Stable transformation of peanut callus via Agrobacterium-mediated DNA transfer

CHANDRA I. FRANKLIN*, KATHY M. SHORROSH, ANTHONY N. TRIEU, BRANDT G. CASSIDY and RICHARD S. NELSON

Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73401, USA
(Fax: +1 405 221 7380)

Received 26 October 1992; revised 15 March 1993; accepted 18 March 1993

Transformed callus was produced from peanut (Arachis hypogaea L. cv. Okrun) hypocotyl explants after four days of co-cultivation with Agrobacterium tumefaciens strains EHA101, LBA4404 or ASE1 carrying the binary vector pKYLX71GUS on a defined medium followed by selection with kanamycin (200 mg l⁻¹). Transformed calluses were cultured as independent cell lines potentially derived from a single transformation event. Stable integration and expression of foreign gene(s) in the callus was confirmed by Southern and western blot analyses and enzyme assays. A few cell lines showed a single insert of the foreign gene. Using the above protocol, transformed peanut callus expressing the peanut stripe virus coat protein gene was obtained.

Keywords: Peanut; Arachis hypogaea; transformation; callus; Agrobacterium tumefaciens; peanut stripe virus coat protein

Introduction

Diseases of peanut limit the yield of this crop worldwide (Wynne and Beute, 1991). Peanut stripe virus (PStV), a member of the potyvirus group of plant viruses, has been shown to reduce peanut yields up to 24% (Demski and Reddy, 1988). Thus far, a peanut cultivar resistant to PStV infection has not been identified (Culver and Sherwood, 1987). In an attempt to engineer resistance to PStV in peanut, coat protein (CP)-mediated protection (Beachy et al., 1990) is being investigated. As an initial step towards this goal, the PStV gene has been cloned (Cassidy et al., 1992) and is being tested for CP-mediated protection in different Nicotiana spp. (B.G. Cassidy and R.S. Nelson, unpublished results). The lack of an efficient genetic transformation system in peanut has hindered the progress of efforts to produce genetically engineered peanut plants protected against PStV. Attempts to produce transgenic peanut plants, using published regeneration protocols (Mroginski et al., 1981; McKently et al., 1991) are now in progress. In the meantime, transformed callus cultures, which can be used for studying the mechanism(s) of CP-mediated protection (Hanley-Bowdoin and Hemenway, 1992; Register and Nelson, 1992) in an homologous system, were obtained. In this report, a protocol for obtaining transformed peanut callus via Agrobacterium-mediated DNA transfer is described. Results from molecular and biochemical analyses that confirm the stable integration and expression of the foreign gene(s) in the transformed callus cultures are presented.

Results and discussion

Callus initiation and culture

Hypocotyl segments 2–3 mm in thickness from 8-day-old aseptically-grown peanut (Arachis hypogaea L. cv. Okrun) seedlings were used as explants. Hypocotyl segments were isolated 2–3 mm below the cotyledonal node, and 3–5 segments were excised from each hypocotyl. The seeds were surface-sterilized as described previously (Franklin et al., 1991). The explants were co-cultivated with Agrobacterium tumefaciens strains EHA101 (Hood et al., 1986), LBA4404 (Hoekema et al., 1983) or ASE1 (referred to as ASE, see McCormick et al., 1986) carrying the binary vector pKYLX71GUS (Franklin et al., 1992) on a defined medium referred to as peanut culture (PC) medium. The binary vector pKYLX71GUS contains the β-glucuronidase reporter gene (uidA or gus) (Jefferson, 1987) driven by the CaMV
Fig. 1. Proliferation of transformed callus clumps from peanut hypocotyl explants on kanamycin selection medium (A) and histochemical assay for β-glucuronidase (GUS) in transformed peanut callus (B). Blue dye indicating GUS activity is represented as a dark patch in this black and white photomicrograph. Bars indicate 2 mm in A and 0.5 mm in B.

35S promoter and the neomycin phosphotransferase (NPTII) selectable marker gene driven by the nopaline synthase promoter. The PC medium contained MS salts and vitamins (Murashige and Skoog, 1962), 10 μM 2,4-dichlorophenoxyacetic acid, 1 μM 6-benzylaminopurine, 3% sucrose and 0.8% agar. After four days of co-cultivation, the explants were transferred to fresh PC medium containing antibiotics carbenicillin (500 mg l⁻¹), vancomycin (50 mg l⁻¹) and kanamycin (200 mg l⁻¹). Results from kanamycin dose response experiments indicated that selection of transformed callus could be performed successfully with 200 mg l⁻¹ of kanamycin (data not shown). Callus initiation occurred from the upper side (cut surface of the hypocotyl segment not touching the medium) of all hypocotyl explants 2-4 weeks after culture initiation, irrespective of the thickness (2-3 mm) of the explant. However, further growth of callus on selection medium containing kanamycin occurred only from those explants co-cultivated with A. tumefaciens carrying the binary vector pKYLX71GUS. Kanamycin-resistant callus was produced as small clumps, as seen in Fig. 1A. Each clump may have been clonally produced from a single transformed cell. These callus clumps were separated from each other 8-12 weeks after culture initiation and cultured as independent cell lines. Proliferation of transformed callus on kanamycin-selection medium occurred 12-16 weeks after culture initiation and the callus doubled in volume during a 4-week subculture period. All three A. tumefaciens strains tested produced transformed callus clumps similar in morphology and size. A. tumefaciens strains ASE1 and LBA4404 produced kanamycin-resistant callus clumps (clones) from hypocotyl explants at more or less equal frequencies. However, the number of kanamycin-resistant callus clumps produced from explants co-cultivated with EHA101 was approximately 3 times more than the other two strains.

Enzyme assays
Using a NPTII ELISA kit (5 Prime → 3 Prime, Inc., Boulder, CO, USA) the amount of NPTII protein detected (on a g fresh weight basis) from extracts of kanamycin resistant calluses was at least 10 times more than the background level present in the untransformed callus (data not shown). Results from GUS histochemical assays (Jefferson, 1987) confirmed the expression of the gus reporter gene in transformed calluses (Fig. 1B).

Southern blot analysis
Genomic DNA from untransformed callus and from callus transformed with pKYLX71GUS was isolated following the protocol described by White and Kaper (1989). Digested and undigested DNA (see Fig. 2 legend for details) were electrophoresed, transferred to a nylon membrane (GeneScreenPlus, NEN Research Products, Boston, Massachusetts, USA) and probed with a [³²P]-labelled gus fragment from the plasmid pKYLX71GUS following the procedure described by the supplier of the nylon membrane.

Genomic DNA from kanamycin-resistant calluses hybridized with the probe (Fig. 2A, lanes 2–9). Genomic DNA from the untransformed callus did not hybridize with the probe (Fig. 2A, lane 1). This result confirms the stable integration of the foreign DNA into the plant genome. Digestion of pKYLX71GUS with Hin dIII and