

Hybrid Bermudagrass Improvement by Genetic Transformation

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Executive Summary

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The ultimate goal of this research direction is to improve bermudagrass cultivars for the golf courses through biotechnology. The specific goals of the project include: to optimize tissue culture conditions for inducing embryogenic calli and regenerating plantlets of bermudagrass; to develop procedure to transform the embryogenic calli by the biolistic method and to recover transgenic plants, and to obtain transgenic plants of bermudagrass that express potential nematode resistant genes. Bermudagrass is a recalcitrant species for plant tissue culture. Thus, so far most of the efforts have been concentrated on optimizing tissue culture conditions, especially at the callus induction stage to improve the callus quality and the regeneration ability.

Various tissues, culture media and supplements to the media have been tested in order to optimize tissue culture conditions of bermudagrass. It was found that approximately 20% of the calli induced from young inflorescence (0.5 to 1 cm) of 'Tifgreen' and 'Savannah' (a common bermudagrass cultivar) had an embryogenic structure when cultured in MS medium (1 mg/L 2,4-D) supplemented with 0.01 mg/L 6-benzylaminopurine (BAP). No such structure were found in media without BAP. The calli were slow-growing, compact, pale or off-white in color, and highly regenerable. The regeneration rate of the calli with embryogenic structure was higher than 50% while calli without this structure had the regeneration rate about 1-5%. In addition, the callus induction rate was raised from 21-33% to over 60% by excising young inflorescence into pieces before the culture inoculation.

It was very difficult to induce callus from young inflorescence of 'Tifway' due to the quick browning of the explants in culture medium. The situation can be improved by pretreatment of explants with 0.2% ascorbic acid, an anti-oxidant.

Pilot experiments were performed to determine the pressure parameter of the biolistic apparatus. It was found by transient assay of GUS reporter gene that bombardment at 1550 psi on osmotically treated calli were the best for transformation experiment.

Callus growth inhibition assays were performed with three potential selection agents at various levels. It was found that 250 mg/L was an effective selection level for both antibiotics, kanamycin and hygromycin B, while 5 mg/L was appropriate for selection with bialaphos, an herbicide.

Hybrid bermudagrass (*Cynodon transvaalensis* x *C. dactylon*) is an important warm-season grass species for the greens, tees and fairways of golf courses in the South of the United States. The ultimate goal of the research direction is to improve bermudagrass cultivars for the golf courses through biotechnology. The specific goals of this project are as the following:

1. To develop and optimize tissue culture conditions in order to obtain embryogenic calli and to regenerate plantlets of bermudagrass.
2. To develop procedure to transform the embryogenic calli by the biolistic (particle bombardment) method and to recover transgenic plants.
3. To obtain transgenic plants of bermudagrass that express potential nematode resistant genes.

This project was initiated in 1997 and has been supported by a USGA grant since July 1, 1998. So far most of the efforts have been concentrated on optimizing tissue culture conditions, especially at the callus induction stage to improve the regeneration ability of the calli. In addition, some pilot experiments have been carried out for developing transformation procedures for bermudagrass plants.

I. Optimizing Tissue Culture Conditions

A. Culture of vegetative tissues of 'Tifgreen' and 'Tifway'

Vegetative tissues of bermudagrass are available year-round from the greenhouse and the field, and thus were tested first for their tissue culture response. Among the tissues tested in pilot experiments of the two major hybrid bermudagrass cultivars, 'Tifgreen' and 'Tifway', nodal segments had the best tissue culture response: over 90% of the nodal segments produced calli in callus induction media. Calli grew fast, looked white or yellowish and 'fluffy', and were not regenerable (Fig. 1a). In winter of 1997 and spring of 1998, a large scale experiment was carried out trying to improve the quality of the calli from nodal segments and their regeneration ability. The media tested were MS, NB, CC and a MS medium supplemented with casein hydrolysate (CH, 1 g/L) and proline (1.16 g/L). Auxins, including 2,4-D, dicamba, NAA, and picloram were tested at various levels. Various level of sucrose were compared. Supplements to callus induction media tested included abscisic acid (ABA), 6-benzylaminopurine (BAP), thidiazuron (TDZ), CH, tryptophan, coconut milk and activated charcoal. It seemed that 1 mg/L 2,4-D with addition of CH had good callus induction. Addition of ABA or BAP reduced the growth rate of callus and thus form more compact calli. Various regeneration media were tested. Over 2,000 explants were cultured and plants were regenerated from only one piece of callus.

In addition, it was attempted to expose the apical meristem tissues to culture medium. Non-regenerable calli similar to the ones from nodal segments were observed. However, it is not clear whether the calli were from meristem tissue or from the surrounding tissues.

B. Culture of young inflorescences of 'Tifgreen' and common bermudagrass

Culture of young inflorescence (0.5 - 1 cm) of 'Tifgreen' was first performed in the summer of 1997 with very limited success. Most of the calli grew fast and still looked quite

'fluffy'. It was observed that calli grew slower at lower auxin level and that may help formation of more compact calli which may be more regenerable. In one experiment using MS medium supplemented with 200 mg/L CH, 2 out of 47 calli produced regenerated plantlets from the callus induction medium containing 1 mg/L 2,4-D whereas none regenerated plant was obtained from 46 calli in the medium of 2 mg/L 2,4-D.

In 1998, BAP, ABA or PVPP (polyvinylpyrrolidone) were added to callus induction medium containing 1 mg/L 2,4-D to compare their effects on callus induction. Among those, 0.01 mg/L BAP had a dramatic effect on inducing embryogenic calli which were very slow-growing, compact, pale or off-white in color, and were recently proven to be highly regenerable (Fig. 1 b-d). Approximately 20% of calli of 'Tifgreen' and a common bermudagrass cultivar 'Savannah' had this structure when cultured in medium containing BAP while none such callus was observed when BAP was absent (Table 1).

Table 1. BAP effect on formation of a regenerable structure in bermudagrass young inflorescence culture (MS medium with 1 mg/L 2,4-D)

Cultivar	BAP (mg/L)	Explant No.	Induced calli No.	Calli with Embryogenic structure
Savannah	0	71	45 (63.4%)	0
	0.01	72	52 (72.2%)	10 (19.2%)
Tifgreen	0	543	350 (64.5%)	0
	0.01	569	388 (68.2%)	82 (21.1%)

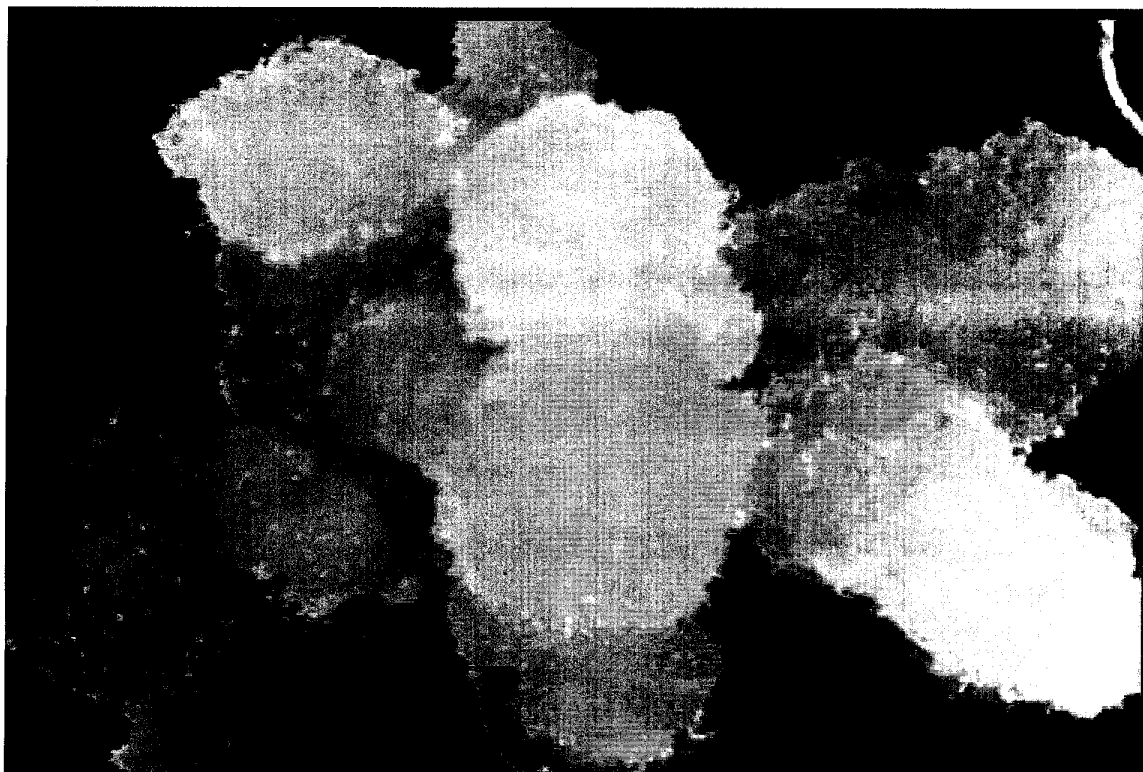
It has been observed that the embryogenic structure is highly associated with the regeneration ability. After four wk in regeneration medium (MS plus 2.5 mg/L BAP), regenerated plantlets can be seen from approximately 50% of such calli although the process was relatively slow. The rate can go up to 80% if younger inflorescences (about 0.5 cm) were used. On the other hand, the regeneration rate of calli without the clear embryogenic structure was only 1-5%.

In 1998, the callus induction rate was also improved from 21% ('Savannah') and 33% ('Tifgreen') to over 60% by excising a young inflorescence into 2 or 3 pieces rather than to culture it as a whole piece.

Fig. 1 Callus morphology and regeneration of bermudagrass

- a. A non-embryogenic, 'fluffy' callus of bermudagrass.
- b. A compact, embryogenic structure formed at the top of a callus cultured in the medium containing 0.01 mg/L BAP.
- c. An embryogenic callus started regeneration on the regeneration medium (MS, 2.5 mg/L BAP). Note the shoot formation surrounding the callus.
- d. Plantlets regenerated from an embryogenic callus.

1a



1b

