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BRIEF COMMUNICATION

The possibility of micropropagation and *Agrobacterium*-mediated transformation of *Kalmia latifolia*

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Abstract

Micropropagation and *Agrobacterium*-mediated transformation were developed in *Kalmia latifolia* cv. Ostbo Red. The transformation of *Kalmia latifolia* plants was carried out by an *Agrobacterium tumefaciens* strain containing the *npt*II and *gus*A genes in its T-DNA. Shoots were regenerated on kanamycin selection medium and the expression of the *gus*A reporter gene was verified by fluorogenic β-glucuronidase (GUS) assay in a few vegetative generations after regeneration. The presence of the *gus*A gene in regenerated kanamycin resistant plants was detected by polymerase chain reaction.

Additional key words: *Ericaceae*, GUS assay, polymerase chain reaction.

The *Ericaceae* include an important group of garden plants which are widely grown in large numbers by commercial nurserymen. From this point of view, it is not surprising that micropropagation has been applied to a number of genera within the group and that genetic transformation is in the forefront of interest. We were interested in species of *Kalmia*, because their micropropagation, especially of the cultivar Ostbo Red, is difficult. The genetic transformation of *Kalmia* plants has not been previously described, although new properties, for example alterations of pigment biosynthesis, are desirable. The aim of our research was to attempt the successful micropropagation of *Kalmia latifolia* cv. Ostbo Red and to find out the possibility of its transformation *via Agrobacterium tumefaciens*.

The successful micropropagation was achieved on WPM medium (Lloyd and McCown 1981) supplemented with 20 g dm^{-3} sucrose, 40 mg dm^{-3} adenin sulfate and

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5 mg dm^{-3} isopentenyladenine (2iP). The concentration of cytokinin used was shown to be sufficient for some others cultivars of *Kalmia latifolia*, too. Stem segments (5 - 10 mm long) were placed horizontally on agar containing WPM medium, the cut edges being in contact with the medium. Abundant regeneration of shoots was usually observed during 4 - 6 weeks. The culture conditions were a 16-h photoperiod, a temperature of 22 °C and irradiance of 90 μmol $m^{-2} s^{-1}$.

The transformation of stem segments of *K. latifolia* cv. Ostbo Red was carried out with *Agrobacterium tumefaciens* strain LBA4404 containing the helper plasmid pAL4404, the GUS-intron chimeric gene and the *npt*II gene (Vancanneyt *et al.* 1990), similarly as described previously for *Agrobacterium*-mediated transformation of *Rhododendron* cultivars (Pavingerová *et al.* 1997). The segments were placed on selection WPM medium containing 200 mg dm^{-3} cefotaxime and 500 mg dm^{-3} ticarcilline to stop bacterial growth and 50 mg dm^{-3} kanamycin for selection. Using lower concentrations of kanamycin (20 or 50 mg dm^{-3}), the shoot regeneration from non-transformed cells predominated, when selection was conducted with higher concentration of kanamycin (100 mg dm^{-3}) the shoot regeneration was arrested. Thereafter, the regenerated shoots were transferred to fresh selection medium with 200 mg dm^{-3} kanamycin.

Table 1. Example of variability of GUS activity [pmol(4-methylumbelliferone) mg^{-1}(protein) min^{-1}] in two vegetative generations of *Kalmia latifolia* cv. Ostbo Red transgenic plants. Means ± SE, $n = 3$.

Clones	GUS activity	Subclones	GUS activity
71	42.52 ± 3.47	71/1	12470.10 ± 32.06
		71/2	72.71 ± 7.17
		71/8	313.75 ± 11.65
78	228.90 ± 6.18	78/4	2147.62 ± 18.13
		78/5	13.57 ± 0.92
		78/7	291.38 ± 6.78
80	89.12 ± 1.89	80/1	825.89 ± 9.63
		80/4	1384.36 ± 11.89
		80/5	95.34 ± 8.47
105	775.55 ± 5.82	105/1	2395.02 ± 12.36
		105/2	4740.45 ± 21.67
107	117.01 ± 5.11	107/1	36.29 ± 4.05
		107/2	74.12 ± 3.12
		107/3	21.99 ± 0.88
108	304.36 ± 6.03	108/1	659.76 ± 8.13
		108/2	31.25 ± 2.75
Control	7.43 ± 0.61		

After selection of regenerated shoots the β-glucuronidase (GUS) activity was determined using a fluorimetric assay (Jefferson 1987) and subsequently, GUS-positive shoots were PCR assayed for the presence of the GUS fragment (Fig. 1). PCR was performed on isolated DNA using primers

5'-TCGATGCGGTCACTCATTAC-3' and 5'-CCACGGTGATATCGTCCAC-3' which amplify a 495 bp fragment. This fragment consists of a part of the *gus*A gene including intron in nucleotide position 263 - 757. The samples were heated to 94 °C for 4 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 30 s, 72 °C for 2 min, with a final extension step of 72 °C for 2 min in a *Perkin Elmer* thermal cycler. The absence of residual bacterial contaminants was shown with PCR in all tested plants by using the plasmid probe containing the vir zone A, located outside of the T-DNA.

After transformation of 50 stem segments, 208 kanamycin resistant plants were obtained, 126 of them were used for fluorimetric GUS assay and 90 plants were found to be GUS-positive. But during the vegetative propagation of transformed plants a big fluctuation of GUS activity in subclones was observed (Table 1). A similar phenomenon was also found in further vegetative generations. Only a very small number of clones were found with stable GUS expression during 12 vegetative propagations (9 % of GUS-positive regenerants). The presence of *gus*A gene in these regenerants was again confirmed with PCR.

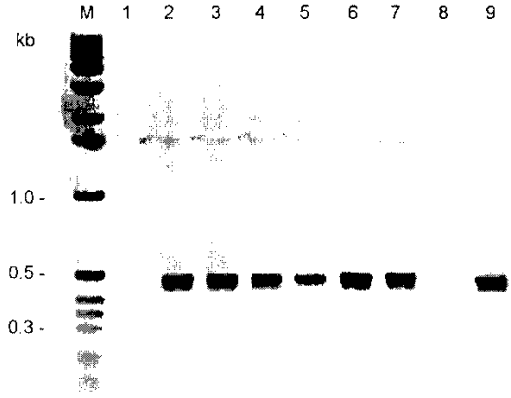

Fig. 1. Representative samples of PCR amplified fragments, showing 495-bp GUS fragment in *Kalmia latifolia* cv. Ostbo Red transgenic plants. *Lane 1* - negative control without DNA, *lanes 2 to 7* - transformed plants, *lane 8* - nontransformed plant, *lane 9* - plasmid p35SGUSint, *lane M* - marker DNA (1 kb ladder).

Thus the possibility of *Agrobacterium*-mediated transformation of *Kalmia latifolia* is evident. Since we showed both the expression of the *gus*A gene with the fluorimetric GUS assay and its presence in plant cells by PCR during 12 vegetative generations in two years, we can infer the integration of transgenes into the *Kalmia* genome, as in the case of five cultivars of *Rhododendron* (Pavingerová *et al.* 1997). The main problem of the results presented is the gradual loss or significant changes in GUS activity during vegetative propagation of the plants. There are at least two causes which could explain this phenomenon. The first one is chimerism of regenerated plants, when the part with minor contents or without transformed cells is

used for propagation. The second possibility applies to cases of different levels of gene silencing in plants (Stam *et al.* 1997).

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