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Plant regeneration by somatic embryogenesis from callus cultures of sweet sorghum

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ABSTRACT

Tissue culture methods were developed for the induction, maintenance, and regeneration of embryogenic callus in sweet sorghum (Sorghum bicolor) cultivars Keller, Rio, and Wray. No significant differences were observed in production of embryogenic callus in cultures established from developmentally immature or mature embryo explants cultured on LS medium with 2 mg/l 2,4-D plus 0.5 mg/l kinetin. Prolific callus production did not occur until the third four-week culture period. Long-term maintenance of embryogenic callus was dependent upon the selective transfer of embryogenic callus, with other callus types discarded. High-frequency plant regeneration was achieved and quantified on a fresh weight basis of embryogenic callus.

ABBREVIATIONS

2,4-D = 2,4-dichlorophenoxyacetic acid, BA = benzyladenine, IAA = indoleacetic acid, IBA = indolebutyric acid, LS = Linsmaier and Skoog basal medium (Linsmaier and Skoog, 1965).

INTRODUCTION

The development of tissue culture methods for the reliable induction, and maintenance of callus, with regeneration of plants, is imperative for the successful application of tissue culture to crop improvement. Conventionally, developmentally immature tissues, such as immature embryos, have been the explants of choice because embryogenic callus is readily induced. However, growing cereal crops such as sorghum to the flowering stage in a glasshouse or growth chamber can be a space and labor consuming task. Methods which employ mature caryopses as explants would add considerable convenience to tissue culture investigations.

Sorghum is an important crop which has special agronomic importance because of its multi-product usage as food, fiber, feed, and fuel (Miller and Creelman, 1980). An emerging fuel product of sweet sorghum is biogas, produced from the anaerobic digestion of the sugar residues present in the grain and stem. Sweet sorghums are especially suited for gas production because of the large accumulation of sucrose in the stem.

Several reports have been made on the in vitro culture and regeneration of grain sorghum (Smith et al., 1983), Bhaskaran et al., 1983, review by Tomes, 1985). The limitations of this technology are the inconsistent production of embryogenic callus, especially from developmentally mature explants, and the lack of reliable, long-term whole plant regeneration. No reports for sweet sorghums have been made on whole plant regeneration from embryogenic callus cultures. This paper describes the culturability of some sweet sorghums, and outlines methods for the consistent production of callus from developmentally mature embryos with capability for plant regeneration

MATERIALS AND METHODS

The cultivars Keller, Rio, and Wray of Sorghum bicolor (L.) Moench were generously supplied by Dr. Fred R. Miller, Texas A & M, College Station, TX. Opaque immature embryos (1-1.5 mm length) were collected from greenhouse grown plants (temperate conditions, natural light) ca. 21 days after inflorescence emergence, when the endosperm was between the milk and dough stage.

Dried mature seeds were surface sterilized with a rinse of 95% ethanol, followed by a 20-25 minute wash with vigorous stirring using a magnetic stirrer, under vacuum in a solution of 20% Chlorox plus 2-3 drops Tween 20. Under sterile conditions, seeds were rinsed six times with sterile distilled water, and soaked 20-24 hrs in sterile distilled water. Immature caryopses were sterilized as above, with the overnight soak in sterile water omitted.

Embryos were dissected out of the imbibed seeds; mature embryos were placed on the medium randomly with respect to the orientation of the scutellum, while immature embryos were planted with the scutellum facing away from the medium. The standard callus initiation and maintenance medium contained LS salts and vitamins (Linsmaier and Skoog, 1965), 4% sucrose, 1% agar, 2 mg/l 2,4-D plus 0.5 mg/l kinetin, and pH at 5.5 prior to autoclaving.

After the initiation passage, each subculture was inoculated with 0.2 gm of callus. At the beginning of second passage, all callus types were transferred. Thereafter, visual selection of the callus was made, and 80% or more of the callus transferred was embryogenic. Weights of embryogenic callus from individual cultures were not determined. Only total embryogenic weights per treatment per passage were obtained in order to reduce time, labor, and contamination. Whole plant regeneration was achieved in two stages. Embryogenic callus (0.1 gm)

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was inoculated on 20 ml of regeneration medium which was composed of LS plus 1 mg/l IAA and 0.5 mg/l BA. Regenerated shoots were transferred to a rooting medium of LS plus 3 mg/l IBA. Callus and regeneration cultures were incubated at 25°C, under continuous light of ca. 2000 lux, and subcultured at four week intervals. The total number of regenerates was scored at the end of a specified four week passage on rooting medium. Green shoot(s) with well developed root systems, that appeared as a discrete transplantable entity, was scored as one plant.

Assessment of culturability in the three cultivars was made by initially testing both immature and mature embryos on four media (1 or 2 mg/l 2,4-D, each in combination with 0.5 mg/l kinetin, or 1 mg/l BA) in continuous light or dark.

RESULTS AND DISCUSSION

For both explant sources in all cultivars, callus production was low (generally less than 0.05 gm total callus per embryo) during the first culture passage regardless of medium or light treatment.

The major callus type produced was a watery, friable nonembryogenic callus which often exuded a reddish-orange pigment. A creamy white, compact-nodular callus was also produced, and was identified as embryogenic (E) based on its gross morphological appearance (Figures 1 and 2).

This callus type, produced by both immature and mature embryos, resembled callus recognized as embryogenic in grain sorghum (Wernicke and Brettel 1980), and other cereals (Nabors et al., 1983). Rarely, a dark green, smooth-compact callus was produced which was also competent for somatic embryogenesis.

The percentage of cultures yielding E callus during the first and second passages was very low although callus quantity increased during the thi and fourth passages with visual selection of E ca (Table 1). Media composition, or light versus da conditions, did not influence the percentage of cultures with E callus (data not shown). Callus subcultured on 1 mg/l 2,4-D did not increase in fresh weight (from a total of 4.2 gm at second passage to 3.6 gm at third passage). However, ca subcultured on 2 mg/l 2,4-D doubled in fresh weig (from a total of 4.6 gm at second passage to 9.0 at third passage). It was difficult to assess cultivar (genotypic) differences in E callus prod tion since neither donor plants, nor callus cultu were grown under identical environmental conditio

In separate experiments, long-term productio and maintenance of callus was assessed in Keller and Rio cultures established from mature embryos (Table 2). During the first and second passages the frequency of cultures with E callus, again, w low. However, these few cultures produced large quantities (more than 5 gms) of E callus during t

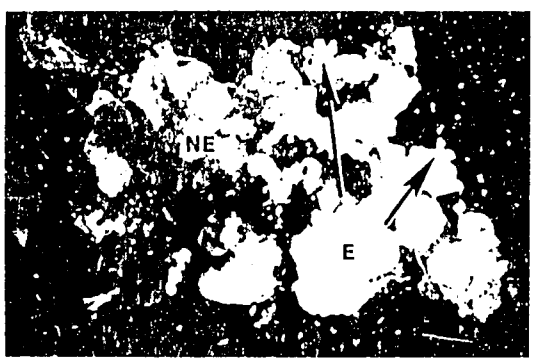

Figure 1. A typical callus mass (at third passage) containing embryogenic (E) and nonembryogenic (NE) regions. Arrows point to small embryoid clusters. Magnification ca. 12x.

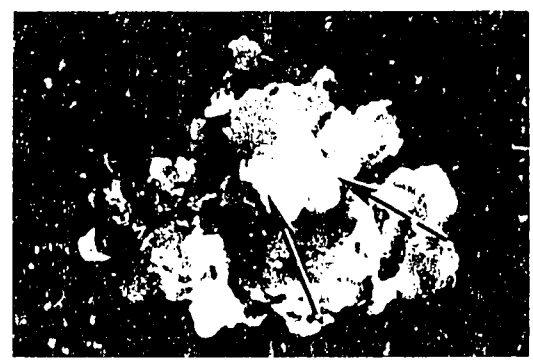

Figure 2. An embryogenic callus mass (several passages old) with embryoids (arrows) at different developmental stages. Magnification ca. 19x.

Table 1. Production of embryogenic (E) callus from cultures established from immature and mature embryo explants.

Cultivar	Cultures with E callus per total cultures		Total E callus (gm) from all cultures	
	Passage 1	2	3	4
Immature Embryo Derived				
Keller	8/180 (0.1 gm)[a]	8/19 (0.4 gm)[a]	0.9	1.8
Rio	6/80 (0.1)[a]	4/7 (0.2 gm)[a]	(terminated)	---
Wray	0/80	2/15 (0.05 gm)[a]	0.2	1.3
Mature Embryo Derived				
Keller	3/143 (0.05 gm)[a]	6/23 (0.4 gm)[a]	0.9	2.6
Rio	0/64	0/15	(terminated)	---
Wray	1/67 (0.01 gm)[a]	1/14 (0.01 gm)[a]	0.3	1.1

[a]total weight of E callus from all embryos

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next several subcultures, provided that E callus was selectively transferred, and other callus types discarded. With our seed source, Rio appeared to be better for E callus production.

Table 2. Total Production of embryogenic callus over time (callus established from developmentally mature embryos).

Passage	Keller	Rio
1[a]	0/400[a]	27/600[a]
2	26/144[a]	19/63[a]
3	0.28 gm	4.5 gm
4	0.8 gm	11.0 gm
5	2.0 gm	17.0 gm
6	2.4 gm	10.6 gm[b]
7	6.6 gm	6.0 gm[b]

[a]cultures with embryogenic callus per total cultures. Total embryogenic amount less than 0.2 gm.

[b]decrease in callus quantity due to high temperature on culture shelf, and 6 week culture interval (apparent phenolic build-up inhibited growth).

The regeneration capacity of the E callus six to seven months old was tested on a medium that had been determined to be optimal for grain sorghum regeneration. In about 100% of the cultures, at least one plant was produced from the E callus. No large differences were observed in plant regeneration from E callus established from either immature or mature embryo explants (Table 3).

Table 3. Regeneration of plants from embryogenic (E) callus derived from immature and mature embryo explants. Callus was first cultured on regeneration medium of LS plus 1 mg/l IAA and 0.5 mg/l BA. Regenerated shoots were then transferred to LS plus 3 mg/l IBA for one passage, and then scored.

Cultivar	Passage	Total plants/ total gm E callus	Plants/gm
Immature Embryo Derived			
Keller	6	83/1.05	79
Wray	6	832/5.2	160
Mature Embryo Derived			
Keller	6	149/0.7	213
Rio	7	175/1.4	125
Wray	6	196/2.3	85

Regenerates were potted in a soil-less mix, and were quickly established in the greenhouse (Figure 3). Seed set of regenerates was comparable to that set by seed-sown plants grown under greenhouse conditions.

Results obtained with sweet sorghums are consistent with that obtained with grain sorghums (MacKinnon et. al., 1986). The E callus type is similar in both sorghums, and several subcultures are required for enhanced production. Regeneration of plants from E callus of grain sorghum is also readily achieved, although a second subculture on IBA-containing medium is necessary for vigorous rooting. The only major difference observed in callus cultures of sweet and grain sorghum is the color of the pigment exuded into the medium; sweet sorghum callus produces a reddish-orange pigment, while grain sorghum callus exudes a purplish-black pigment.

Figure 3. Sweet sorghum regenerates established in a glasshouse.

In conclusion, it was determined that the key factors for obtaining highly regenerable callus in sweet sorghums were: (1) culturing isolated mature or immature embryos on 2,4-D containing medium for at least two four-week passages, (2) identification of the compact-nodular callus, and (3) selective transfer of the compact callus to ensure maximum growth. While the production of embryogenic callus initially is low, recurrent subculture of this callus type results in increased production over time.

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