Preliminary study of the establishment of callus regeneration systems and its application on genetic transformation in *Lilium longiflorum* Thunb.[1]

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Summary

Two regeneration systems based on callus were established in *Lilium longiflorum* Thunb., which were utilized for propagating sufficient material for genetic transformation. The immature self-crossed ovules were cultured *in vitro* to induce callus formation. A vigorous grown callus line LG41, yellowish with friable or granular appearance, was selected thereafter on MS medium supplemented with 2,4-Dichlorophenoxy acetic acid (3.0 mg/l) and benzyl aminopurine (0.25 mg/l). The callus could be proliferated continuously. After subcultured on a medium without plant growth regulators, the callus were able to form plantlets. Besides, the filaments were cultured in vitro to induce callus, and the successful rate was 91.8%. Since it was easy to establish a callus regeneration system, the filaments were also applicable for studying gene transformation. The *Agrobacterium*-mediated transformation experiments were conducted by utilizing filaments cultured system. An *Agrobacterium*, which contains a plasmid harboring a GUS gene together with a hygromycin resistance gene, was investigated in the experiments. Young flower filaments were immersed in *Agrobacterium* solution for 10, 20, 30 minutes, respectively. The co-cultured period on a semi-solid medium were 1, 2, 3 days, respectively. The best results showed that the immersion time with *Agrobacterium* was 30 minutes. The best co-cultured period was two days. The filaments could form green calluses on the selective medium containing 40 mg/l of hygromycin, however, they turned brown 51 days after incubation. Another transformation experiment was conducted through the PDS-1000/He particle delivery system. The purified plasmid DNA molecules were extracted and coated on gold particles in advance, then the gold particles were bombarded into friable calluses. After bombardment, these callus samples were collected to do histochemical assays for GUS expression. A part of the calluses were observed with blue-staining spots, and which appeared constantly during sub-culture period. It indicated that GUS gene could be expressed in *Lilium longiflorum* callus.

(Key words: Easter lily, filament culture, callus, genetic transformation)

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