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Production of herbicide-resistant and colored creeping Bentgrass by *Agrobacterium*-mediated transformation

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Objectives

We have performed *Agrobacterium*-mediated transformation of creeping bentgrass to obtain high-valued creeping bentgrass.

Materials and Methods

1. Materials

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

Agrobacterium strain/vector - EHA105/pCambia3301 (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 vector was activated by incubating with 100 M acetosyringone for 4 hr at 28?. 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, and various molecular analysis methods.

Results and Discussion

Creeping bentgrass (*Agrostis stolonifera* L.) is the principal turfgrass species used on golf course greens and is a crop of economic importance. We have produced phosphinothricin (PPT)-resistant creeping bentgrass cv. Crenshaw and Penncross by *Agrobacterium*-mediated transformation. Various factors were checked to improve the transformation efficiency, including phytohormone, pH, acetosyringone concentration, infection time, and co-cultivation period. Results showed that the use of 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 (Average transformation efficiency~8.1%). After the transformation, the putative transgenic plants were selected from BASTA resistance test at the concentration of 0.8 %. Genomic integration of *bar* gene was confirmed by genomic PCR and Southern blot analysis, and the expression of *bar* was also validated by Northern blot analysis. Thus, we have successfully established a stable genetic transformation system through *Agrobacterium* with creeping bentgrass cv. Crenshaw and Penncross. In the presentation, the development of colored bentgrass that we have recently obtained by introducing genes in flavonoids biosynthetic pathway will also be included.

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