Transformation of the monocot Alstroemeria by Agrobacterium rhizogenes

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Abstract

An efficient procedure is described for transformation of calli of the monocotyledonous plant Alstroemeria by Agrobacterium rhizogenes. Calli were co-cultivated with A. rhizogenes strain A13 that harbored both a wild-type Ri-plasmid and the binary vector plasmid pIG121Hm, which included a gene for neomycin phosphotransferase II (NPTII) under the control of the nopaline synthase (NOS) promoter, a gene for hygromycin phosphotransferase (HPT) under the control of the cauliflower mosaic virus (CaMV) 35S promoter, and a gene for β-glucuronidase (GUS) with an intron fused to the CaMV 35S promoter. Inoculated calli were plated on medium that contained cefotaxime to eliminate bacteria. Four weeks later, transformed cells were selected on medium that contained 20 mg L–1 hygromycin. A histochemical assay for GUS activity revealed that selection by hygromycin was complete after eight weeks. The integration of the T-DNA of the Ri-plasmid and pIG121Hm into the plant genome was confirmed by PCR. Plants derived from transformed calli were produced on half-strength MS medium supplemented with 0.1 mg L–1 GA3 after about 5 months of culture. The presence of the gusA, nptII, and rol genes in the genomic DNA of regenerated plants was detected by PCR and Southern hybridization, and the expression of these transgenes was verified by RT-PCR.

Abbreviations: NPTII – neomycin phosphotransferase II; NOS – nopaline synthase; HPT – hygromycin phosphotransferase; CaMV – cauliflower mosaic virus; GUS – β-glucuronidase; MS – Murashige and Skoog medium; X-Gluc – 5-bromo-4-chloro-3-indolyl-β-d-glucuronide; RT-PCR – reverse transcription = polymerase chain reaction

Introduction

Agrobacterium rhizogenes, a soil-borne bacterium, induces the development of hairy roots when its Ri T-DNA is integrated into a plant genome (Chilton et al. 1982). Ri-transformed plants of several plant species have a characteristic phenotype, with shortened internodes, wrinkled leaves, and an abundant root mass with extensive lateral branching (Tepfer 1984). The rol genes in Ri T-DNA induce changes in sensitivity to plant hormones and/or in the metabolism of plant hormones (Maurel et al. 1994; Moritz and Schmülling 1998; Nilsson et al.; Shen et al. 1988). Furthermore, transformation of plant tissues by infection with A. rhizogenes increases the production of certain metabolites (Ermayanti et al. 1994; Mano et al. 1986; Sim et al. 1994). Since A. rhizogenes can also transfer the T-DNA of binary vectors ‘in trans’, the Ri plasmid has been widely used as a vector for
the introduction of foreign DNA into a broad range of dicotyledonous plant species (Simpson et al. 1986). Particle bombardment is a popular choice for transformation of monocots, because there is no host range limitation with this method, and the transformation efficiency is relatively high when combined with an efficient selection method (Christou 1995; Jähne et al. 1995). In many instances, this is now a routine technology. Recently, Lin et al. (2000) reported a system for genetic transformation of Alstroemeria by this method. On the other hand, establishment of a system for transformation of monocots mediated by Agrobacterium had been considered difficult, since infection of monocots by Agrobacterium is a very rare event. However, by the use of ‘super-virulent’ A. tumefaciens strains and/or acetosyringone, a phenolic compound inducing expression of vir genes on the Ti-plasmid, transformation via A. tumefaciens has become a major method in monocots (Belarmino and Mitani 2000; Eady et al. 2000; Hiei et al. 1994; Smith and Hood 1995; Wilmink et al. 1992). However, there are no reports of transformation of monocots mediated by A. rhizogenes. Establishment of a system for transformation of Alstroemeria mediated by A. rhizogenes should facilitate not only the introduction of foreign genes but also the production of dwarf cultivars.

Several systems for culture of Alstroemeria have been developed (Buitendijk et al. 1995; De Jeu and Jacobsen 1995; Gonzalez-Benito and Alderson 1990; Ishikawa et al. 1997; Lin et al. 1998), and attempts at transformation of Alstroemeria by particle bombardment have been made (Lin et al. 2000). However, to our knowledge, transformation by A. rhizogenes has not been reported. We describe here the establishment of transgenic plants of Alstroemeria by A. rhizogenes-mediated transformation.

**Materials and Methods**

**Plant material**

Ovaries of interspecific hybrids of Alstroemeria pelegrina (L.) var. alba and A. magenta were surface-sterilized by soaking them in a 1% (v/v) solution of sodium hypochlorite for 10 min, and then they were rinsed three times with sterilized water. Ovules were excised from the sterilized ovaries and plated on solidified Murashige and Skoog (MS) medium (Murashige and Skoog 1962) that had been supplemented with 3% sucrose and 2% gellan gum (Wako, Osaka, Japan). The calli that developed from the ovules were transferred to 100-mL Erlenmeyer flasks that contained 40 mL of liquid MS medium supplemented with 3% sucrose and 1 mg L⁻¹ picloram (Wako) and were agitated at 100 rpm on a gyratory shaker. The pH of the medium had been adjusted to between 5.6 and 5.8 before autoclaving. Calli were subcultured at two-week intervals for use in subsequent experiments.

Cultures were incubated at 20 °C under continuous illumination at 50 μmol m⁻² s⁻¹ from cool-white fluorescent lamps throughout the experiments.

**Strain of Agrobacterium**

The mikimotine-type A. rhizogenes strain A13 [MAFF02–10266] (Daimon et al. 1990), harboring both a wild-type Ri plasmid and the binary vector pIG121Hm (Ohta et al. 1990), was used in this study. pIG121Hm included a gene for neomycin phosphotransferase II (NPTII) under the control of the nopaline synthase (NOS) promoter, a gene for hygromycin phosphotransferase (HPT) under the control of the cauliflower mosaic virus (CaMV) 35S promoter (Jefferson et al. 1987), and a gene for β-glucuronidase (GUS) with an intron fused to the CaMV 35S promoter. Before co-cultivation, bacterial cultures were prepared by incubation of bacteria in YEB liquid medium (Herrera-Estrella and Simpson 1988) that contained 50 mg L⁻¹ kanamycin and 20 mg L⁻¹ hygromycin overnight at 28 °C with reciprocal shaking at 120 rpm.

**Transformation of calli by A. rhizogenes**

Calli were inoculated by exposure to a suspension culture of freshly grown bacteria for 10 min. For cocultivation, inoculated calli were placed on MS medium that contained 3% sucrose and 2% gellan gum with or without 1 mg L⁻¹ picloram, or on half-strength MS medium that contained 2% sucrose and 3% gellan gum with or without 1 mg L⁻¹ picloram. After co-cultivation for 0, 1, 2, 3, 4, or 5 days, calli were transferred to the same fresh medium supplemented with 500 mg L⁻¹ ceftotaxime (Claforan; Aventis Pharma, Tokyo, Japan), which was added after autoclaving, to eliminate bacteria. After two weeks, calli were transferred to the same medium supplemented with 250 mg L⁻¹ ceftotaxime. After an additional two weeks of culture, calli were transferred...