Transformation of 12 different plasmids into soybean via particle bombardment

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Abstract. Particle bombardment offers a simple method for the introduction of DNA into plant cells. Multiple DNA fragments may be introduced on a single plasmid or on separate plasmids (co-transformation). To investigate some of the properties and limits of co-transformation, 12 different plasmids were introduced into embryogenic suspension culture tissue of soybean [Glycine max (L.) Merrill] via particle bombardment. The DNAs used for co-transformation included 10 plasmids containing RFLP markers for maize and 2 plasmids separately encoding hygromycin-resistance and β-glucuronidase. Two weeks following bombardment with the 12 different plasmids, suspension culture tissue was placed under hygromycin selection. Hygromycin-resistant clones were isolated after an additional 5 to 6 weeks. Southern hybridization analysis of 26 hygromycin-resistant embryogenic clones verified the presence of introduced plasmid DNAs. All of the co-transferring plasmids were present in most of the transgenic soybean clones and there was no preferential uptake and integration of any of the plasmids. The copy number of individual plasmids was approximately equal within clones but highly variable between clones. While some clones contained as few as zero to three copies of each plasmid, others clones contained as many as 10 to 15 copies of each of the 12 different plasmids.

Key Words: Glycine max - Recombination - Soybean - Transformation

Introduction

Production of transgenic plants has become routine for many plant species, permitting modifications to basic metabolism and introduction of characteristics that could not be incorporated using conventional breeding techniques. Although different procedures exist for the introduction of foreign DNA, particle bombardment has become the method of choice for transformation of many plant tissues. With particle bombardment, the difficulties of using fragile protoplasts and host-range limitations associated with Agrobacterium can be avoided.

Although success in particle bombardment-mediated transformation of plants has increased tremendously in recent years, there is limited information available on the fate of introduced DNA. In order for the DNA to be integrated into the genome, the introduced DNA must be physically modified. These modifications are important in understanding the organization, integration and expression of the introduced DNA.

Southern hybridization analysis (Southern 1975) of DNA from stably transformed plants produced by naked DNA-mediated transformation reveals complex hybridization patterns (Bates et al. 1990). These hybridization patterns give clues as to the nature of plasmid DNA processing following introduction into the cell. Although integration at single sites in the genome is most common (Christou et al. 1989), multiple integration sites have also been observed (Potrykus et al. 1987). Southern hybridization analysis of DNA from transgenic tobacco obtained by electroporation of plasmid protoplasts (Riggs et al. 1980), and from transgenic cotton and soybean (Finer et al. 1990, 1991) obtained via particle bombardment, indicates that the introduced plasmid forms head-to-tail concatemers. This suggests that the plasmids underwent homologous recombination during the transformation process. In addition to homologous recombination, plasmid DNA(s) could also recombine via illegitimate recombination or, alternatively, the plasmid(s) could be linearized and ligated to other plasmid fragments during the integration process. The high efficiency of co-transformation and the observation of a genetically-linked integration of calf thymus DNA used as a carrier provides evidence for this type of recombination process in electroporated plant protoplasts (Pecharolis et al. 1985).
In order to gain an understanding of plastid DNA integration and determine some of the limitations of particle bombardment in plants, we co-transformed 12 different plastids (as a single cocktail) into embryogenic soybean tissues. One of these plastids contained a scarable 8-glucuronidase gene, another contained a selectable hygromycin-resistance gene, and ten other plastids contained different RFLP markers from maize. Southern hybridization analyses of stably transformed tissue revealed the following: 1) All 12 of the plastids could be taken up and incorporated, 2) There was no preferential uptake or integration of any one of the plastids, 3) Plasmid amplification may have occurred in some clones, 4) Concatemer formation, indicative of homologous recombination, was observed to a limited extent, 5) Ligation of plasmid fragments also occurred at a high rate.

Materials and Methods

Plasmid DNA: The plasmid pUCDLS (Fiori et al., 1995), which encodes 8-glucuronidase (GUS), and pCB10 '9 (Esteban 1987), which encodes resistance to the antibiotic hygromycin-B, were used as the scarable and selectable marker genes, respectively. The 2 regulatory elements for both genes were the CaMV35S promoter. Plasmids had 14.2 kb, and were 39, 34, 38, 39, 42, 84, 107, 115, and 119 (obtained from David Hingston, University of Missouri, Columbia, MO) contained RFLP markers for maize. Plasmid DNA was prepared using the alkaline lysis method and were purified by centrifugation in a cesium chloride-ethidium bromide gradients as described by Barberok et al. (1977).

Soybean Transformation: Embryogenic suspension cultures of soybean [Glycine max (L.) Merritt cv Fayette were initiated and maintained as described previously (Fiori et al., 1988). Cultures were bombarded according to Fiori et al. (1991). Briefly, 1 g of embryogenic suspension culture was transferred to a 1.5 cm Petri dish. The ovary medium was removed with a pipet tip and the tissue was allowed to air dry in a laminar flow hood for 10 to 15 min. Immediately prior to bombardment, the tissue was covered with a 200 μm pore size nylon screen (Tevo Tec, Elmhurst, N.Y.).

The 2 plastids were mixed together in equal amounts (equal mass) to obtain a final total DNA concentration of 1 μg/ml in TE. As the plastids were >5 kb, equal mass was easily equivalent to equimolar. Plasmid DNA was precipitated onto target 10 μg of DNA/ml. TE) ovary medium and the tissue was allowed to air dry in a laminar

Selection for Transformed Tissues: One to 2 h following bombardment, embryogenic tissue was transferred to liquid FN maintenance medium (Fiori and Nagasawa 1988). After 2 weeks, embryogenic tissue was then transferred to fresh FN medium containing 50 μg/ml hygromycin-B (Calbiochem, LaJolla, CA). Tissues were placed in fresh hygromycin-containing maintenance medium every week for 3 additional weeks.

Seven to 8 weeks post bombardment, brown t-tips of transformed embryogenic tissue containing green shoots of T-DNA-resistant tissues were removed and separately cultured in fresh hygromycin-containing maintenance medium. Tissues were removed from these cultures for histochemical GUS assay (Jefferson 1987). DNA extraction and Southern hybridization analysis (Southern 1975).

Molecular Analysis: DNA was extracted from transgenic embryogenic tissues 1 year after selection using the CTAB procedure (Mogel-Marcel et al., 1994). DNA concentration was determined using a TKO 180 microdensitometer (Labbortechnik 1980) and equal amounts of genomic DNA were digested with the appropriate restriction enzymes. DNA were then electroeluted through either a 0.4% or 0.8% agarose gel following digestion with either one or two restriction enzymes, respectively. The DNA was recovered from the gels to nuclease-mad and as described previously (Fiori et al., 1991).

DNA probes consisted of the 1 kb coding region of the hygromycin-resistance gene, the coding region for GUS and the maize DNA inserts from the left and right clones. Clones were random primer labeled (Feinberg and Vogelstein 1983) and hybridized with membrane containing soybean genomic DNA for 24 to 48 h. Following hybridization, membranes were washed five times each with 2 X SSC, 0.1% SDS and then with 0.1 X SSC, 0.1% SDS at 65°C for 10 min each. Membranes were then placed on Kodak XAR-5 film with intensifying screens at -70°C to visualize the hybridization pattern.

Results and Discussion

Over 75 soybean clones that grew in the presence of 50 μg/ml of hygromycin-B were obtained. Twenty sixty of these clones were randomly selected for further analysis; the remainder were discarded due to the large efforts required to separately maintain this amount of tissue. Each clone, derived from a single transformed cell (Fiori et al., 1991), was recovered prior to clone duplication from the original piece of transformed tissue. The timing of clone selection from the original bombarded tissue was important in the recovery of non-duplicated clones. Southern hybridization analysis of HmInIII-digested DNA from these transgenic clones (HmInIII) recognizes only one site on each of the 12 introduced plasmids, the site was not present within the DNA fragments used as probes) revealed unique and complex banding patterns (Fig. 1). The unique hybridization patterns displayed by each of the clones indicated that all clones were unique.

DNA from all of the clones hybridized with the coding region for hygromycin-resistance gene, indicating efficient selection for transformed tissue. Hybridization of the maize RFLP markers to HmInIII-digested maize leaf tissue DNA resulted in 1 to 2 single copy intensity fragments, while there was no hybridization signal with genomic DNA from non-transformed soybean tissue. A hybridization signal was present at unit-plasmid-length (Fig. 1, arrows) in some of the clones, giving some evidence for concatenation of the introduced plasmid DNAs. Clearly, fragments other than unit plasmid length were more common than unit length. None of the clones displayed the hybridization pattern which would result primarily or exclusively from the formation of head-to-tail concatemers. Although plasmid concatenation, which is indicative of homologues recombination either before or after integration (Folgers et al., 1982) was observed, most of the recombination events (as indicated by the complex banding pattern) appear to be illegitimate. Homologous recombination between plastids may only occur if a region of homology exists between those plastids. Because all of the plastids used in this study were pUC based, they contain approximately 2680 bp of common sequence, where ho-
Figure 1 Representative samples of autoradiographs of Southern hybridization analysis. Hind III-digested genomic DNA (10 μg) from non-transformed soybean (wtab), corn, and 26 transformed soybean clones hybridized with DNAs from umc34 (top panel), umc38 (middle panel) and pUCGUS (bottom panel). The arrows designate unit-length of the plasmid DNA which contained the region being used as a probe. The "+" sign designates clear unit-length fragments for Clone 34.
mologous recombination could take place. Plasmid con-
catenation has been observed with electroporated tobacco cells (Riggs et al. 1986) and bombardered cotton and soy-
bean embryogenic cultures (Fitter et al. 1990, 1991). In
these studies, as well as most others involving DNA in-
tructions, only one or two different plasmids are typi-
cally used. Homologous recombination may be more
common between identical plasmids than with similar
plasmids. The use of 12 different plasmids, as reported
here, makes analysis of recombination events more diffi-
cult.

The copy number of the introduced plasmids was
estimated based exclusively on the number of bands on the
autoradiographs using signal from maize eaf DNA as a
single copy reference (Table 1). Copy number was
determined from the original film as some signal defini-
tion was lost in the photographic copies (Fig. 1). There
was low variability in plasmid copy number within clones
but higher variability among clones. The copy
number for individual plasmids varied greatly, from zero
in some clones to 16 for plasmid uncle 38 in clone 30. All
of the clones contained at least some introduced plasmid
DNA. Clones 32, 33, 35 and 50 were classified as low
copy number clones and contained an average of 2 or
fewer copies of each plasmid. Clones 9, 24, 37, 59, 63
and 64 were high copy number clones and these clones
contained an average of 10 or more copies of each intro-
duced plasmid. Most of the clones that contained high
copies of one plasmid had high copy numbers for all
other plasmids, while the low copy number clones gen-
erally had low copy numbers for all plasmids. Four
clones (clones 8, 12, 30 and 65) that varied widely in
copy number of introduced plasmid DNA, were
termed variable copy clones, while the remaining clones
were intermediate copy number clones. The complex
hybridization patterns observed in all of the clones indi-
cate that the plasmids primarily recombined with each
other at random. The consistency of plasmid copy num-
ber suggests that there was no preferential uptake of any
of the plasmids.

The presence of intense hybridization signals at po-
sitions other than unit-length indicates that plasmid
data was amplified in some clones. Amplification of
introduced plasmid DNA was clearly observed in clones
9, 24, 34, 49, and 63. Amplification of plasmid DNA
results in either partial or full amplification of the hy-
bridization signal. This is noticeable as some of the hy-
bridization signals are intense while others are present at
low intensity. Amplification of resistance genes in re-
response to both antibiotic (Czezinski et al. 1986) and
herbicidal (Wang et al. 1991) selection has been reported.
Selection of transformed clones using high levels of hy-
gramycin-B may have contributed to amplification of the
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Table 1. Plasmid copy number, GUS activity and intactness of the GUS gene in transformed soybean tissue (0 indicates high copy number clone; 0 indicates low copy number clone; ○ indicates variable copy number clone; clones not labeled are intermediate copy number clones) following transformation with 12 different plasmids.
recombinant integration unit) in some of the clones. When the copy number and size of the introduced plasmids are considered, approximately 600 kb of plasmid DNA was introduced into the soybean genome for high copy clones. The amount of introduced plasmid DNA in this report is considerably higher than reported earlier for other bombardment and naked DNA-mediated transformation systems. Around three to five plasmid copies were introduced with a liposome mediated trans- fer to protoplasts (Desthay et al. 1985). Transgenic soybean obtained via particle bombardment contained approximatley five copies of a single plasmid (McCabe et al. 1988). While in transgenic tobacco obtained via microinjection, some cell lines contained less than one copy of the plasmid (Crossway et al. 1980). The large amount of introduced DNA in this report may result from either the use of a large number of different plasmids or an enhanced "transformation competence" (the ability to incorporate foreign DNA) of these soybean cells. The results of an 11-way co-transformation in embryogenic cotton cultures gave similar hybridization patterns to those reported here (data not shown). Although extensive analyses were not undertaken, approximately 10 copies of each of the 11 plasmids were introduced into these embryogenic cotton cultures (Finer, unpublished).

In general, particle bombardment results in low num- bers of integration events in soybean (Christou et al. 1989). The number of integration events (separate sites of integration) was not determined in the present study. Although plants were recovered from some of the cultures obtained in this study (unpublished), they showed severely reduced fertility and progeny analysis could not be used to determined the number of integration events. This reduced fertility was a result of the extended cell culture period and was not related to the introduction of large amounts of DNA. Plants recovered from non-transformed cultures showed the same phenotype as the transformed plants.

GUS assays were performed on embryogenic cultures that were morphologically similar and were grown under identical conditions. All the clones were tested for GUS activity once at 8 weeks and again at 16 weeks after transformation. The presence of the intact GUS coding unit did not correlate with the expression of the GUS gene. Of the 11 clones that contained the intact expres- sion unit for GUS, 4 clones never showed histochemical GUS activity and 4 clone displayed GUS activity only after 8 weeks (and not at 16 weeks). Only six of the 26 clones showed GUS activity at both time points (Table 1). Four of the clones that were GUS-positive at the 8 week period were negative at the 16 week test. All clones that were initially GUS-negative remained GUS- negative at the 16 week point. Southern hybridization analysis indicated the presence of an intact GUS expression unit in all but one clone that at some point ex- pressed GUS. Clone 8, the single clone that did not con- tain the intact expression unit (Table 1) but expressed GUS, may have lost non-essential elements of the expres- sion unit during recombination (Finer and McMullen 1991) or the coding region may have integrated into a nonhomologous sequence, resulting in transcriptional inactivation or translational fusion between the GUS coding re- gion and an endogenous gene (Halfter et al. 1992, Koszegi et al. 1988, Mayerhofer et al. 1991). In addition, some of the clones that never expressed GUS also contained the proper size restriction length fragment associated with the intact GUS expression unit. In clones that contained the intact expression unit but did not stably express GUS, there was no relationship between copy number and expression. Co-suppression, which is a suppression of gene expression resulting from introduction of multiple copies of the same gene (Napoli et al. 1990), was not clearly observed in this study. Co-suppression has been suggested to occur in transformed soybean tissue (Finer et al. 1991), where multiple copies of the intact GUS gene were present but the gene was not expressed. The tandemization of DNA giving rise to more than one promoter per integration site could lead to transcriptional interference (Czaum et al. 1986), resulting in the absence of GUS activity.

In most cases of naked DNA-mediated transformations, only one or two different plasmid DNAs are used. Multiple copy integrations, resulting from the in- troduction of one or two different plasmids, can lead to formation of large concatemers, where the plasmid DNAs can lie directly adjacent to one another. The proximity of these introduced plasmid DNAs to one an- other or their position within the genomic DNA may influence expression of the genes carried on this DNA (position effect). In the case of multiple plasmid co- transformations, the introduced plasmid DNAs are sepa- rated by each other, thus creating a type of "buffer" re- gion for gene expression. If the introduced genes are adjacent to or associated with regions of introduced DNA that do not reduce or possibly enhance gene ex- pression, it should be possible to modify or stabilize transgene expression thereby reducing position effect. The use of RFLP markers, which are typically low copy number and unmethylated, for co-transformation ex- periments may actually enhance gene expression, trans- formation efficiencies and serve as an additional tool for detecing the introduced DNA in the progeny. Scaflford Attachment Regions (Brennan et al. 1992) and Transfor- mation Boostor Sequences (TBS) (Meyers et al. 1988), which enhance transformation rates and gene expres- sion, may act in somewhat similar ways.

Study of co-transformations can provide much in- formation on the process of recombination. Co-transforma- tion can also be used to introduce large numbers of genes to different plasmids without the labor intensive and inefficient process of repeated transformations. If these plasmids recombine to form large chains of mixed
plasmid DNAs, multiple co-transformations may also be useful for construction of subchromosomal regions where the genes of interest could be flanked by expanses of defined DNAs.

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References.

Batte OW, Sigrid AC, Piirle WC (1990) Plant Mol Biol 14: 899-905

Riggs CD, Barlow GW (1986) Proc Natl Acad Sci USA 83: 5400-5406