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Inheritance of foreign genes in transgenic bean (*Phaseolus vulgaris* L.) co-transformed via particle bombardment

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Abstract Exploiting the biolistic process we have generated stable transgenic bean (*Phaseolus vulgaris* L.) plants with unlinked and linked foreign genes. Co-transformation was conducted using plasmid constructions containing a fusion of the *gus* and *neo* genes, which were co-introduced with the methionine-rich *2S* albumin gene isolated from the Brazil nut and the antisense sequence of *AC1*, *AC2*, *AC3* and *BC1* genes from the bean golden mosaic geminivirus. The results revealed a co-transformation frequency ranging from 40% to 50% when using unlinked genes and 100% for linked genes. The introduced foreign genes were inherited in a Mendelian fashion in most of the transgenic bean lines. PCR and Southern blot hybridization confirmed the integration of the foreign genes in the plant genome.

Key words Transgenic bean · *Phaseolus vulgaris* · Co-transformation · Biolistic · Inheritance

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the developing world. In Latin America, beans are a very important source of protein and calories. Despite its nutritional importance, production growth rates have been declining in Brazil and Andean regions. The main reasons for this are diseases, insects, nutritional deficiencies, and a lack of drought tolerance. Consequently, there is considerable interest in the introduction of agronomically useful traits into beans by breeding and genetic engineering.

Early efforts to produce transgenic bean plants failed due to poor DNA delivery and regeneration systems. While McClean et al. (1991) were able to introduce genes into beans using the *Agrobacterium* system, they were unable to regenerate transgenic plants. Several protocols have been described for shoot organogenesis from apical or axillary meristems in beans (McClean and Grafton 1989; Malik and Saxena 1992; Mohamed et al. 1992). Recently, Russel et al. (1993) utilized these information to achieve transgenic bean plants, using an electrical-discharge particle acceleration device.

Over the last decade, several investigators have utilized the co-transformation process to introduce unlinked and linked genes into plants, mainly by the electroporation of protoplasts (Schocher et al. 1986; Tagu et al. 1988; Damm et al. 1989; Christou and Swain 1990; Hervé et al. 1993). Co-transformation frequencies varied from 18% to 88% for unlinked genes, versus 50% to 100% for linked genes (Klein et al. 1989; Christou and Swain 1990). Using the biolistic process, Spencer et al. (1990) observed a co-transformation frequency of 50% in maize cells with two separate plasmids, while Gordon-Kamm et al. (1990) found a co-transformation frequency ranging from 68% to 86% in a transformed cell suspension of maize. To date, the efficiencies of co-transformation in plants achieved by the apical cells particle bombardment system have not been evaluated.

Studies analyzing the progeny of transgenic plants, such as soybean (Hinchee et al. 1988; Christou et al. 1989), tobacco (Uchimiya et al. 1986), *Arabidopsis* (Damm et al. 1989), rape (Hervé et al. 1993) and cotton (McCabe and Martinell 1993), transformed by distinct transformation systems have shown that the transgenes are inherited as Mendelian genes. This has been interpreted as being single locus integration. Nevertheless, in some cases a non-Mendelian inheritance has been observed, with segregation ratios of more than three transformed plants for one

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non-transformed in the R1 generation (Cooley et al. 1995; Damm et al. 1989).

In this study, we have utilized different plasmids containing the neomycin phosphotransferase II (*neo*) and β-glucoronidase (*gus*) marker genes, the methionine-rich *2S* albumin gene from Brazil nut (Gander et al. 1991; Aragão et al. 1992) and the antisense sequences of *AC1*, *AC2*, *AC3* and *BC1* genes from the bean golden mosaic geminivirus (Gilbertson et al. 1993) to evaluate the frequency of co-transformation and inheritance of linked and unlinked genes over three generations of transgenic bean plants.

Material and methods

Plasmids

The plasmid pBI426 containing the *gus-neo* gene fusion (Datla et al. 1991) driven by a double 35S cauliflower mosaic virus (CaMV) promoter plus a leader sequence from alfalfa mosaic virus (AMV) was provided by Dr. W. Crosby (Plant Biotechnology Institute, Saskatoon, Canada). The plasmid pEA23 contains the *gus* coding region under control of the 35S CaMV promoter and the *2S* albumin gene from the Brazil nut (Gander et al. 1991) driven by the double 35S CaMV promoter plus the AMV leader sequence. The plasmid pAC123BC1-BZ contains the bean golden mosaic geminivirus coding sequences of genes *AC1*, *AC2*, *AC3* (linked) and *BC1* (Gilbertson et al. 1993), in antisense orientation, and the *gus* coding sequence, both driven by the 35S CaMV promoter. All plasmids have a pUC19 backbone (see Fig. 1).

Embryonic axes preparation

Explant preparation was carried out essentially as described by Aragão et al., (1993). Basically, mature seeds of common bean (cv 'Olathe') were surface-sterilized in 1% sodium hypochlorite for 20 min, and then rinsed three times in sterile distilled water. The seeds were soaked in distilled water for 16–18 h. Then, the embryonic axes were excised from the seeds and the apical meristems were exposed by removing their primary leaves. Subsequently, the radicules were excised, and the embryonic axes were surface-sterilized again in 0.1% sodium hypochlorite for 10 min. The prepared embryonic axes were rinsed three times with sterile distilled water. Ten to fifteen embryonic axes were placed with the apical region directed upwards in 5-cm dishes containing the bombardment medium, MS medium (Murashige and Skoog 1962) supplemented with 44.3 μ*M* benzylaminopurine (BAP), 3% sucrose and 0.7% phytagel (Sigma), immediately prior to the bombardment.

Preparation of microparticles and bombardment

We followed a protocol based on Smith et al. (1992), with modifications. Basically, DNA was bound to 1.2-μm-diameter tungsten particles (M10, Sylvania Inc.) by mixing sequentially in an Eppendorf tube 50 μl microparticles (60 mg.ml^{-1} in 50% glycerol), 4 μl (1 mg ml^{-1}) of each plasmid (pBI426 in combination with either pEA23 or pAC123BC1-BZ), 50 μl $CaCl_2$ (2.5 *M*) and 20 ml spermidine free-base (100 m*M*). After 10 min of incubation, the DNA-coated microparticles were centrifuged at 15 000 *g* for 10 s, and the supernatant was removed. The pellet was washed with 150 μl 70% ethanol and then with absolute ethanol, respectively. The final pellet was re-suspended in 24 ml of absolute ethanol and sonicated for 2 s, just before use. Aliquots of 3 μl were spread onto the carrier membranes (Kapton, 2 mil, DuPont), which were allowed to evaporate in a dessicator under 12% relative humidity. The bombardment was conducted utilizing a high pressure helium-driven particle acceleration device built in our laboratory, essentially as described by Sanford et al. (1991), connected to a computer-controller system (Rumsey & Loomis, Ithaca, N.Y.). The relative humidity in the biolistic laboratory was 50%, the gap distance from shock wave generator to the carrier membrane was 8 mm, the carrier membrane flying distance to the stopping screen was 13 mm, the DNA-coated microparticles flying distance to the target was 80 mm, the vacuum in the chamber was 27 inches of Hg and the helium pressure utilized in all experiments was 1 200 psi.

Plant growth: selection, shoot culture and acclimatization

After bombardment, the embryonic axes were cultivated for 1 week at 28°C with a 16-h photoperiod (50 μmols $m^{-2}s^{-1}$) in the bombardment medium. The germinated embryonic axes were then transferred to MS medium containing 44.3 μ*M* BAP, 100 mg l^{-1} kanamycin and 0.8% agar (Fisher). After 2 weeks the embryonic axes developing shoots were transferred to MS medium without kanamycin and BAP to allow shoot elongation. As soon as the shoots achieved 2–4 cm in length, they were excised at the stem basal level. A 1-mm section of the basal stem and the leaf tip were assayed for expression of the *gus* gene, as described by McCabe et al. (1988). Shoots expressing the

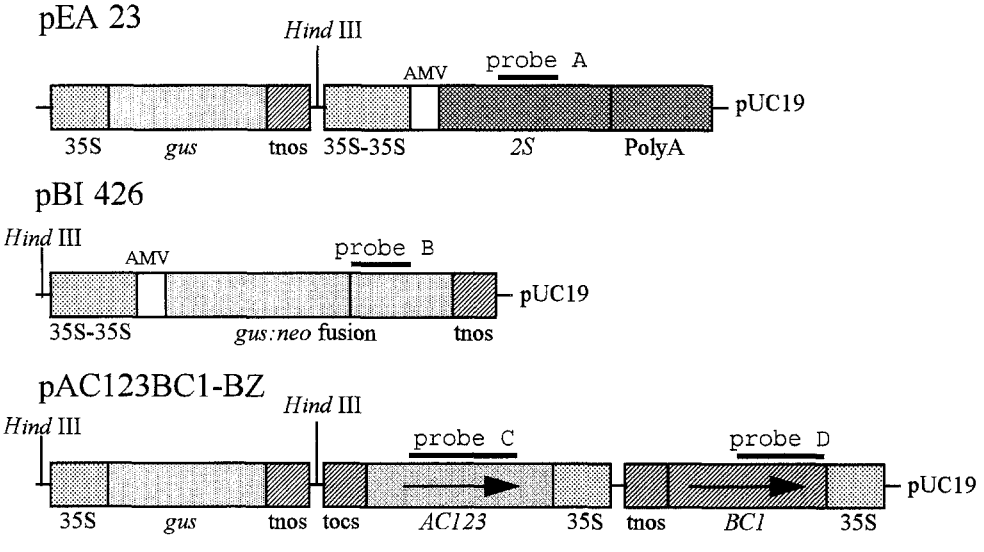

Fig. 1 Diagram of the expression cassettes utilized for co-bombardment. *Bars* indicate the probes for Southern DNA analysis

gus gene were individually cultured to MS medium plus 0.8% agar (Fisher) with no growth regulators and the sucrose level reduced to 1%. Once the plantlets rooted, they were transferred to a plastic pot containing autoclaved soil:vermiculite (1:1) and covered with a plastic bag for 1 week. Plantlets were transferred to soil and allow to set seeds. The temperature in the greenhouse was 25°C, and the relative humidity was above 80%.

Polymerase chain reaction (PCR)

For PCR analysis of the transformed plants, DNA was isolated from leaf disks according to Edwards et al. (1991). Each PCR reaction was carried out in 25 µl containing 10 m*M* Tris-HCl (pH 8.4), 50 m*M* KCl, 2 m*M* $MgCl_2$, 160 µ*M* of each dNTP, 200 n*M* of each primer, 2 U of *Taq* polymerase (GIBCO BRL) and about 20 ng of genomic DNA. Table 1 shows the primers utilized to amplify each sequence. The mixture was overlaid with mineral oil and denatured for 5 min at 95°C in a Hybaid thermal cycler (Hybaid, UK) and then subjected to 35 cycles of amplification (95°C for 1 min, 55°C for 1 min, 72°C for 2.5 min) with a final cycle of 7 min at 72°C. One half of the reaction was then loaded directly onto 1% agarose gel and visualized under UV light by ethidium bromide staining.

Co-transformation determination

The co-transformation frequencies were evaluated by PCR analysis based on the presence of (1) the *neo* and *2S* gene sequences (in plants derived from co-transformation with plasmids pBI426 and pEA23) and (2) the *neo*, *AC123* and *BC1* sequences (in plants derived from the co-transformation with plasmids pBI426 and pAC123BC1-BZ) in the plant genome.

Progeny analysis

The analysis of the R0, R1, R2 and R3 generations was conducted by detecting the introduced foreign genes (*neo*, *AC123*, *BC1*, *2S*, *gus*) by PCR and by the GUS histochemical assay in leaves of self-pollinated plants.

All of the seeds of each mother plant (R1) and 50 seeds of each plant in the following generations (R2 and R3) were planted and analyzed. Seeds from the R1 generation were harvested and their position on the mother plant was recorded.

Chi-square (χ^2) analysis was performed to determine if the observed segregation ratio was in agreement with a Mendelian ratio in the R1 generation.

Southern blot analysis

Genomic DNA was isolated according to Dellaporta et al., (1983). Southern blotting and hybridization were carried out as previously described (Sambrook et al. 1989). Genomic DNA (15 µg), digested with *Hin*dIII, was separated on an 1% agarose gel, blotted onto a nylon membrane (Hybond) and hybridized with the specific probes (see Fig. 1), labelled with $\alpha[^{32}P]$dCTP (1.1×10^{13} Bq/mol) using a random primer DNA labelling kit (Pharmacia Biotech) according to the manufacturer.

Table 1 Sequence of the oligonucleotides utilized as primers for the PCR analysis

Gene	Position	Sequence (5′ → 3′)	Expected size of fragment (bp)
neo	60	GAGGCTATTCGGCTATGACTG	410
	470c	TCGACAAGACCGGCTTCCATC	
2S	145	CACAGTGGTGGAGGAGGAGAA	373
	518c	TCGGCCAGCCTCATCATCCTT	
gus	251	TTGGGCAGGCCAGCGTATCGT	420
	671c	ATCACGCAGTTCAACGCTGAC	
AC123	1369	AGGTGGTATACTCTGGTCGTT	900
	2249c	GGAGGTCAACAGACAGCTAAT	
BC1	1546	GAATACTTGTGTCCAATCTATG	710
	2227c	GAATCTAACCGTGATGAATATC	

Table 2 Summary of 21 independent experiments of co-transformation with the plasmid pBI426 in combination with pAC123BC1-BZ or pEA23

Experiments	Plasmid combined with pBI426	Bombarded embryonic axes	Obtained shoots	Transgenic plants
1	pAC123BC1-BZ	181	34	1
2	pAC123BC1-BZ	113	3	1
3	pAC123BC1-BZ	105	37	3
4	pAC123BC1-BZ	101	37	2
5	pAC123BC1-BZ	187	8	0
6	pAC123BC1-BZ	180	3	1
7	pAC123BC1-BZ	116	12	1
8	pAC123BC1-BZ	179	18	1
9	pAC123BC1-BZ	116	26	3
10	pAC123BC1-BZ	116	29	2
11	pAC123BC1-BZ	114	18	1
12	pAC123BC1-BZ	111	33	0
13	pAC123BC1-BZ	125	27	3
14	pEA23	197	5	1
15	pEA23	212	11	2
16	pEA23	176	6	2
17	pEA23	175	10	0
18	pEA23	121	4	1
19	pEA23	126	41	2
20	pEA23	176	11	0
21	pEA23	152	4	0
	Total	3079	377	27

Results

Transgenic plants

The apical region of bean embryonic axes were co-bombarded with a DNA mixture of pBI426 in combination with pEA23 or pAC123BC1-BZ (Fig. 1). Table 2 summarizes the results of 21 independent experiments. From the 3,079 bombarded embryonic axes (Fig. 2a), 377 (9%) apical shoots were obtained. After histochemical analysis of the basal stem and leaf tip for expression of the *gus* gene, 27 (7%) potentially transgenic shoots were identified (Fig. 2b). The average frequency of transformation (the total number of putative transgenic plants divided by the total number of bombarded embryonic axes) was 0.9%.

All GUS-positive shoots (Fig. 2b) were transferred to MS with 1% sucrose as described in the Material and methods. As soon as the plantlets developed vigorous roots, they

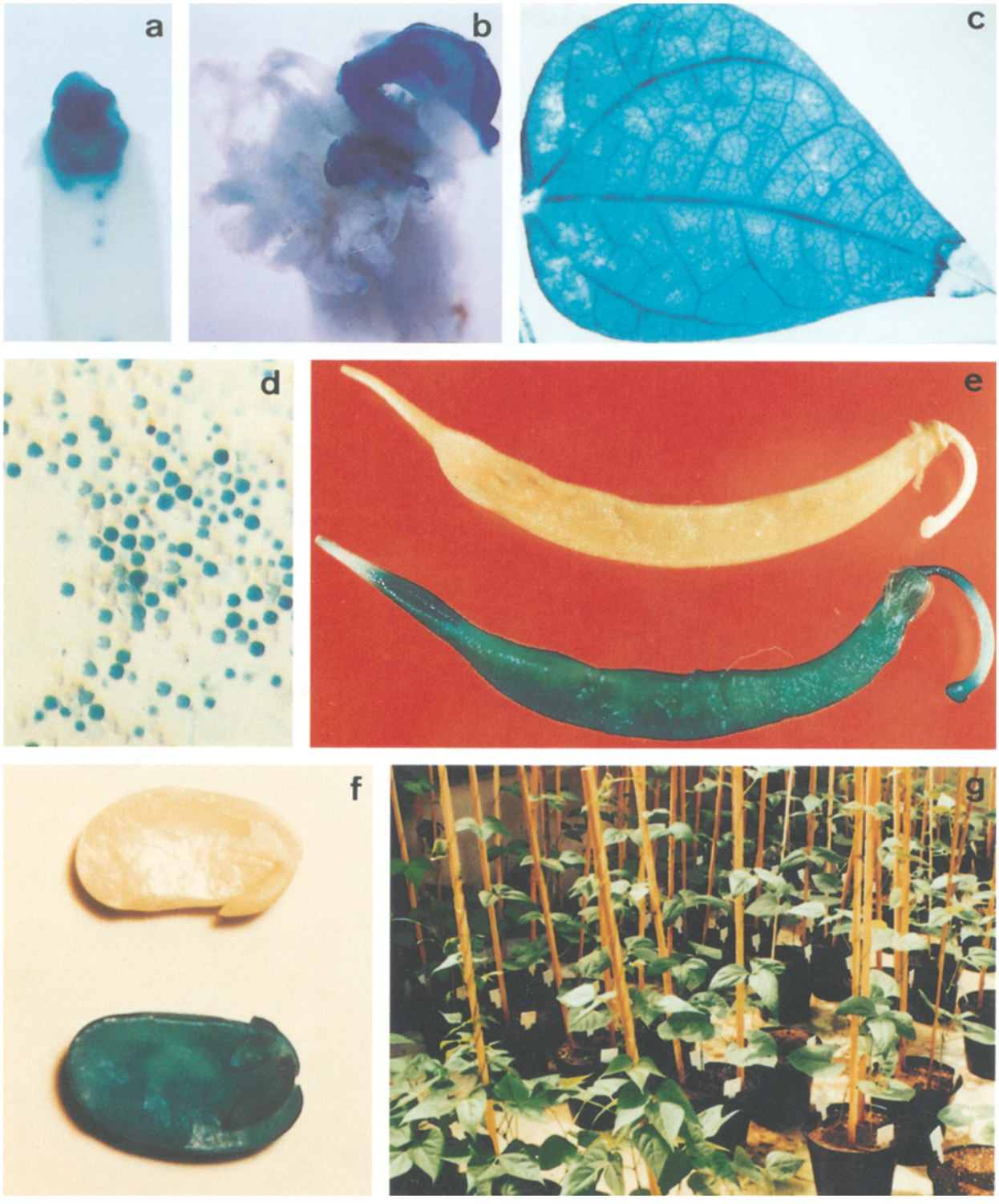

Fig. 2a–g Bean transformation. **a** Embryonic axes expressing the *gus* gene 24 h after bombardment, **b** transgenic shoot from a multiple-shooting apical meristem, **c** leaf from an acclimatized plant expressing the *gus* gene, **d** microspores showing *gus* segregation, **e** pods and **f** seeds from transgenic plants (*below*) and control (*above*), **g** R1 progeny of transgenic bean plants growing in the greenhouse

were acclimatized and transferred to soil. All plants presented a normal phenotype, were fertile and set pods and seeds (Fig. 2d–f). Histochemical GUS assays performed on leaves of all transgenic plants revealed intense enzymatic activity (Fig. 2c).

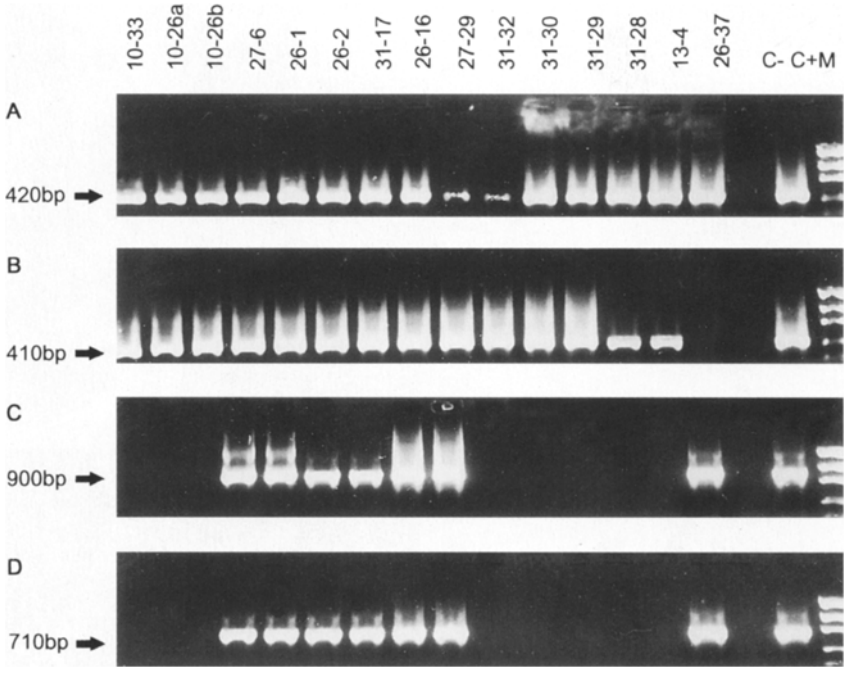

Fig. 3A–D PCR analysis of co-transformed bean plants with the plasmid pBI426 in combination with pAC123BC1-BZ: **A** *gus*, **B** *neo*, **C** *AC123* **D** *BC1* gene. Plant numbers are indicated *above* the lanes, *C*– Non-transformed plants, *C*+ positive control (pBI426 in **A** and **B**; pAC123BC1-BZ in **C** and **D**), *M* molecular size standard (DNA mass-BRL). The expected fragments are indicated by an *arrow*

Table 3 Summary of the PCR analysis of co-transformed plants using the plasmid pBI426 in combination with the plasmid pAC123BC1-BZ

R0 plants	*gus*	*neo*	*AC123*	*BC1*
26–37	+	–	+	+
13–4	+	+	–	–
31–28	+	+	–	–
31–29[a]	+	+	–	–
31–30	+	+	–	–
31–32	+	+	–	–
27–29	+	+	+	+
26–16[a]	+	+	+	+
31–17	+	+	+	+
26–2[a]	+	+	+	+
26–1	+	+	+	+
27–6	+	+	+	+
10–26a	+	+	–	–
10–26b	+	+	–	–
10–33[a]	+	+	–	–

[a] Transgenic bean plants which have transmitted the introduced genes to the R1 generation

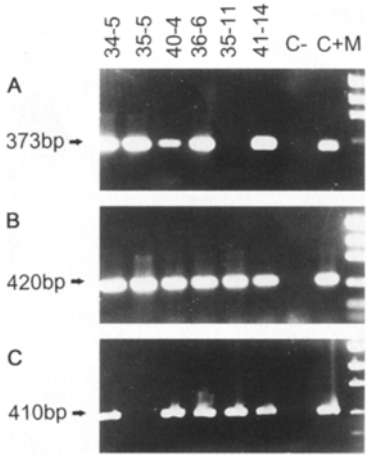

Fig. 4A–C PCR analysis of co-transformed bean plants with the plasmid pBI426 in combination with pEA23: **A** *2S*, **B** *gus*, **C** *neo* gene. The plant numbers are indicated above the lanes. *C*– Non-transformed plants, *C*+ positive control (pEA23 in **A** and **B**; pBI426 in **C**), *M* molecular size standard (DNA mass-BRL). The expected fragments are indicated by an *arrow*

Frequencies of co-transformation

Co-transformation of unlinked genes was determined by PCR analysis. Fifteen independent plants transformed with pBI426 in combination with pAC123BC1-BZ were assessed for the presence of the introduced gene sequences (Table 3; Fig. 3). Six plants revealed the presence of the *neo* (from pBI246), *AC123* and *BC1* sequences (from pAC123BC1-BZ), reflecting a co-transformation frequency of 40%. All of the plants revealed the presence of the *gus* and *neo* sequences, except plant 26–37, which lacked the *neo* sequence (Fig. 3).

Table 4 shows the PCR analysis of 6 independently transformed plants from experiments with pBI426 co-bombarded in combination with pEA23. Four plants contained the *neo* sequence (from pBI426) and the *2S* sequence (from pEA23) reflecting a co-transformation frequency of approximately 66%. All of the plants analyzed showed the presence of the *gus* and *neo* sequences, except the plant 35–5, in which the *neo* sequence was not present (Fig. 4).

Co-transformation of the linked genes was evaluated using the sequences of *AC123* and *BC1* present in plasmid pAC123BC1-BZ. On the basis of R0 plant analysis, the co-transformation frequency in this case was 100% (Table 3).

Table 4 Summary of the PCR analysis of co-transformed plants using the plasmid pBI426 in combination with the plasmid pEA23

R0 plants	*gus*	*neo*	*2S*
34–5[a]	+	+	+
35–5	+	–	+
35–11[a]	+	+	–
36–6[a]	+	+	+
40–4[a]	+	+	+
41–14[a]	+	+	+

[a] Transgenic bean plants which have transmitted the introduced genes to the R1 generation

Table 5 Segregation analysis of self-fertilized transgenic plants in the R1 generation

R0 plants	R1 generation[a]		Segregation ratio	χ^2	P [b]
	Positive	Negative			
34–5	29	9	3:1	0.03	>0.8
35–11	20	6	3:1	0.05	>0.8
36–6	34	11	3:1	0.03	>0.8
40–4	15	6	3:1	0.14	>0.7
41–14	32	10	3:1	0.03	>0.8
26–16	6	51	3:1	126.3	<0.001
26–2	6	66	3:1	170.6	<0.001
10–33	28	8	3:1	0.14	>0.7
31–29	23	7	3:1	0.04	>0.8

[a] Data are based on histochemical assay for *gus* gene expression and PCR analysis

[b] P is the probability that the observed ratios reflect the expected segregation ratio

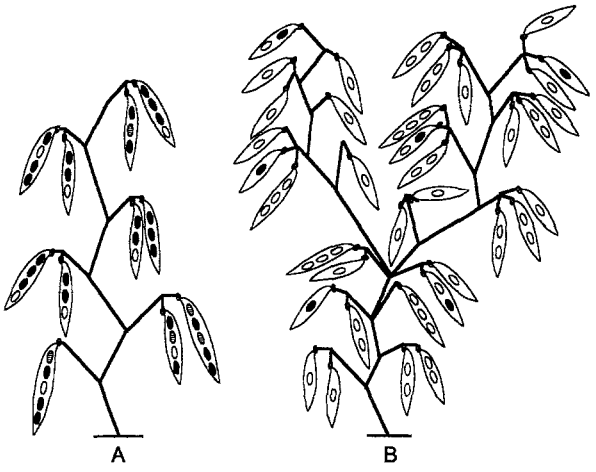

Fig. 5 Distribution of transformed R1 seeds in primary transgenic bean plants 10–33 (**A**) and 26–16 (**B**) showing a Mendelian (3:1) and non-Mendelian segregation ratio, respectively. Seeds were germinated and analyzed by the GUS histochemical assay and PCR for all introduced genes (Table 3). *Solid circles* Positive seeds, *open circles* negative seeds, and *hatched circles* non-germinated or aborted seeds

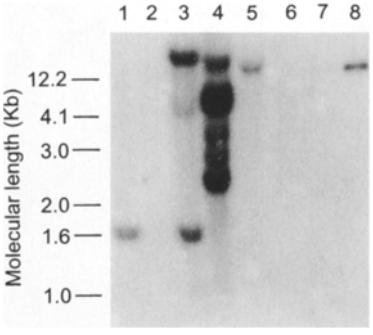

Fig. 6 Southern blot analysis of representative putative transformed lines in the R3 generation. DNAs were digested with *Hin*dIII, transferred to a nylon membrane and probed with an internal fragment of the *2S* gene (probe A, Fig. 1). *Lanes 1–5* transformed lines: 34–5 (*lane 1*), 35–11 (*lane 2*), 36–6 (*lane 3*), 40–4 (*lane 4*), 41–14 (*lane 5*). *Lane 6* non-transformed plant, *lane 7* pEA23 (10 pg), *lane 8* pEA23 (50 pg). Molecular size markers are indicated on the *left*

Progeny analysis

The progeny of the 21 self-fertilized transgenic plants were screened by PCR analysis. Only 9 R0 plants (marked with a superscript "a" in Tables 3 and 4) transferred the foreign genes to the R1 generation (Fig. 2 g). Chi-square (χ^2) analysis indicated that 7 plants segregated in a Mendelian fashion (3:1) (Table 5). The other 2 plants (26–16 and 26–2), had a non-Mendelian ratio (Table 5).

The position of each seed from the R1 generation in all primary transgenic plants was recorded. Figure 5 shows a random distribution of transgenic seeds in the 10–33 and 26–16 R1 transgenic lines.

DNA analysis

In order to evaluate the integration of the introduced foreign genes *2S*, *AC123*, *BC1* and *neo*, Southern blot analysis of genomic DNA from the R3 generation of the transgenic bean plants was conducted. The results indicated the presence of the *2S* and *neo* sequences in R3 generation lines 34–5, 36–6, 40–4 and 41–14 (Fig. 6). Line 10–33 showed the presence of the *neo* sequence, while line 26–16 revealed the presence of the *neo*, *AC123* and *BC1* sequences (Figs. 7 and 8). Line 31–29 did not show any hybridization signal with the AC123 or BC1 probe.

Since plasmids pBI426 and pEA23 have an unique *Hin*dIII restriction site (Fig. 1), Southern blot analysis allows us to confirm the integration as well as to determine the copy number of the *neo* and *2S* genes present in transgenic bean plants (Figs. 7 and 8). Southern analysis performed on 9 R3 transgenic lines revealed the presence of a small number of integrated copies of the *neo* gene. Except for transgenic line 40–4, which revealed at least 5 copies, the number of integrated copies was between 1 and 2 (Fig. 7).

With respect to the *2S* gene, the number of integrated copies was between 1 and 2 for 3 R3 lines analyzed; line 40–4 revealed at least 5 copies (Fig. 6). Lines 34–5 and

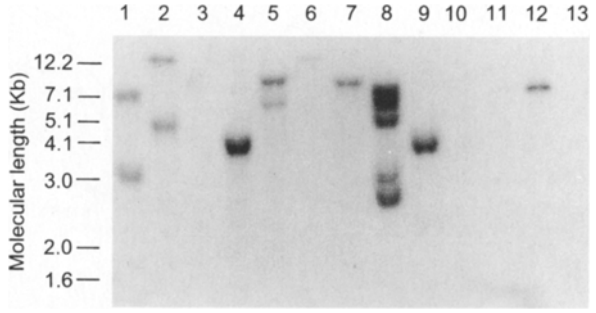

Fig. 7 Southern blot analysis of representative putative transformed lines in the R3 generation. DNAs were digested with *Hin*dIII, transferred to a nylon membrane and probed with an internal fragment of the *neo* gene (probe B, Fig. 1). *Lanes 1–9* transformed lines: 10–33 (*lane 1*),26–16 (*lane 2*), 26–1 (*lane 3*), 31–29 (*lane 4*), 34–5 (*lane 5*), 35–11 (*lane 6*), 36–6 (*lane 7*), 40–4 (*lane 8*), 41–14 (*lane 9*). *Lane 10* Non-transformed plant, *lane 11* pBI426 (10 pg), *lane 12* pBI426 (50 pg), *lane 13* pAC123BC1-BZ (50 pg) Molecular size markers are indicated on the *left*

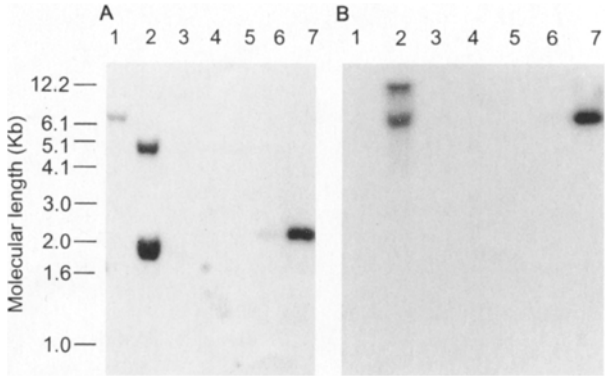

Fig. 8A, B Southern blot analysis of representative putative transformed lines in the R3 generation. DNAs were digested with *Hin*dIII, transferred to a nylon membrane and probed with an internal fragment of the **A** *AC123* gene (probe C, Fig. 1) **B** *BC1* gene (probe D, Fig. 1). *Lanes 1–4* transformed lines: 10–33 (*lane 1*), 26–16 (*lane 2*), 26–1 (*lane 3*), 31–29 (*lane 4*). *Lane 5* Non-transformed plant, *lane 6* pAC123BC1-BZ (10 pg), *lane 7* pAC123BC1-BZ (50 pg). Molecular size markers are indicated on the *left*

36–6 presented fragments smaller (1.6 kb) than the *35S–35S–2S* gene cassette (Fig. 1; Fig. 6). Line 35–11 did not present any hybridization signal with the *2S* probe. DNA isolated from non-transformed plants did not hybridize with any probe.

Discussion

In this study we utilized an efficient and reproducible system to routinely achieve transgenic bean plants and evaluated the co-transformation frequencies and inheritance of unlinked and linked foreign genes.

The bean transformation system was based upon the development of a tissue culture protocol of multiple shooting induction, shoot elongation and rooting. Using a high pressure helium-driven device to mediate the introduction of the foreign genes, we achieved an average transformation frequency of 0.9%. Similar frequencies of transgenic bean plants were achieved by Smith et al. (1992) on the basis of the number of bombarded embryonic axes. Recently, Russel et al. (1993) were able to obtain transgenic bean plants using an electrical particle acceleration device. However, the frequency of transgenic plants they obtained was much lower (0.03%). In addition, their tissue culture protocol was time consuming, involving several temperatures treatments and medium transfers of the bombarded embryos prior to achieving transgenic shoots.

In the experiments utilizing unlinked genes we obtained a co-transformation frequency ranging from 40% to 66%. Similar results were achieved by Lyznik et al. (1989) electroporating maize protoplasts (47%) and by Spencer et al. (1990) bombarding a maize cell suspension culture (50%). In rape seed electroporation-mediated co-transformation frequencies ranging from 33% to 60% (Hervé et al. 1993) were obtained. Other studies have described frequencies of co-transformation ranging from 18% to 88% using the electroporation of protoplasts (Christou and Swain 1990; Hayakawa et al. 1992). Interestingly, the data described above show that the co-transformation frequencies obtained was not dependent on the foreign genes and gene delivery systems employed.

As expected, we have found a frequency of co-transformation of 100% for the genes cloned in the same plasmid (pAC123BC1-BZ). These results are consistent with those previously described (Klein et al. 1989; Christou and Swain 1990).

Some studies have supported the concept that all foreign genes introduced by the biolistic process are normally transmitted to the progeny (Hinchee et al. 1988; Christou et al. 1989; Hervé et al. 1993; McCabe and Martinell 1993). In contrast, the results obtained by Register et al.(1994) in transgenic maize plants, produced by means of the biolistic process, and Ulian et al.(1994), utilizing *Agrobacterium*-mediated petunia transformation, have shown that progeny (R1) of some transgenic plants (R0) were negative for the presence of the foreign genes. Indeed, from the total number of transgenic bean plants obtained in our study, 12 plants (44%) did not transfer the introduced foreign genes to the R1 generation, and 2 plants showed a poor transmission of the transgenes (1:10) to the R1 generation line. Chimerism is frequent in transgenic plants obtained by the biolistic process (Christou 1990). However, this non-Mendelian ratio was not due to chimerism, since the position of the transgenic seeds were randomly distributed in the mother plant (R0). These results might suggest that the inserted foreign genes may cause some de-stabilization of the chromosome structure and a poor transgene transmission to the progeny. Moreover, an insertion mutation of an essential gene required for ovule fecundation and/or development might account for this aberrant inheritance. Surprisingly, despite similar frequencies of co-

transformation in the R0 generation of the transgenic bean plants using both plasmid combination (pBI426/pEA23 and pBI426/pAC123BC1-BZ), the transmission of the foreign introduced genes to the R1 (in each plasmid combination) was not similar. In this respect, more studies are necessary to elucidate the results obtained.

Progeny analysis of the R1, R2 and R3 generations of the co-transformed bean plants showed that the foreign genes transmitted to R1 generation were co-maintained in the following generations. This fact, associated with the Mendelian segregation ratio presented, may suggest that the foreign genes were integrated at the same locus despite being located in separated plasmids.

Southern blot analysis revealed a simple integration pattern of all introduced genes. Line 10–33 did not show any amplification of the *AC123* or *BC1* genes in the PCR analysis. However in the Southern blot analysis there appeared a large fragment (9 kb) hybridized with the AC123 probe. Two lines, containing the *2S* gene (34–5 and 36–6) contained fragments smaller (1.6 kb) than the 35S–35S–2S gene cassette. Both unexpected results could be explained by a partial integration of the plasmids. The fragmentation of the plasmid DNA can occur during the transformation process, integration and/or stabilization of the insert (De Block 1993).

Experiments are now in progress to evaluate the methionine expression levels in transgenic bean seeds containing the *2S* albumin methionine gene and the susceptibility to the bean golden mosaic geminivirus (BGMV) in those transgenic plants containing the antisense sequences *AC123* and *BC1*.

These results are of particularly importance to the understanding of the basic process of integration of foreign genes in bean. This in turn, may form the foundation to the effective and practical application of genetic engineering to introduce important traits such as protein quality and disease resistance into this important legume.

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