Particle-mediated transformation of Oriental hybrid lily 'Star Gazer'

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Introduction

Lilium species are monocots which are less susceptible to *Agrobacterium* transformation. Particle bombardment for DNA delivery is an alternative transformation way for monocots. Transgenic plants of *Lilium longiflorum* were reported (Van der Leede-Plegt et al. 1995; Watad et al. 1998). There are three main commercial lily groups, namely *Lilium longiflorum*, Oriental lily and Asiatic lily. Oriental hybrid 'Star Gazer', which is widely cultivated, was selected for bombardment transformation experiments.

Materials and methods

Young scales excised from *in vitro* bulblets of 'Star Gazer' were used for callus and bombardment experiments. They were sliced horizontally into slices with a thickness of 2~3 mm and incubated for 3 weeks on callus induction medium in the dark. Picloram 1.5 mg/l appeared the optimal concentration for callus induction. Plasmid pPG5 containing the PAT and GUS genes was used to optimise the transformation system. All transformation experiments were conducted with Biolistics PDS 1000/He system using gold particles. Macrocarrier travel distance was 1.0 cm and target distance was 11 cm. Bombardment pressure was 1800psi. Selection took place on regeneration medium containing 1.5 mg/l Basta.

Results and discussion

Scale age is a main factor for callus induction. The youngest scales (pit) have strong capacity to produce callus. After 4 weeks' incubation, 100% of pit slices produced callus with an average 2.5 times of original explant size. Old scale slices were less active for callus induction. Different concentrations of picloram showed large effect on callus formation of scale slices incubated in dark. Picloram 1.5 mg/L appeared to be the best concentration for callus induction. The supplement of BAP did not show positive effects on callus formation.

Among the different types of tissue tested (scale slice, pit slice, slice of white pit, callus and low part of scale), pit slice was most susceptible to bombardment. Callus induction time has large effects on bombardment susceptibility of pit and scale slices (Fig 1). The optimum time is between 3 to 5 weeks.

The effect of herbicide is cumulative in explants. Growth loss increases with longer incubation (Fig. 2). PPT 1.5 mg/L and Basta 1.0 mg/L were found as optimum selection concentration.

Conclusions

- Callus can be induced from scale slices and be propagated on MS with picloram 1.5 mg/L.
- Explant type and callus induction time are important factors to bombardment.
- Oriental hybrid lily 'Star Gazer can be transformed by particle bombardment (Photo 4).

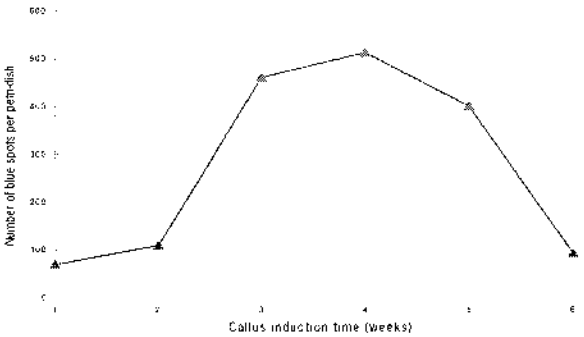

Fig. 1 Effect of callus induction time on bombardment efficiency of pit and scale slice

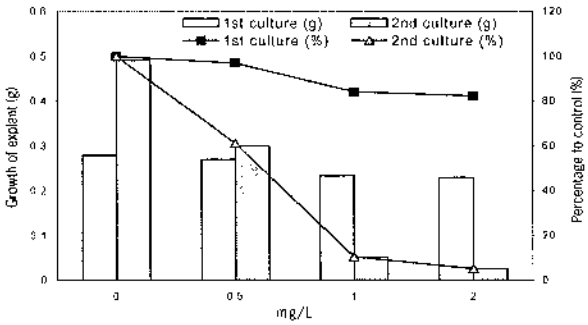

Fig. 2 Growth of pit slices at different PPT concentrations (each culture 4 weeks)

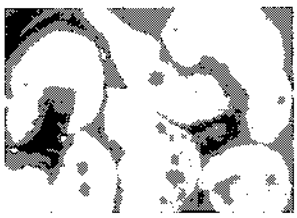

Photo 1 Transient expression of GUS in pit slice

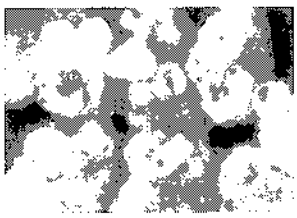

Photo 2 Transient expression of callus

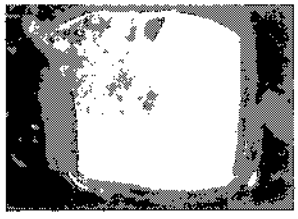

Photo 3 Transient expression of low part of scale

Photo 4 Leaf of Star Gazer transgenic plant

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PLANT RESEARCH INTERNATIONAL

A greenhouse screening assay for *B. tulipae* resistance in tulips

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Introduction

Botrytis tulipae, as a leaf pathogen, threatens bulb production of tulip seriously. Chemical control of *B. tulipae* is not desired for environmental reasons. Resistant cultivars can play an important role in the control of this disease. To select cultivars resistant to *B. tulipae* a reliable screening assay is desired. A greenhouse test was developed to establish resistance at clonal level.

Materials and methods

Planted bulbs of 20 cultivars were cooled for several weeks at 9-2°C and forced to bloom in the greenhouse. Conidia of *B. tulipae* were sprayed (2.10^5 conidia/ml) on the first and second leaf of the plants. Removal of the wax layer by soft hand rubbing of the leaves was essential to obtain a homogeneous distribution of the conidia. Long periods of leaf wetness, achieved by regular water evaporation, was essential for getting spreading lesions. Plant material was incubated in the greenhouse during 7-9 days at 18°C and 100% humidity (Fig. 1). Disease ratings were scored according a scale of 1-5 depending on the affected area (Fig. 2).

Figure 1. Incubation

Figure 2. Disease rating

Results

Large differences in resistance were found within the tulip cultivars tested (Table 1).

Table 1. Average disease ratings of various tulip genotypes tested for B. tulipae resistance.

Cultivar	ADR	Cultivar	ADR	Cultivar	ADR
Tarda	1.0	I. de France	3.0	Ad Rem	3.8
Flair	1.2	Pax	3.3	Gander	4.0
J. Strauss	1.3	Bonanza	3.4	M. Carlo	4.1
Br. Star	1.3	E.Yellow	3.4	Parade	4.3
G. de Wit	1.5	C.Cardinal	3.7	Renown	4.5
Princeps	1.8	L v.d. Mark	3.8	Chr.Marvel	4.7
Bellona	2.6	Thule	3.8		

Repeating the screening tests over two successive years showed very similar results (Fig. 3, $r^2 = 0.93$).

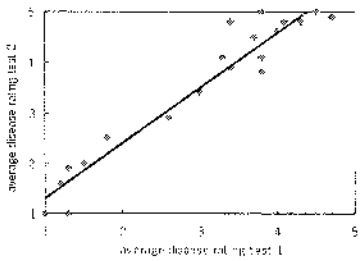

Figure 3. Correlation between two greenhouse assays

Addition of nutrients (0.067 KH_2PO_4, 0.11M glucose, pH 5.0) to the inoculum enhances the infection process but did not change the susceptibility rating of the cultivars (Fig. 4).

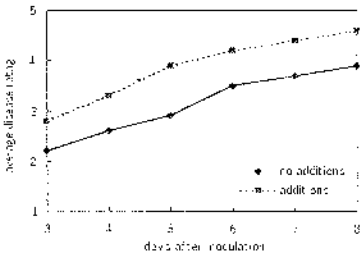

Figure 4. Disease development in time with and without additions

To determine whether the developmental stage of the plant influences disease sensitivity we tested three different plant stages: (1) before flowering, (2) during flowering and (3) after flowering (Fig. 5).

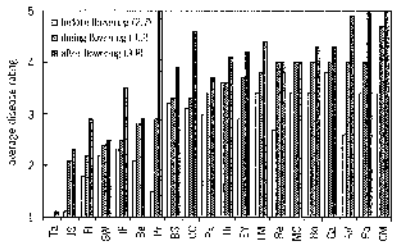

Figure 5. Disease rating after inoculation before, during and after flowering

A significant plant stage effect was found. Plants before flowering are most resistant, after flowering most sensitive. Some cultivars did not rank identically in susceptibility when different plant stages were compared. We therefore advise to inoculate at flowering stage (simple to determine) and retest the selected genotypes during the pre- and post-flowering period.

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