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Refined glufosinate selection in *Agrobacterium*-mediated transformation of soybean [*Glycine max* (L.) Merrill]

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Abstract Modern genetic analysis and manipulation of soybean (*Glycine max*) depend heavily on an efficient and dependable transformation process, especially in public genotypes from which expressed sequence tag (EST), bacterial artificial chromosome and microarray data have been derived. Williams 82 is the subject of EST and functional genomics analyses. However, it has not previously been transformed successfully using either somatic embryogenesis-based or cotyledonary-node transformation methods, the two predominant soybean transformation systems. An advance has recently been made in using antioxidants to enhance *Agrobacterium* infection of soybean. Nonetheless, an undesirable effect of using these antioxidants is the compromised recovery of transgenic soybean when combined with the use of the herbicide glufosinate as a selective agent. Therefore, we optimized both *Agrobacterium* infection and glufosinate selection in the presence of L-cysteine for Williams 82. We have recovered transgenic lines of this genotype with an enhanced transformation efficiency using this herbicide selection system.

Keywords Antioxidant · Glufosinate · *Glycine max* · Regeneration · T-DNA

Abbreviations DTT: Dithiothreitol · EST: Expressed sequence tag · GUS: β -Glucuronidase

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Introduction

Soybean, one of the world's leading protein and oil crops, ranks with model plants in importance in functional genomics studies. For example, the soybean expressed sequence tag (EST) collection, recovered mainly from genotype Williams 82, ranks among the top EST collections of major crop plants (Shoemaker et al. 2002). Soybean investigators face the challenge of dealing with this prodigious amount of information. Additionally, a number of isolines containing variant alleles have been constructed in the Williams 82 background (Lohnes et al. 1993). To extend genetic analysis to RNAi, activation tags, enhancer traps, etc. in Williams 82 and other public genotypes, it is necessary to be able to transform them efficiently.

Published transformation techniques do not currently serve high-throughput analyses in soybean functional genomics, though significant improvements have been made in the two most frequently used transformation systems over the past decade. One is biolistic-mediated transformation of soybean embryogenic cultures (Finer and McMullen 1991; Finer et al. 1992; Parrott et al. 1994; Hadi et al. 1996; Stewart et al. 1996; Hazel et al. 1998; Santarem and Finer 1999). The other is *Agrobacterium*-mediated transformation of soybean cotyledonary-nodes through direct organogenesis (Hinchee et al. 1988; Di et al. 1996; Zhang et al. 1999; Clemente et al. 2000; Xing et al. 2000). However, routine recovery of transgenic soybean plants using either of these two transformation systems has been restricted to a few genotypes with no reports of transformation of Williams 82. Therefore, development of an efficient and consistent transformation protocol for Williams 82, and other public genotypes, will greatly aid soybean functional genomics and soybean transgenic technology in general.

Antioxidants have been used to improve *Agrobacterium* T-DNA transfer, for example: dithiothreitol (DTT) coupled with phenol-absorbing polyvinylpyrrolidone in grape (*Vitis vinifera* L.) (Perl et al. 1996), L-cysteine in sugarcane (*Saccharum officinarum* L.) (Enriquez-Obre-

gon et al. 1998), and ascorbic acid and L-cysteine in rice [Japonica rice (cv. R321)] (Enriquez-Obregon et al. 1999). Recently, T-DNA transfer to soybean cotyledonary-node cells has also been improved employing L-cysteine (Olhoft and Somers 2001; Olhoft et al. 2001). L-Cysteine was added to the co-cultivation medium and enhanced infection was achieved in both non-differentiating and differentiating tissues. Nonetheless, a drawback associated with the use of L-cysteine is its alleviation of selective pressure by the herbicide glufosinate, leading to a potential decrease in the recovery of truly transgenic soybean plants.

To overcome the inefficient recovery associated with the glufosinate selection following cocultivation in the presence of three antioxidants, L-cysteine, DTT, and N-thiosulfate, Olhoft et al. (2003) employed hygromycin B as a selective agent in the genotype 'Bert', leading to a dramatic increase in transformation efficiency. However, neither recovery of transgenic Williams 82 nor efficient recovery of other soybean genotypes has been reported to date. Also, it is not known whether hygromycin B selection is effective in recovery of Williams 82 and other public genotypes. Indeed, our preliminary results have indicated that the efficacy of hygromycin B selection could be genotype-dependent (unpublished data).

Here, we report the optimization of both *Agrobacterium* infection and glufosinate selection schemes in the presence of L-cysteine to accomplish efficient transgenic recovery of a large number of Williams 82 transgenic plants. We maximized transformation efficiency by using optimized levels of glufosinate. Thus, glufosinate selection still has merit in most soybean genotypes including those in which hygromycin B or other selective agents may work more efficiently.

Materials and methods

Transformation materials

Agrobacterium strain EHA101 (Hood et al. 1986), carrying either binary vector pPTN140 (Zhang et al. 1999) or PTF102 (Frame et al. 2002), was used exclusively. Each binary vector contains a β -glucuronidase (GUS) reporter gene and a plant-selectable *bar* gene cassette within the T-DNA region. Each gene is driven by a double cauliflower mosaic virus 35S promoter. The vector PTF102, also named pZY102, was used exclusively in preliminary screening for transformants, since it contains an intron (Vancanneyt et al. 1990) within the GUS ORF, thus eliminating any possible GUS staining of *Agrobacterium* harbored in assayed tissues. Williams 82 seed was obtained from Illinois Foundation Seeds (Champaign, Ill.).

Transformation and regeneration

Agrobacterium-mediated transformation of soybean (*Glycine max*) using the *bar* gene as a selectable marker coupled with the herbicide glufosinate as a selective agent followed the procedure of Zhang et al. (1999) with a few modifications: (1) seeds were germinated on B₅ basal medium (Gamborg et al. 1968) solidified with 3 g/l Phytigel (Sigma-Aldrich, St. Louis, Mo.); (2) L-cysteine was added to the co-cultivation medium at 400 mg/l and co-cultivation lasted 5 days (Olhoft and Somers 2001); (3) shoot

initiation, shoot elongation, and rooting media were amended with 50 mg/l cefotaxime, 50 mg/l timentin, 50 mg/l vancomycin, and solidified with 3 g/l Phytigel; (4) upon transferring to rooting medium, elongated shoots (3–5 cm) were excised and basal portions were dipped in 1 mg/l indole 3-butyric acid for 1–3 min to promote rooting (Khan et al. 1994); (5) rooting was in 25×100 mm glass culture tubes containing rooting medium; (6) soil mix for acclimatization of plantlets was Metro-mix 200 (Hummert International, Earth City, Mo.). Glufosinate, the active ingredient of Liberty, was used for selection during shoot initiation and elongation and was a generous gift from Aventis CropScience (Research Triangle Park, N.C.).

Transformation efficiencies

Transformants were defined as GUS-positive plants that also demonstrated tolerance to Liberty (Aventis), and transformation efficiency as the number of both GUS⁺ and Liberty-resistant plants divided by the total number of explants at the start.

Assay of putatively transformed plants, and progeny analysis

Young putatively transformed plants (T₀) were assayed by leaf-painting (Zhang et al. 1999) with Liberty, histochemical GUS staining (Jefferson et al. 1987) and Southern blot analysis (Southern 1975) for incorporation of the GUS gene. GUS staining and leaf-painting assays were conducted on young, fully expanded, leaves. For Southern blot analysis, 10 μ g DNA from a leaf sample of each plant was digested with *Hind*III, electrophoresed on a 1% agarose gel, and blotted onto a Zeta-probe GT genomic blotting membrane (Bio-Rad, Hercules, Calif.). The GUS ORF isolated from the vector pPTN140 as a *Hind*III-*Eco*RI fragment was used as a probe and labeled with the ECL random prime labeling and detection kit (RPN 3040/3041, Amersham Pharmacia, Piscataway, N.J.). Pre-hybridization, hybridization, washes, signal generation and detection followed the manufacturer's instructions.

For progeny analysis, leaf samples from progeny (T₁) of 11 Williams 82 primary transformants (T₀) were assayed using the above methods. Fertility was determined by seed set, i.e., total number of seeds per plant.

Experimental design and statistical analysis

Varying levels of glufosinate as experimental treatments for the selection during shoot initiation were compared to a standard treatment. All treatments were arranged in a randomly complete block design (RCBD) in separate independent experiments rather than in a single unmanageable RCBD experiment. Our strategy was to make step-wise comparisons, that is, earlier experiments dealt with selection schemes at low glufosinate concentrations and later experiments with higher glufosinate concentrations.

Variance analysis and mean separation were performed using SAS Proc Mixed using pooled errors for all experiments as standard errors of means (Bowley 1999). Experimental units suffering contamination in some experiments were treated as missing data.

Results and discussion

Selection schemes

Since Williams 82 is the subject genotype of the public soybean functional genomics project, it was chosen for the optimization of selection of transgenic events. To this end we varied glufosinate levels during the two stages of shoot initiation following cocultivation in the presence of

Table 1 Optimization of selection schemes for Williams 82 transformation. Data are from five independent experiments. All remaining treatments included 400 mg/l L-cysteine in the co-cultivation medium, in addition to various glufosinate levels as listed. The glufosinate level during shoot elongation was either 3 or 4 mg/l (not shown). *GUS* β -Glucuronidase

Selection scheme ^c	Total number of explants	Regenerated explants	Recovered plants	GUS ⁺ plants	% GUS ⁺ plants ^d
5/5 ^e	295	207	3	1	0.2 a
8/4	634	163	14	8	0.1 a
8/8	1,318	578	139	79	5.9 b
10/5	907	271	24	9	0.9 a
10/10	841	235	36	13	1.4 a

^cThe numbers in the first column for each selection scheme stands for mg/l glufosinate levels during the first and second shoot initiation stages, respectively

^dMeans representing the number of GUS positive plants divided by the total number of explants per replication. Pooled SE =0.40, $n=22$. Means followed by the same letter are not significantly different at $\alpha=0.05$ based on single degree of freedom contrasts from within an analysis of variance

^eStandard co-cultivation in the absence of L-cysteine in the semi-solid co-cultivation medium

Table 2 Progeny analysis of T₀ Williams 82 transformants based on an expected 3:1 segregation ratio

T ₀ plant codes	Number of progeny assayed	Segregation pattern										Southern blot analysis of T ₀	T ₀ fertility seed set
		GUS gene expression					<i>bar</i> gene expression						
		GUS+	GUS-	df	χ^2	<i>P</i> -value	<i>bar</i> +	<i>bar</i> -	df	χ^2	<i>P</i> -value		
W-5-3 ^a	15	7	8	1	6.4222	0.0113	9	6	1	1.8000	0.1797	+ ^b	85
W-5-4	10	5	5	1	3.3333	0.0679	7	3	1	0.1333	0.7150	NT ^c	159
W-6-6	11	9	3	1	0.0000	1.0000	7	4	1	0.7576	0.3841	+	178
W-8-10	14	11	3	1	0.0952	0.7576	12	2	1	0.8571	0.3545	+	82
W-9-1	15	10	5	1	0.5556	0.4561	8	7	1	3.7556	0.0526	NT	NT
W-11-1	5	5	0	1	1.6664	0.1967	5	0	1	1.6664	0.1967	+	169
W-11-19	8	6	2	1	0.0000	1.0000	4	4	1	2.6667	0.1025	+	145
W-11-25	10	7	3	1	0.1333	0.7150	7	3	1	0.1333	0.7150	+	108
W-11-34	4	3	1	1	0.0000	1.0000	3	1	1	0.0000	1.0000	NT	NT
W-12-8 ^a	15	6	9	1	9.8000	0.0017	12	3	1	0.2000	0.6547	+	219
W-13-10	15	10	5	1	0.5556	0.4561	14	1	1	2.6889	0.1011	NT	238
Total				11	22.5616	0.0204			11	14.6589	0.1987		
Pooled	122	79	44	1	7.6125	0.0058	88	34	1	0.5355	0.4643		
Heterogeneity				10	14.9491	0.1339			10	14.1234	0.1674		

^aDid not fit 3:1 segregation ratio

^bSouthern blot positive

^cNot tested

L-cysteine. A total of four different selection schemes were evaluated at levels 8/5, 8/8, 10/5, or 10/10 mg/l glufosinate during the first/second shoot initiation stages, respectively, and compared to a standard treatment of 5/5 mg/l glufosinate without the addition of L-cysteine to the cocultivation medium. Transgenic Williams 82 plants were recovered in all selection schemes, but the optimal selection scheme was glufosinate at 8 mg/l across the first and second shoot initiation stages and 3–4 mg/l during shoot elongation (Table 1). Recovery at 8/8 mg glufosinate/l was consistent with an average transformation frequency of 5.9%. The final recovery frequency of transgenic plants, defined as greenhouse-established fully grown plants, was 5.4% (data not shown), or about 3–5 times higher than previously reported (Zhang et al. 1999; Clemente et al. 2000; Xing et al. 2000).

Characterization of primary transformants

A total of 110 T₀ GUS-positive Williams 82 plants were characterized by the leaf-painting assay and all displayed resistance to the herbicide Liberty (Table 2). Of these, 17 randomly sampled plants were further subjected to Southern blot analysis (Fig. 1a, b). We digested with *Hind*III since it cleaves both pPTN140 and PTF102 once between the *bar* and GUS gene cassettes within the T-DNA, and independent transformants probed with GUS should give different banding patterns. This was seen in the 17 GUS⁺ and glufosinate-resistant T₀ individuals (Fig. 1a, b). Furthermore, 15 Williams 82 events had 1–3 GUS gene loci and each locus had about 1–5 copies (Fig. 1a, b). Four (W-5-3, W-12-8, W-12-27 and W-13-28) out of 17 events showed multiple loci.

In addition to the 17 transgenic events, two plants (W-8-6, and W-13-4) displaying inconsistent tolerance to Liberty were included in the Southern blot analysis of Fig. 1a and b. Their blots were negative. This result is consistent with reproducible tolerance to Liberty (via

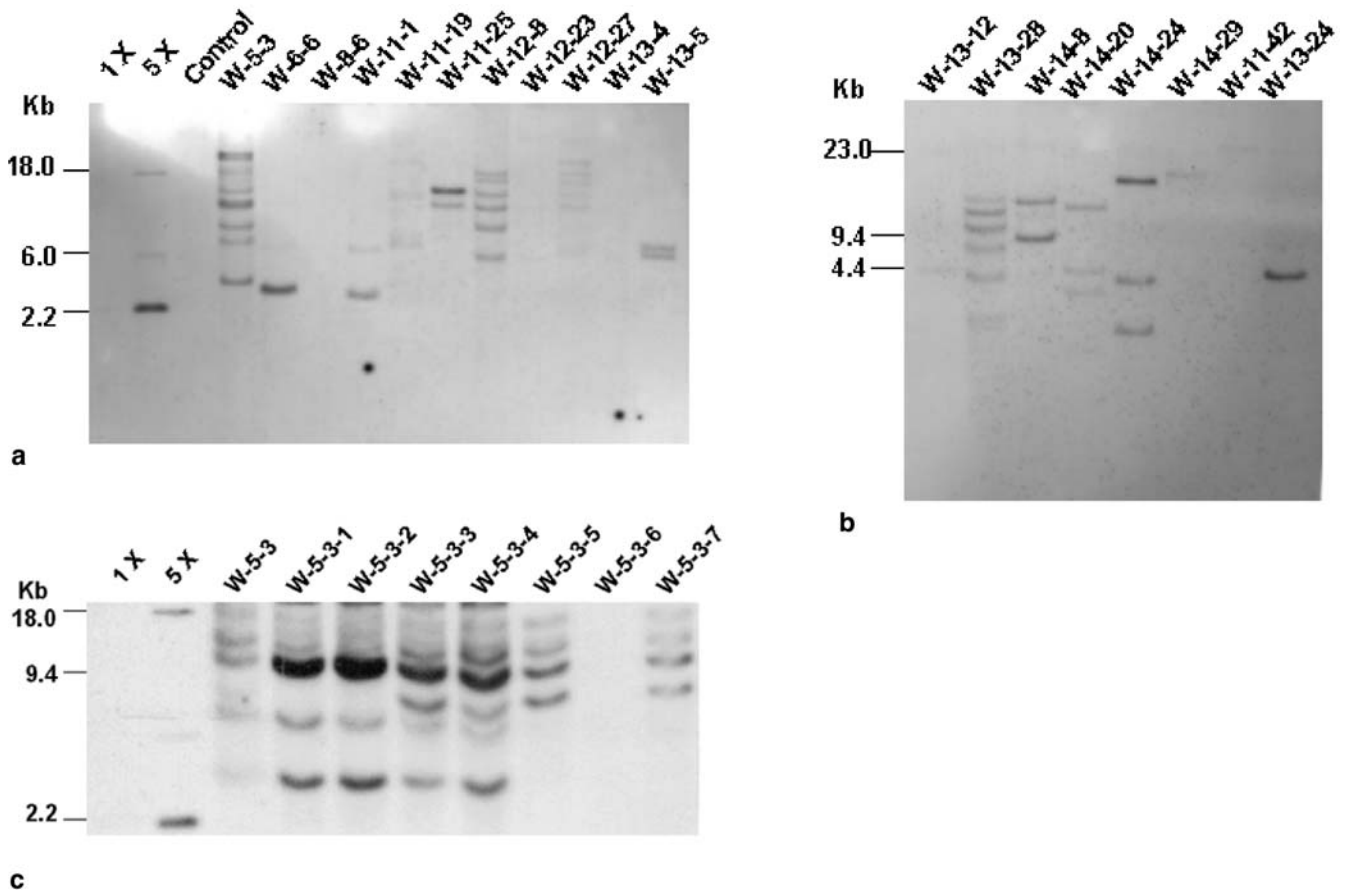


Fig. 1a–c Southern blot analysis of transgenic Williams 82 plants and their progeny. Total genomic DNA (10 μ g) was digested with *Hind*III. The membrane was probed with the β -glucuronidase (GUS) open-reading frame (ORF). **a, b** Primary (T_0) Williams 82 transformants. Lanes: 1, 2 DNA positive control for 1 \times and 5 \times genome equivalents using 18 and 90 pg GUS ORF, 3 wild type

Williams 82, 4, 5, 7–12, 14–22 putative ntransgenic T_0 Williams 82 plants expressing strong GUS activity, 6, 13 plants expressing weak and variable GUS activity. **c** T_1 progeny of event W-5-3. Lanes: 1, 2 1 \times and 5 \times genome equivalents as copy number controls, using GUS ORF DNA, 3 T_0 parental event W-5-3, 4–10 T_1 progeny plants

leaf-painting) being a reliable, quick and simple phenotypic scoring method to verify transgenic events.

Progeny analysis

Progeny analysis was conducted on T_1 seedlings of 11 Williams 82 T_0 events (Table 2). Progeny of most transformants showed segregation for expression of both the GUS and *bar* genes. The less than 50% GUS-positive individuals among the W-5-3 and W-12-8 T_1 families could result from the relatively small number of progeny assayed. However, it is more likely that gene silencing is operative in these two lines since they had a high number of insertion events, evidenced by the large number of bands on the Southern blot (Fig. 1a). Previous studies have clearly demonstrated transgene silencing because of multiple transgene insertions (for a comprehensive review, see Fagard and Vaucheret 2000).

Clearly, chimeras or non-germ-line events were not a concern in this study. There was a discrepancy, however, between herbicide resistance and GUS reaction among the

progeny assayed. That is, a few plants expressing GUS did not tolerate the herbicide at 100 mg/l and others showed herbicide resistance but did not exhibit GUS expression. This may be due to differential expression levels of *bar* or GUS. However, our statistical analysis confirmed segregation of transgene expression among progeny of nine T_0 plants, which fits an expected segregation ratio of 3:1 at the 0.05% significance level (Table 2). To date, Southern blot analyses have been carried out only on two random sets of progeny-derived Williams 82. Figure 1c demonstrates assay results from one event (W-5-3) with clear segregation for the GUS loci. All progeny plants but one (W-5-3-6) carried the GUS gene, as does the parent, indicating a stable transmission of this gene to the progeny.

The fertility of primary transgenic plants was apparently near normal since most produced a large number of seeds (Table 2). Those few plants that had a smaller number of seeds were not healthy during the plantlet stage. Their T_2 progeny, however, yielded about the same number as non-transgenic controls (data not shown).

In summary, we have accomplished a consistent recovery of transgenic plants from the public soybean genotype Williams 82. This success required careful optimization of selection conditions following cocultivation in the presence of L-cysteine. The transgenes have been stably transmitted to the progeny. Our results to date demonstrate that antioxidant-enhanced transformation, once optimized for the appropriate selection, can lead to efficient transformation of Williams 82, for which none has been reported to date. These transgenic recovery frequencies have been increased significantly and should aid soybean functional genomics studies. The strategy deployed here should be applicable to optimizing the selection process for transformation of other public or elite soybean genotypes when the selection process is adversely influenced by the addition of “counter-selection” agents like L-cysteine.

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References

- Bowley SR (1999) A hitchhiker's guide to statistics in plant biology. Any Old Subject Books, Guelph, Ontario
- Clemente TE, LaVallee BJ, Howe AR, Conner-Ward D, Rozman RJ, Hunter PE, Broyles DL, Kasten DS, Hinchee MA (2000) Progeny analysis of glyphosate selected transgenic soybean derived from *Agrobacterium*-mediated transformation. *Crop Sci* 40:797–803
- Di R, Purcell V, Collins GB, Ghabrial SA (1996) Production of transgenic soybean lines expressing the bean pod mottle virus coat protein precursor gene. *Plant Cell Rep* 15:746–750
- Enriquez-Obregon GA, Prieto-Samsonov DL, de la Riva GA, Perez M, Selman-Housein G, Vazquez-Padron RI (1999) *Agrobacterium*-mediated Japonica rice transformation: a procedure assisted by an antineoplastic treatment. *Plant Cell Tissue Organ Cult* 59:159–168
- Enriquez-Obregon GA, Vazquez-Padron RI, Prieto-Samsonov DL, de la Riva GA, Selman-Housein G (1998) Herbicide-resistant sugarcane (*Saccharum officinarum* L.) plants by *Agrobacterium*-mediated transformation. *Planta* 206:20–27
- Fagard M, Vaucheret H (2000) (Trans)gene silencing in plants: how many mechanisms? *Annu Rev Plant Physiol Plant Mol Biol* 51:167–194
- Finer JJ, McMullen MD (1991) Transformation of soybean via particle bombardment of embryogenic suspension culture tissue. *In Vitro Cell Dev Biol Plant* 27:175–182
- Finer JJ, Vain P, Jones MW, McMullen MD (1992) Development of the particle inflow gun for DNA delivery to plant cells. *Plant Cell Rep* 11:323–328
- Frame B, Shou H, Chikwamba R, Zhang Z, Xiang C, Fonger T, Pegg SE, Li B, Nettleton D, Pei D, Wang K (2002) *Agrobacterium*-mediated transformation of maize embryos using a standard binary vector system. *Plant Physiol* 129:13–22
- Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Expt Cell Res* 50:151–158
- Hadi MZ, McMullen MD, Finer JJ (1996) Transformation of 12 different plasmids into soybean via particle bombardment. *Plant Cell Rep* 15:500–505
- Hazel CB, Klein TM, Anis M, Wilde HD, Parrott WA (1998) Growth characteristics and transformability of soybean embryogenic cultures. *Plant Cell Rep* 17:765–772
- Hinchee MA, Connor-Ward DV, Newell CA, McDonnell RE, Sato SJ, Gasser CS, Fischhoff DA, Re DB, Fraley RT, Horsch RB (1988) Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer. *BioTechnology* 6:915–922
- Hood EE, Helmer GL, Fraley RT, Chilton MD (1986) The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiBo542 outside of T-DNA. *J Bacteriol* 168:1291–1301
- Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* 6:3901–3907
- Khan MRI, Table LM, Heath LC, Spencer D, Higgins TJV (1994) *Agrobacterium*-mediated transformation of subterranean clover (*Trifolium subterraneum* L.). *Plant Physiol* 105:81–88
- Lohnes DG, Wagner RE, Bernard RL (1993) Soybean genes *Rj2*, *Rmd*, and *Rps2* in linkage group 19. *J Hered* 84:109–111
- Olhoft PM, Flagel LE, Donovan CM, Somers DA (2003) Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method. *Planta* 216:723–735
- Olhoft PM, Lin K, Galbraith J, Nielsen NC, Somers DA (2001) The role of thiol compounds increasing *Agrobacterium*-mediated transformation of soybean cotyledonary-node cells. *Plant Cell Rep* 20:731–737
- Olhoft PM, Somers DA (2001) L-Cysteine increases *Agrobacterium*-mediated T-DNA delivery into soybean cotyledonary-node cells. *Plant Cell Rep* 20:706–711
- Parrott WA, All JN, Adang MJ, Bailey MA, Boerma HR, Stewart CN Jr (1994) Recovery and evaluation of soybean plants transgenic for a *Bacillus thuringiensis* var. kurstaki insecticidal gene. *In Vitro Cell Dev Biol-Plant* 30:144–149
- Perl A, Lotan O, Abu-Abied M, Holland D (1996) Establishment of an *Agrobacterium*-mediated transformation system for grape (*Vitis vinifera* L.): the role of antioxidants during grape-*Agrobacterium* interactions. *Nat Biotechnol* 14:624–628
- Santarem ER, Finer JJ (1999) Transformation of soybean [*Glycine max* (L.) Merrill] using proliferative embryogenic tissue maintained on semisolid medium. *In Vitro Cell Dev Biol Plant* 35:451–455
- Shoemaker R, Keim P, Vodkin L, Retzel E, Clifton SW, Waterston R, Smoller D, Coryell V, Khanna A, Erpelding J, Gai X, Brendel V, Raph-Schmidt C, Shoop EG, Vielweber CJ, Schmatz M, Pape D, Bowers Y, Theising B, Martin J, Dante M, Wylie T, Granger C (2002) A compilation of soybean ESTs: generation and analysis. *Genome* 45:329–338
- Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517
- Stewart CN Jr, Adang MJ, All JN, Boerma HR, Cardineau G, Tucker D, Parrott WA (1996) Genetic transformation, recovery, and characterization of fertile soybean transgenic for a synthetic *Bacillus thuringiensis* cryIAC gene. *Plant Physiol* 112:121–129
- Vancanneyt G, Schmidt R, O'Connor-Sanchez A, Willmitzer L, Rocha-Sosa M (1990) Construction of an intron-containing marker gene: splicing of the intron in transgenic plants and its use in monitoring early events in *Agrobacterium*-mediated plant transformation. *Mol Gen Genet* 220:245–250
- Xing A, Zhang Z, Sato S, Staswick PE, Clemente TE (2000) The use of the two T-DNA binary system to derive marker-free transgenic soybeans. *In Vitro Cell Dev Biol Plant* 36:456–463
- Zhang Z, Xing A, Staswick PE, Clemente TE (1999) The use of glufosinate as a selective agent in *Agrobacterium*-mediated transformation of soybean. *Plant Cell Tissue Organ Cult* 56:37–46