Transgenic plants of turfgrass (*Agrostis palustris* Huds.) from microprojectile bombardment of embryogenic callus

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ABSTRACT

Transgenic creeping bentgrass (*Agrostis palustris* Huds., cv. Penncross; Poaceae) plants have been obtained by microprojectile bombardment of and regeneration from embryogenic calli with a vector designed to deliver the β-glucuronidase (GUS) gene under the control of rice actin 1 5′ regulatory sequences. Southern analysis of polymerase chain reaction (PCR)-amplified and restriction-digested genomic DNA of four transgenic plants regenerated from these cultures showed the unscrambled integration of the gus fragment. Northern blot analysis confirmed the expression of gus mRNA in one of the transgenic plants. Western blot analysis revealed a high level of accumulation of gus protein. Histochemical assays showed enzymatic activity of β-glucuronidase in all parts of the transgenic turfgrass plant. The order of gus expression level in different tissues of the transgenic plant is as follows: stem node > first young leaf > root tip > second / third / fourth young leaf > stem internode > root hair-zone.

Abbreviation: GUS = β-glucuronidase; MS = Murashige and Skoog (1962) medium; BA = 6-benzyladenine; dicamba = 3, 6-dichloro-o-anisic acid; PCR = polymerase chain reaction.

INTRODUCTION

Creeping bentgrass (*Agrostis palustris* Huds.), the most commonly used turfgrass on golf courses in North America, is highly susceptible to the major turfgrass diseases such as *Ustilago* stripe smut, *Helminthosporium* diseases and *Rhizoctonia* brown patch (James, 1982). In addition, insects, environmental and soil stresses threaten creeping bentgrass, as well as other turfgrasses. Genetic improvement of turfgrass will therefore be of significant value to the turfgrass industry. One approach to improve the tolerance/resistance of turfgrass to diseases, insects and adverse environmental conditions is to introduce precisely and express stably corresponding resistance gene(s) in plants using genetic engineering techniques. Here we report a first important step towards such improvement, the transfer of a foreign gene in creeping bentgrass followed by the analysis of gene expression in different tissues of transgenic plants. The β-glucuronidase (GUS) gene under the control of the 5′ region of the rice actin 1 gene (*Act*; McElroy et al., 1990) was introduced via microprojectile bombardment of embryogenic calli, followed by regeneration of transgenic plants.

MATERIALS AND METHODS

Somatic Embryogenesis and Plant Regeneration. Mature seeds of ‘Penncross’ creeping bentgrass (*Agrostis palustris* Huds.) were surface sterilized and cultured on MS medium supplemented with 500 mg/l enzymatic casein hydrolysate (Sigma), 3% sucrose, 30 μM 3,6-dichloro-o-anisic acid (dicamba) and 2.25 μM 6-benzylaminopurine (BA) to induce embryogenesis (Zhong et al., 1991). Cultures were incubated at 24 ± 2 °C in the dark, and subcultured onto fresh media every eight weeks for four months. Following embryogenesis, plantlets were developed on half-strength MS basal medium under fluorescent light (60 μE m⁻² s⁻¹, 24 h) at 24 ± 2 °C. After rooting, the plants were transferred into clay pots containing a 1:1 mixture of sand:Bacto Pro Plant Mix (Michigan Peat Co.) and maintained in a greenhouse. The regenerated plants were propagated by the separation of their stolons.

Gene Transfer and Screening for Transformed Cells. About 200 mg of embryogenic calli (2-3 mm in diameter; 40-50 pieces/dish) were placed on the surface of agar medium in Petri dish one week after subculture and bombarded twice with 2 μl of 1.2 μm tungsten particles (GTE Sylvania) coated with or without pAct1-F (6.5 kb; Fig. 1; McElroy et al., 1990) DNA as previously described (Cao et al., 1990), using a Biolistic particle acceleration device (PDS 1000, DuPont) under 26 mm Hg vacuum at a distance of 12 cm from the stopping plate. Bombarded calli were cultured as described above and subcultured at 8 week intervals. GUS activity was examined in situ in embryogenic calli one month after bombardment by incubation of the samples for 24 hours in a filter-sterilized buffer (Wang et al., 1988) in the dark at room temperature. To evaluate the transient expression of the GUS gene, one-half of bombarded embryogenic calli from each sample were incubated with the GUS substrate mixture. The number of embryogenic calli with or without a blue coloration (indicative of GUS positive expression) were scored under a stereomicroscope. Embryogenic calli from samples which exhibited GUS...
DNA Isolation and PCR Analysis. Total DNA was isolated from leaf and stem tissues using the CTAB method (Rogers and Bendich, 1985). The DNA was subjected to the polymerase chain reaction (PCR) (Mullis and Faloona, 1987) using a DNA Thermal Cycler (Perkin Elmer Cetus) for 35 cycles of 2 min at 95 °C, 2 min at 65 °C and 5 min at 72 °C. The amplified DNA was electrophoresed in a 1% agarose gel, transferred to Nytran membranes and were probed with the radioactive markers (Amersham International Inc). Quantitative analysis of gus expression was carried out using a computing densitometer (Molecular Dynamics, California) to scan the filters and compare the amounts of purified β-glucuronidase.

In Situ Histochemical Assay for GUS Activity in Transgenic Plants. Tissues from transgenic and control plants were stained for GUS activity as described above. Green tissues were incubated in 75% ethanol for one hour before pictures were taken. Photomicrographs were taken using Kodak Ektachrome 160 tungsten film and a ZEISS SV8 stereomicroscope or a ZEISS Axioskop routine microscope.

RESULTS
Gene Transfer and Regeneration of Putatively Transformed Plants. A preliminary study to determine the best stage of callus growth for DNA delivery showed that embryogenic calli from one-week old subcultures gave the highest gus expression (data not shown). Therefore, embryogenic calli used in all subsequent experiments were grown for one week on MS medium containing 2,250 µM BA and 30 µM dicamba before being bombarded with pAct1-F (Fig. 1) plasmid DNA. Half of each sample from 22 DNA-bombarded and 3 control (bombarded with uncoated tungsten particles) calli in five independent experiments was assayed for GUS activity. Nineteen bombarded