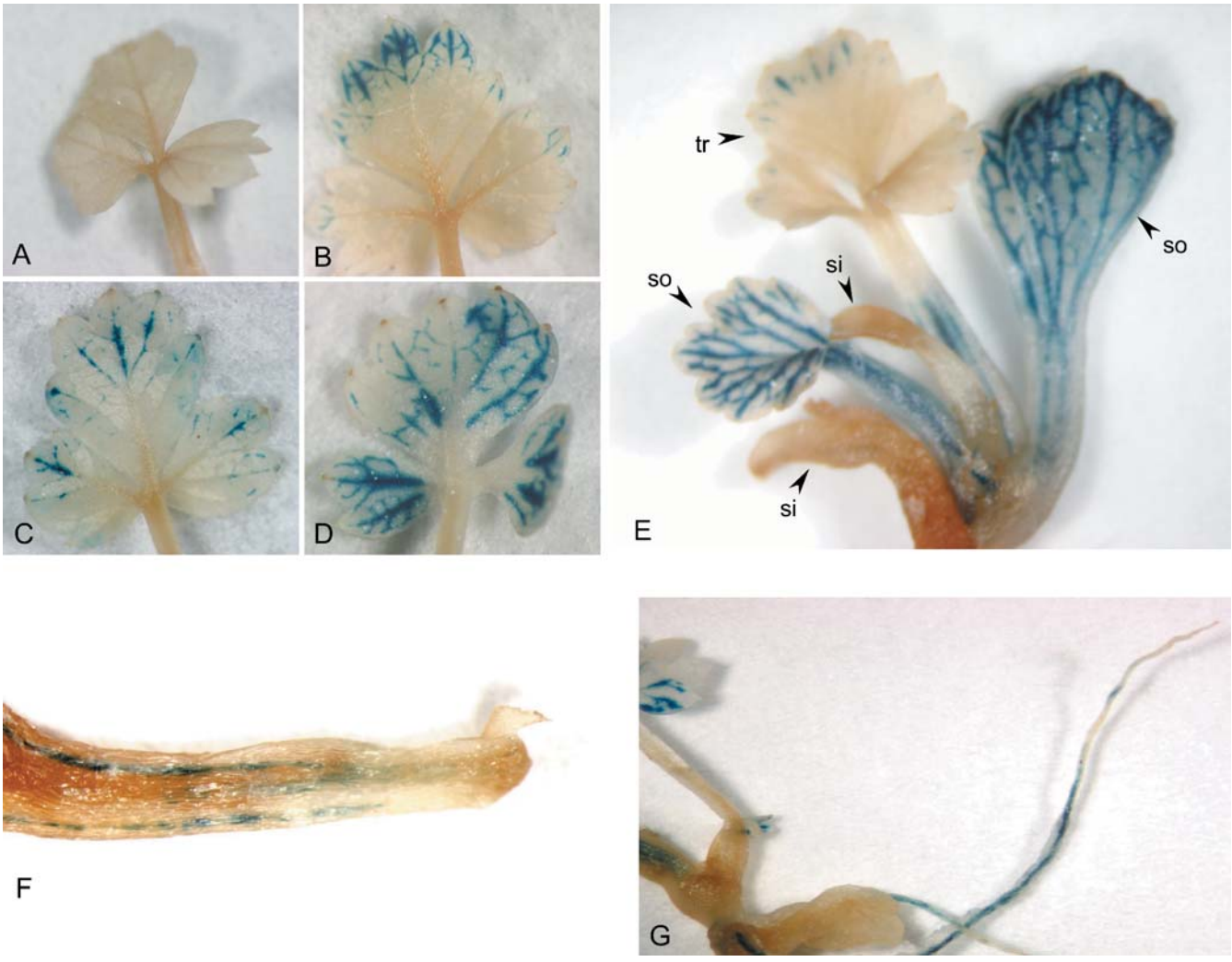

Fig. 4 *AtSUC2*-promoter-directed phloem-specific expression of the GUS reporter gene in transgenic strawberry lines. GUS activity, indicated by histochemical staining, was observed in the vascular cylinders of both diploid (**b**–**d**, **g**) and octoploid (**e**, **f**) transgenic strawberry plants. GUS staining was absent in sink leaves (**a**, **e** *si*), emerged in the tip of transitional leaves (**b**, **e** *tr*), expanded to midrib and branch veins of the source part of transitional leaves (**c**), and was most intense in major and minor veins of source leaves (**d**, **e** *so*). The appearance of GUS staining in the peripheral vascular cylinder of longitudinally sectioned petioles (**f**) indicated that reporter gene expression was localized exclusively in the phloem. GUS staining was also detected in roots (**g**)

O_1–O_3). Genetic transformation of the six strawberry lines was further verified by genomic Southern blot analysis. When probed with a DIG-labeled *uidA* gene fragment, gel blots of *Xba*I-digested genomic DNA gave either one or two hybridization band(s) (Fig. 3b), confirming the presence of the reporter gene in the host strawberry genomes. An additional gel blot analysis was performed, in which genomic DNA was doubly digested with *Hin*dIII and *Sst*I. All six transgenic lines gave a single 2.8-kb hybridization band (data not shown). Since, in the construct pBISPG, the *Hin*dIII and *Xba*I restriction recognition sites delineate the *AtSUC2* promoter and the *uidA* gene, this 2.8-kb single band hybridization pattern suggested that, during the process of *Agrobacterium*-mediated T-DNA transfer and host chromosome integration, no genetic rearrangement had occurred within the promoter-reporter region. Thus, these transgenic lines were suitable for further gene expression studies.

Phloem-specific expression of the reporter gene

Regardless of genome ploidy level (diploid or octoploid), transgenic strawberry plants carrying the *AtSUC2-uidA* fusion construct exhibited strong GUS activities in the vascular tissues of leaves, petioles, and roots (Fig. 4). In the leaves, the intensity and distribution of GUS activities were regulated during leaf development. GUS activity was absent in young leaves (sink leaves, where active growth requires import of sugars to support development). On the other hand, in fully expanded photosynthetic leaves (source leaves, where sugars are produced by photosynthesis and subsequently transported to areas of active

growth and/or photosynthate storage), GUS activity was found in all major and minor veins, as well as in petioles, and extended to the center of the rosette. In leaves undergoing the transition from sink to source, GUS activity was first seen in the tip and then in the midrib and branch veins of the top half of a transitional leaf as the source region expanded. These results are in line with previous reports showing that *AtSUC2* promoter-driven reporter gene expression was confined to the phloem of transgenic *Arabidopsis* and tobacco, and that the promoter activity was regulated developmentally in relation to sink/source transition and to structural and functional vein maturation (Imlau et al. 1999; Wright et al. 2003). Microscopic analysis of petiole sections from these plants showed that GUS staining appeared in the peripheral vascular cylinder, suggesting that this expression was restricted to the phloem of the vascular tissue.

Histochemical analysis of transgenic strawberry plants also revealed strong GUS activity in the vascular cylinder of roots, which is a classical sink tissue (Fig. 4g, see also discussion below). Not unexpectedly, GUS activity was absent in root tips, where phloem tissue has not yet been differentiated from apical meristem. No GUS staining was found in any ripe "fruit", the red fleshy tissue derived from the receptacle or, in botanical terms, pseudocarp. A total of 27 independent transgenic plants (20 with diploid genotype and 7 with octoploid genotype) were used in the histochemical assay. The overall GUS staining pattern was similar in all cases.

Relative transcript levels of the reporter gene in developing reproductive organs

As no GUS staining was observed in ripe pseudocarps by histological assay, we further investigated the GUS reporter gene (*uidA*) transcript levels in the RNA population from flower buds, developing pseudocarps, flower petals, and ripe pseudocarps of both diploid and octoploid transgenic strawberries. Figure 5 shows the results from a representative transgenic line derived from octoploid *Fragaria* × *ananassa* ever-bearing cultivar 'Hecker'. As assessed by RT-PCR analysis, steady-state reporter gene transcript levels in flower buds and developing pseudocarps were comparable to those of typical source leaves (Fig. 5, lanes L, B, and dpC). The reporter gene transcript was undetectable in flower petals or ripe pseudocarps (Fig. 5, lanes P and rpC). Results from diploid transgenic lines were similar (data not shown).

Among the reproductive structures examined for GUS reporter transcript, white flower petals and red ripe pseudocarps are photosynthetically inactive and are considered as permanent sink tissues. The absence of the reporter transcript in these tissues is not unexpected, since *AtSUC2* promoter activity is generally associated with source tissues. On the other hand, the presence of the reporter transcript in flower buds and developing pseudocarps seems to be peculiar, as flower buds and developing pseudocarps are also classified as sink tissues. In-

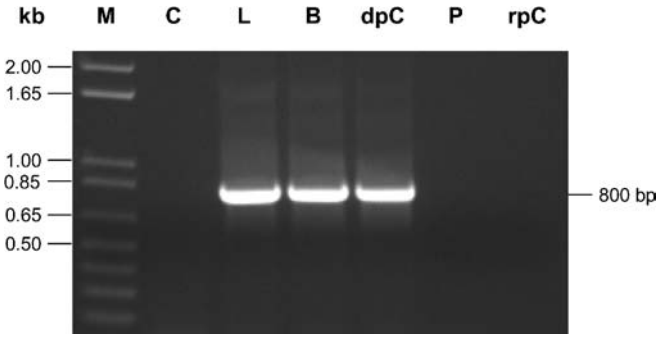

Fig. 5 Reverse transcriptase (RT)-PCR assay of reporter gene expression. Total cellular RNA samples were extracted from a representative transgenic line derived from octoploid ever-bearing cultivar 'Hecker'. Steady-state *uidA* gene transcript levels from different plant parts were compared based on relative intensities of the 800-bp cDNA band amplified from total RNA samples. Transcript levels were similar in source leaves (*L*), flower buds (*B*), and developing pseudocarps (*dpC*), and were undetectable in flower petals (*P*) or ripe pseudocarps (*rpC*). *M* DNA molecular size marker (Invitrogen, #10787), *C* untransformed plant (control)

terestingly, similar results were reported from the study of transgenic *Arabidopsis* plants carrying an AtSUC2-GUS fusion construct. GUS activity was found in sink tissues such as roots and developing pods (Truernit and Sauer 1995). These and our results suggest that the *AtSUC2* promoter activity profile may be more complex than simply mirroring the source-strength of tissues.

For many plant biotechnological studies, transgenic plants are usually created with a functional gene under the control of the 35S promoter from CaMV. Although the constitutive CaMV 35S promoter is active in phloem tissue, it is also expressed in other tissues. Since the present work is part of a study aimed at eventual targeted delivery of anti-microbial agents against phytoplasmas, it would be desirable to use a promoter that directs gene expression in a phloem-specific manner. This could minimize or avoid unnecessary exposure of non-target plant tissues and consumers to the anti-microbial agents. The present study showed that the *AtSUC2* promoter is able to direct phloem-specific gene expression in vegetative parts and developing reproductive organs of genetically modified strawberry plants, with the advantage that expression was absent or undetectable in ripe pseudocarps, the part consumed by humans. The successful targeted expression of the GUS reporter gene in both diploid and octoploid strawberry genotypes has encouraged further work aimed at engineering phytoplasma-resistant transgenic strawberry plants. In experiments using the *AtSUC2* promoter and the modified transformation protocol, we are currently analyzing the expression profiles of an anti-microbial peptide gene and an anti-apoptotic gene in transgenic strawberry lines.

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