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## Production of Herbicide Resistant Creeping Bentgrass by *Agrobacterium tumefaciens*-mediated Transformation

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### Objectives

We have performed *Agrobacterium*-mediated transformation of creeping bentgrass and obtained herbicide-resistant creeping bentgrass.

### Materials and Methods

#### 1. Materials

Plant - Creeping bentgrass (*Agrostis palustris*) cv. Crenshaw and Penncross

*Agrobacterium* strain/vector - EHA105/E9 (*gus* as a reporter gene and *bar* as a selective marker)

#### 2. Methods

Embryogenic calli were induced from mature seeds on callus induction media (MS salts + 3 % sucrose + 2 mg/L 2,4-D + 0.3 % gelrite, pH 5.8), and precultured on 0.5 mg/L kinetin-containing media for 5 days before transformation. *Agrobacterium* harboring E9 vector was activated by incubating with 100  $\mu$ M acetosyringone for 4 hr at 28°C. Transformation efficiency was checked by GUS staining, and transformed calli were selected on media containing 10 mg/L PPT and 250 mg/L cefotaxime. The plantlets with well-developed roots were transferred onto soil and analyzed by BASTA painting test, genomic PCR and Southern blot analyses.

### Results and Discussion

We have produced phosphinothricin (PPT)-resistant creeping bentgrass cv. Crenshaw and Penncross by *Agrobacterium*-mediated transformation. Various factors were checked to improve the transformation conditions, such as phytohormone, pH, acetosyringone concentration, infection time, and co-cultivation period. The results showed that phytohormone is a key factor and others are minor factors. When kinetin was added into callus pre-culture media, the embryogenic calli showed good transformation efficiency about 3 folds. To confirm the putative transgenic plants, BASTA painting test, genomic PCR and Southern blot analyses were performed. Several putative transgenic plants were shown strong resistance to BASTA at the concentration of 0.8 % and genomic PCR and Southern blot analyses confirmed the presence of *bar* gene in the plant genome. Then, we have successfully established a stable genetic transformation system through *Agrobacterium* with creeping bentgrass cv. Crenshaw and Penncross. We are now introducing other genes into creeping bentgrass, such as shade-tolerant genes, stress-tolerant genes, and disease-resistant genes.