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ENTER INFORMATION ONLY IF NOT INCLUDED ON COVER OR TITLE PAGE OF DOCUMENT

1. Project/Subproject Number
931-1254

2. Contract/Grant Number
DAN-1254-G-SS-5065-00

3. Publication Date
1987 February

4. Document Title/Translated Title
Callus induction and plant regeneration from shoot portions of mature embryos of high tannin sorghums

5. Author(s)
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6. Contributing Organization(s)
USAID INTSORMIL grant PRF-4B

7. Pagination
10p.

8. Report Number
Journal Paper No. 10946

9. Sponsoring A.I.D. Office
S&T Bureau

10. Abstract (optional - 250 word limit)

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11. Subject Keywords (optional)
1. cereal tissue culture
2. plant regeneration
3. Sorghum bicolor
4.
5.
6.

12. Supplementary Notes
Plant Cell, Tissue and Organ Culture 9: 245-252 (1987)

13. Submitting Official
John M. Yohe, Director-INTSORMIL

14. Telephone Number
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16. DOCID

17. Document Disposition
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WORK SHEET

Plant Cell, Tissue and Organ Culture 9: 245–252 (1987)
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Short communication

Callus induction and plant regeneration from shoot portions of mature embryos of high tannin sorghums

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Received 7 November 1986; accepted in revised form 24 February 1987

Key words: cereal tissue culture, plant regeneration, *Sorghum bicolor*

Abstract. Callus and plant regenertion were induced from shoot portions of mature embryos (dry seeds) of five high tannin *Sorghum bicolor* (L.) Moench cultivars. The explants were cultured on Murashige and Skoog medium with altered concentrations of 5 salts, supplemented with 150 mg/L L-asparagine, 5 mg/L 2,4-Dichlorophenoxyacetic acid and 0.05 mg/L kinetin. Calli which were yellow and globular were formed with 70–90% frequencies. The subculture medium which gave best results was MS with 2 mg/L 2,4-Dichlorophenoxyacetic acid and 0.5 mg/L kinetin. Plants were regenerated on MS medium supplemented with 150 mg/L L-asparagine and 0.2 mg/L kinetin with regeneration frequencies of 11–48%.

Abbreviations. 2,4-D: dichlorophenoxyacetic acid

Introduction

Callus induction and plant regeneration in sorghum (*Sorghum bicolor* L. Moench) was first reported from cultures of seedling tissue by Masteller and Holden [1]. Subsequent reports have confirmed the use of seedlings [2, 3] and extended the source of the callus to immature embryos [2, 4–7], scutellum of mature embryos [5], inflorescences [8], leaves [9], germinating seeds [10] and anthers [11]. Shoot parts of the mature embryo (from dry seeds) have not been reported as a source of explants. Although immature embryos and young inflorescences are the most readily cultured explants in the cereals, they must be obtained from field or grenhouse grown plants during a limited period. Mature embryos therefore offer a great advantage for readily obtaining explants. Moreover, the procedure for induction of callus from mature embryos is more convenient than from seedlings. A few examples of mature embryos used as explants for callus culture in cereals have been reported. Olien et al. [12] established a procedure for initiation of callus from rye and barley embryo pieces, and plants have been regenerated from the calli

derived from mature embryos of pearl [13] and Hiproly [14] barley. We now report induction of calli and plant regeneration from shoot portions of mature sorghum embryos.

Materials and methods

Because of our continuing interest in the nutritional [15] and agronomic [16] characteristics of high tannin sorghums, as well as the effects of possible synthesis [17] of tannins in cultured cells, cultivars selected for this investigation all contain relatively high amounts of tannins in the grain, and most of the cultivars are also relatively rich in total phenols, protein precipitable phenols, flavan-4-ol monomers, and pigments (Table 1) [18]. Included in Table 1 for comparison are examples of well-known high and low tannin sorghums. We have observed that high tannin sorghums have a propensity to produce large amounts of toxic phenolics in culture and are especially difficult to culture as callus in comparison to low tannin sorghums. The selected cultivars represent a variety of agronomic types.

Sorghum grain from open-pollinated heads was produced at the Purdue Agronomy Farm, West Lafayette, Indiana, under the supervision of Dr. John Axtell. Grain was stored for 2–4 years at room temperature before use. Seeds selected for absence of damage and for cleanliness were surface-sterilized in 70% ethanol and 50% commercial bleach for 2 and 20 minutes respectively, followed by washing, then soaked in sterile distilled water and/or placed in a dish with wet filter paper (Whatman No. 1) up to 24 hr. At the stage where the coleoptiles just break through the seed coat, sprout tops or shoot portions of the embryo (1–1.5 mm in size) were excised by either peeling off the coleoptile or bisecting the shoot longitudinally into two pieces. The explants were plated on agar-solidified medium and incubated in the dark for callus initiation. The induction medium contained the inorganic, vitamin, and amino acid components of Murashige and Skoog's medium [19] but the concentrations of some salts were changed to (in mg/L) 880 calcium chloride $2 \cdot H_2O$, 41.775 ferrous sulfate, 55.95 disodium EDTA, 29.43 manganese sulfate $4 \cdot H_2O$, 9.0 zinc sulfate $7 \cdot H_2O$, and was supplemented with 150 mg/L L-asparagine, 5 mg/L 2,4-D, 0.05 mg/L kinetin and 15 g/L sucrose. After 3 to 4 weeks, cultures were transferred to weak diffuse light (about 500 Lux) and were transferred at 20–30 day intervals. The subculture medium was the same as MS medium [19] but with 2 or 3 mg/L 2,4-D, 0.1 or 0.5 mg/L kinetin or 10% (v/v) coconut water and 30 g/L sucrose. Plant regeneration was conducted with a 14-hour day (light provided by Sylvania white F40 fluorescent tubes, 1,500–2,000 lux) and

Table 1. Polyphenol analyses of sorghum cultivars studied.[a]

Cultivar	Group	Seed color	Testa color	Pigment A_{510}	Tannin assays			F-4-ols A_{550}	Total phenols Klett units
					Vanillin A_{510}	Proanthocyanidins A_{550}	Protein precipitable phenols A_{510}		
IS2830	III	Brown	Brown	0.04	0.51	0.73	0.08	0.028	94
IS3150	III	Brown	Brown	0.51	1.18	1.74	0.422	0.84	324
IS4225	III	Brown	Brown	0.67	1.38	1.42	0.198	0.485	188
IS6881	III	Buff	Brown	0.12	1.9	2.4	0.52	0.11	390
IS8260	III	Reddish Brown	Brown	0.86	5+	6+	0.32	0.85	518
IS8768	II	Gray	Purple	0.09	1.03	1.16	0.29	0.037	235
Standard high tannin line for comparison									
BR64	III	Brown	Brown	0.14	1.45	3.12	0.475	0.19	372
Standard low tannin line for comparison									
IS0469	I	Lemon Yellow	None	0	0	0	0.02	0	17

[a]For definitions of groups and for assay methods see [18].

10-hour night photoperiod after 1–2 subculture times. Temperatures for the whole process were kept between 25–27 C. The regeneration medium was also MS supplemented with 150 mg/L-asparagine, 0.1 or 0.2 mg/L kinetin, 0 or 5 mg/L NAA, and with no 2,4-D. The media were adjusted to pH 5.7 with 0.1N NaOH and autoclaved for 15 min at a pressure of 1.5 kg/cm^2.

Results

About 7–10 days after inoculation, callus formed on the swelled shoot parts of the mature embryo, even on the blackened explants taken from seeds containing high levels of tannins and/or pigments (Fig. 1). The calli were yellow and globular. Based on a minimum of 100 explants for each cultivar, the callus induction frequencies ranged from 70–90% (Table 2) except for IS8260 which produced much more purple pigment. Culturing the mesocotyl and root sections of mature embryos taken from the same seed lots gave no yellow callus; the explants swelled and then blackened, producing only very small dark slow growing callus which eventually died. During the subculture stage callus proliferated well (Fig. 2) although genotypic differences were apparent. For example, the callus from IS4225 grew quickly and was generally green-yellow (Fig. 3) but that from IS8260 callus grew slowly and was grey-black. In most cases, the callus was originally yellow and gradually turned white or green-yellow. More of the calli blackened (Fig. 4) and died after the second subculture time. Roots usually appeared on

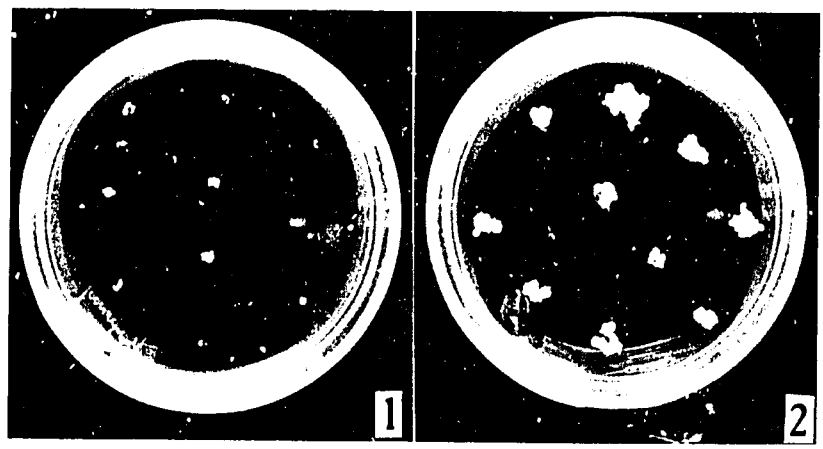
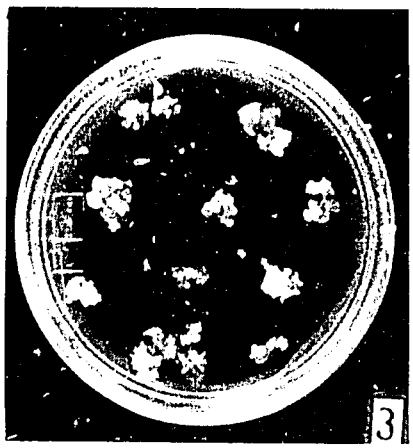


Fig. 1. Callus initiated from shoot parts of mature sorghum embryos after 12 days inoculation (var. IS8768).

Fig. 2. The first subculture of sorghum callus showing yellow globular structure (0.5 mg/L kinetin, 3 mg/2,4-D) (IS4225).

Table 2. Frequencies of callus induction from shoot portions of mature embryos of high tannin sorghum.

Genotypes	No. of embryos inoculated	No. of calli formed	Freq. of calli formation (%)
IS8260	150	64	42.7
IS8768	100	72	72.0
IS4225	200	166	83.0
IS3150	160	139	86.9
IS2830	200	182	91.0
IS6881	160	125	78.1



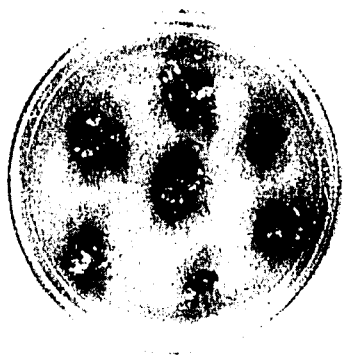


Fig. 3. The second subculture showing white and green-structure (0.5 mg/L kinetin, 2 mg/L 2,4-D) (IS4225).

Fig. 4. White callus maintained 84 days (IS8768).

subculture, and sometimes green shoots were seen on medium containing 0.5 mg/L kinetin. The callus cultured on subculture medium containing 0.1 mg/L kinetin gave fewer shoots. In most cultures purple or brown pigments (referred to as blackening by Mastellar and Holden, 1970) diffused into the medium; some lines such as IS2830 or IS3150 produced less. When transferred to regeneration medium five of the six cultivars regenerated plantlets (Fig. 5). IS8260 gave rise to roots but no shoots under these conditions. Of the three regeneration media, that containing 0.2 mg/L kinetin gave more regenerated plants than that with 5 mg/L NAA with or without 0.1 mg/L kinetin. In suitable conditions the frequency* of callus differentiating shoots and the frequency** of plants regenerated from the

*Freq. of calli differentiating shoots: No. of calli differentiating shoots/No. of calli transferred to regenertion culture.

**Freq. of plants regenerated: No. of plants regenerated/No. of calli transferred to regeneration culture.

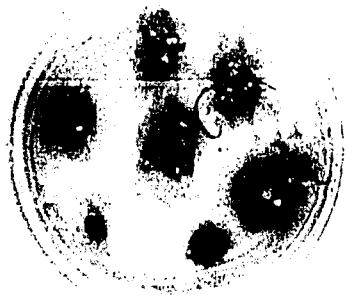


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Fig. 5. Shoot formation (0.2 mg/L kinetin, 0/2,4-D) (IS8768).

Table 3. Regeneration frequencies of calli induced from shoot portions of mature embryos of high tannin sorghum.

Genotypes	No. of calli* transferred to regeneration medium I	No. of calli differentiating shoots II	No. of plants regenerated III	Freq. of regeneration (%) II/I	III/I
IS4225	132	39	64	29.5	48.5
IS3150	118	40	37	33.9	31.4
IS8768	145	31	65	21.4	44.9
IS6881	61	16	14	26.2	22.9
IS2830	82	9	9	11.0	11.0

*The calli were subcultured on media containing 0.5 mg/L kinetin and were transferred to the regeneration medium containing 0.2 mg/L kinetin.

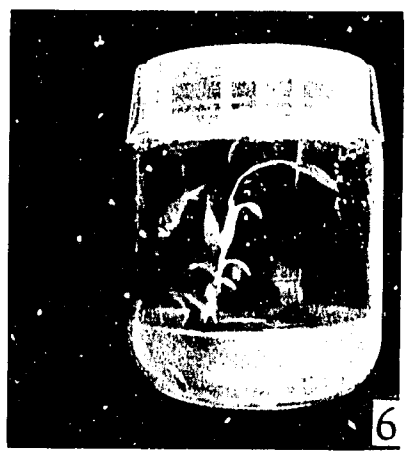

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Fig. 6. A cluster of sorghum plantlets with one albino (IS8768).

five cultivars varied from 11–48% (Table 3). These calli have been maintained on subculture medium for as long as 9 months, and so far over 700 plants have been obtained. An albino plant which appeared in plants regenerated from IS8768 is shown in Fig. 6.

These results show that using mature embryos as explants for callus cultures of sorghum may not only enable us to simplify the culture procedure, but also to get regenerable cultures, even from high tannin cultivars.

Acknowledgements

We gratefully acknowledge help from K. Landen for polyphenol assays, S. Short for tissue culture and W. Dai for photographic work. Supported by USAID Intsormil grant PRF-4B. Journal Paper No. 10,946 Agricultural Experiment Station, Purdue University.

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