DIFFERENTIATION PATTERNS IN INFLORESCENCE CULTURES OF EUROPEAN SLOUGHGRASS (*BECKMANNIA ERUCIFORMIS* (L.) HOST)

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ABSTRACT

The objectives of this research were to investigate patterns of differentiation and to regenerate plants in callus cultures of young inflorescences of European sloughgrass. Embryonic calli were proliferated from inflorescence segments inoculated on RM medium with 5 mg 2,4-D per L. These calli differentiated profusely into plants on RM medium supplemented with 0.1 mg benzylaminopurine. Plantlets sporadically evolved directly from explants on medium with 2 to 5 mg 2,4-D per L or 5 mg NAA per L, while rooty calli initiated on RM medium with 2 or 5 mg NAA per L. Plant regeneration was completed after the cultures were transferred onto hormone-free RM basal medium. The regenerated plants grew to maturity in the greenhouse.

INTRODUCTION

European sloughgrass (*Beckmannia eruciformis* (L.) Host) is a perennial, rhizomatous, creeping grass adapted to wet meadows and river banks. It produces satisfactory yields of hay, has good regrowth characteristics, and approaches foxtail (*Alopecurus pratensis*) and timothy (*Phleum pratense*) in forage quality.

The objectives of this research were to investigate patterns of differentiation and to regenerate plants from callus cultures of young inflorescences of this species.

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MATERIALS AND METHODS

A Turkish accession (P.I.#383067) of European sloughgrass obtained from the Regional Plant Induction Station at Experiment, Georgia, was used in this research. This accession was propagated in a field nursery at Brookings, S. D. Tillers containing young unemerged inflorescences were collected prior to emergence of the flag leaf and were used as explants.

Linsmaier and Skoog's RM basal medium (Linsmaier and Skoog, 1965) supplemented with 0, 1, 2, and 5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthaleneacetic acid (NAA) was used for callus induction. RM medium containing 0, 0.1, or 1 mg/L benzylaminopurine (BAP) was prepared for testing morphogenetic quality of the calli. All media were adjusted to pH 5.7 and solidified with 8 g agar per L. Ten mL of the medium, after being autoclaved, were dispensed aseptically into individual 60 X 15 mm culture dishes, which were used for primary culture, callus maintenance and differentiation. For plant regeneration, 40 mL of hormone-free RM basal medium were dispensed into 125-mL Erlenmeyer flasks which were covered with a double-layered aluminum foil and then autoclaved for 15 min. Methods of culturing were essentially the same as described by Chen and Boe (1988) and Chen et al. (1988).

Calli were sampled from morphogenetic medium at 5-day intervals for 25 days. The calli were fixed in Nawaschin fluid for 48 hr., dehydrated in an ethanol/butanol series, and then embedded in paraffin blocks. Specimens were serially sectioned at a thickness of 8 μm and stained with a safranin-fast green combination. Detailed procedures of histological studies were described by Sass (1964).

RESULTS AND DISCUSSION

Response of European sloughgrass primary cultures to RM medium supplemented with 0 to 5 mg 2,4-D or NAA per L are shown in Table 1. Few or no calli were initiated from explants cultured on RM medium containing 2,4-D or NAA at concentration lower than 2 mg/L. Direct plant regeneration sporadically occurred in explants cultured on RM medium containing 2 to 5 mg 2,4-D per L or 5 mg NAA per L. This might have resulted from de-repression of presumptive shoot primordia carried over from explants, as a shoot primordium was histologically observed in the later stage of a primary culture on RM medium with 5 mg 2,4-D per L (Figure 1). Microtillering has been reported in many instances in primary and secondary cultures of gramineous plants (Chen et al., 1982; Chen et al., 1988; Morrish et al., 1987). Since most calli initiated from RM medium containing 2 or 5 mg NAA per L were rooty, calli isolated from primary cultures grown on RM medium supplemented with 2 mg and

5 mg 2,4-D per L were transferred onto RM basal medium for morphogenetic induction. Plant regenerability on the morphogenetic medium was much higher in calli isolated from RM medium with 5 mg than with 2 mg 2,4-D per L (Table 2). An improvement of regenerability was obtained by growing calli on RM medium containing 0.1 mg BAP per L (Table 3).

Table 1. Differences in response of European sloughgrass inflorescence cultured on RM medium supplemented with 2,4-D or NAA.

	No. dishes cultured	No. calli initiated	No. plantlets regenerated
2,4-D (mg/L)			
5	36	85	4
2	11	17	12
1	11	0	0
0	11	0	0
NAA (mg/L)			
5	11	19	15
2	11	7	0
1	11	0	0

Table 2. Effects of 2,4-D concentration in callus initiation medium on shoot differentiation in an European sloughgrass callus cultured on morphogenetic medium.

2,4-D concentration in callus initiation medium (mg/L)	Average number of plants per dish in morphgenetic medium
5	18.6
2	4.8

Table 3. Shoot differentiation in European sloughgrass calli grown on RM medium supplemented with three levels of BAP.

	BAP concentration (mg/L)		
	0	0.1	1
Shoot counts in 8 flasks	44	136	84
Average	5.5	17.0	10.5
Range	1-13	7-31	3-19

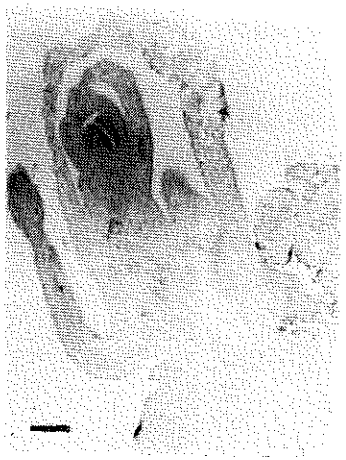

Figure 1. (Above) A shoot primordium developing in primary culture. Scale is 100 μm.

In serial sections of opaque calli sampled at the end of primary culturing on RM medium supplemented with 5 mg 2,4-D per L, meristematic cell masses with large nuclei and dense cytoplasm were observed (Figure 2A). Transfer of the callus onto morphogenetic medium induced differentiation. The cell mass appeared to differentiate into heart-shaped embryoid after the callus was subcultured for 10 days (Figure 2B). A young embryoid complete with scutellum-like structure was found in a callus sampled 15 days after subculture (Figure 2C). Thus, both organogenesis and embryogenesis occurred in the European sloughgrass inflorescence cultures.

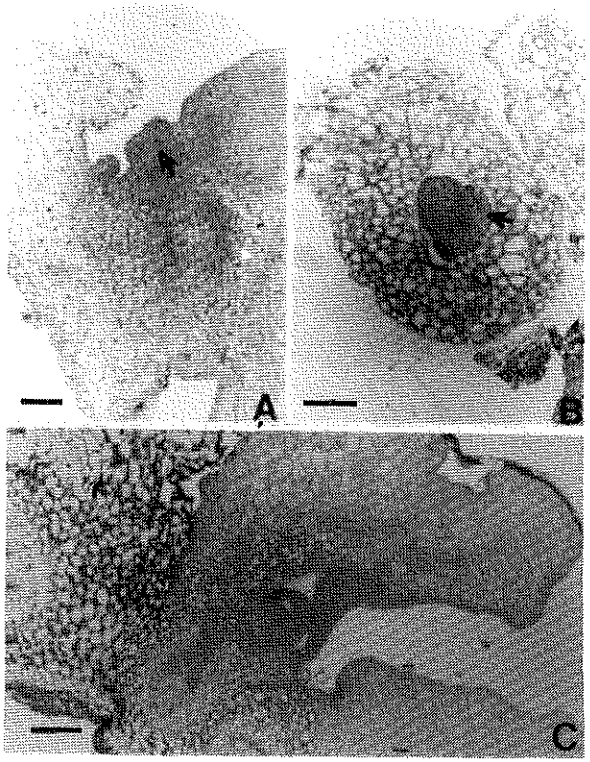

Figure 2. (Right) Embryoid development in calli. (A) Meristematic cell mass (Arrow). (B) Heart stage (Arrow). (C) A developed embryoid. Scale is 100 μm.

Plant regeneration was completed after the differentiating calli were grown on hormone-free RM medium. The plants grew to maturity in the greenhouse (Figure 3).

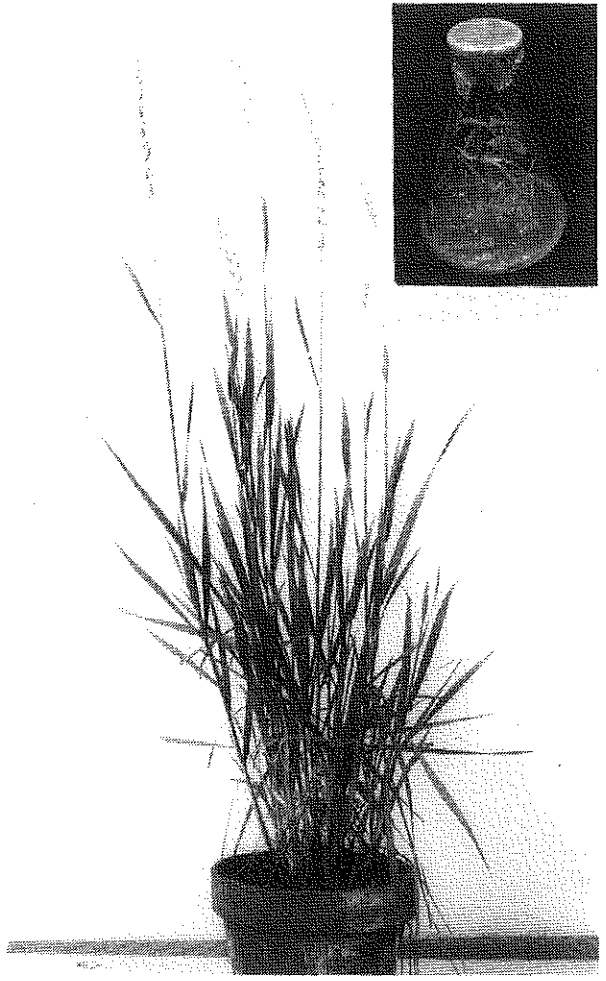

Figure 3. A mature plant derived from inflorescence culture (Inset).

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