TRANSGENIC LILIES *VIA* POLLEN-MEDIATED TRANSFORMATION

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Abstract

We have developed a procedure for the production of transgenic lilies by using the pollen grain as vector for DNA delivery. First, a particle gun was used for the introduction of the *NPTII* gene (for kanamycin resistance) into pollen of lily (*Lilium longiflorum*), cv 'Gelria'. Subsequently the bombarded pollen was used for pollination of flowers of cv 'Indian Summer'.

A large number (approx. 400,000) of seeds were harvested, of which 65,000 were tested for *in vitro* germination in the presence of kanamycin. Three plants that developed well on media with 50µg kanamycin were obtained. These plants were grown-on in the greenhouse. Regeneration of bulbscale explants on kanamycin confirmed the resistance of these selected plants. Furthermore, by using PCR the presence of the transgenes in the genome of the putative transformants was established.

To investigate the transfer of the transgenes to the next generation, crosses were made using the three plants as male or female parent. The offspring were tested again for germination on kanamycin-containing medium, and the presence of kanamycin-resistant as well as kanamycin-sensitive seedlings proved that transfer and segregation of the transgenes had occurred. The kanamycin-resistant seedlings of the first generation were positive in the PCR reaction.

The results clearly show that transgenic lily plants have been obtained. However, segregation analysis showed that transmission of the genes to the F1 was not mendelian. This will be investigated by following the transfer of transgenes to future generations.

Introduction

Recently we have shown that DNA can be introduced into *Nicotiana glutinosa* by using mature pollen as a vector (van der Leede-Plegt *et al.*, 1995. Transgenic Research 4: 77-86). This pollen-mediated transformation has now been used for transformation of lily.

Pollen Bombardment

Mature pollen from *Lilium longiflorum* cv 'Gelria' was bombarded using a PDS helium device. Particles were coated with the DNA construct pCPO1.2'*gus*, harboring the glucuronidase reporter gene driven by the TR2' promoter and the kanamycin-resistance gene *aphII* driven by the NOS promoter. expression of GUS was observed in pollen that was also able to germinate

Pollination with Bombarded Pollen

About 2000 emasculated flowers of *L. longiflorum* cv 'Indian Summer' were hand-pollinated with bombarded pollen.

About 400,000 seeds were harvested. 65,000 seeds were tested for kanamycin resistance by using a large-scale in vitro selection procedure.

Selection of Kanamycin-Resistant Seedlings

Three kanamycin resistant seedlings were obtained. Kanamycin-resistant plantlets formed a bulblet. The plants were grown in the greenhouse and further analyzed. Vegetative growth and flower formation was normal. The presence of transgenes was proven by PCR.

Transmission of Transgenes

The three transgenic plants were crossed with cv. 'White American'. Seeds were selected again *in vitro* for kanamycin resistance and analysed by PCR. Kanamycin-resistance was correlated with the presence of the transgene showing the transmission of the trait to the next generation.

Conclusion

This research has resulted in the first transgenic lilies. It shows that pollen is a good vector to introduce DNA via normal pollination and without a regeneration step. The procedure can now be applied for the introduction of interesting genes.