IMPROVEMENT OF FORAGE QUALITY BY MEANS OF BIOTECHNOLOGY: STABLE TRANSFORMATION OF WARM-SEASON GRASSES BY PARTICLE BOMBARDMENT

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ABSTRACT
We have used a simple and inexpensive, self-built particle acceleration apparatus for direct delivery of DNA to cultured cells of warm-season grasses. High levels of transient expression of the b-glucuronidase gene were obtained following bombardment of embryogenic suspension cells and calli of bahiagrass (*Paspalum notatum* Flügge) and dallisgrass (*Paspalum dilatatum* Poir). Furthermore, stable transformed calli of both species have been obtained using this simple particle gun.

KEYWORDS
Bahiagrass, Dallisgrass, Embryogenic suspension culture, Particle bombardment, Stable transformation, Transient expression

INTRODUCTION
Particle bombardment has become a popular technique in transient gene expression and stable transformation studies in plants. The physical nature of DNA introduction permits reliable and effective gene transfer to major agronomic monocotyledonous species. However, full exploration of particle gun technology is still limited due to high cost of the equipment and the complexity of the method. The recent introduction of an inexpensive and simple acceleration device that is easy to build and operate should greatly further the application of this technology.

In the following, we describe the utilization of such a simple system in the transformation of two warm-season grass species by optimizing gun pressure and target distance.

MATERIALS AND METHODS
The particle bombardment apparatus was constructed as described by Finer et al. (1992).

Embryogenic cell suspensions and calli of *Paspalum notatum* (bahiagrass) and *P. dilatatum* (dallisgrass) were obtained as previously reported (Akashi and Adachi 1993a, 1993b, 1994). Suspended cells of tobacco (*N. tabacum* L. cv. Bright Yellow-2, kindly provided by Dr. Shibata) served as controls. Prior to bombardment, cell suspensions of dallisgrass and tobacco (ca. 300 ml PCV) were placed on 35 mm filter paper and the medium removed by vacuum filtration. A single shot with DNA-coated gold particles was applied to the cells at various acceleration pressures under vacuum pressure greater than 10 mm Hg. In bahiagrass, embryogenic calli were planted in the center of 90 mm petri dishes containing solid MS medium supplemented with 2 mg/l 2,4-D. Two DNA constructs were used in our transformation experiments. The plasmid pSLG2 (kindly provided by Dr. Shibata) carries the lipogenase coding region, placed under the control of the CaMV 35S promoter and the nopalin synthase (*nos*) terminator (Kato et al., 1991). The plasmid p*Act*1-F (kindly provided by Dr. Wu) contains the GUS reporter gene under the control of the 1.3 kb 5' region of the rice actin 1 gene (*Act*1) and *nos* terminator (McElroy et al., 1990). Following bombardment, all suspensions were incubated at 27°C for 2 days and assayed for GUS activity. For the *in situ* assay, staining was according to Jefferson et al., (1987).

RESULTS AND DISCUSSION
First, we investigated the effect of helium pressure on delivery and transient gene expression in suspended cells for both plasmids. In dallisgrass, maximum pressure (5.0 kg cm^{-2}) resulted in highest GUS expression when p*Act*1-F was used while for pSLG2, 2.5 kg cm^{-2} turned out to be optimal. For tobacco cells, however, medium pressure (2.5 kg cm^{-2}) was found to be best for both plasmids. Next, we investigated the effect of target distance by using the p*Act*1-F plasmid. For both grass species, 12.5 cm target distance resulted in highest GUS expression. Stable transformed calli of bahiagrass as well as dallisgrass have been obtained under these experimental conditions, originally selected for best transient GUS expression. Using the optimized values for pressure and distance, experiments for the production and selection of herbicide-resistant calli are in progress in both warm-season grass species .

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