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Table 1. Influence of nitrogen sources on growth of embryogenic callus (EC) in prime cultures of young panicles of sorghum cultivar Volzhskoye 2.

Medium	Ion composition (mg L^{-1}) NH_4+	NO_3^-	Organic nitrogen	Total mass of callus[1] (mg)	EC (mg)	EC (%)
MS[2]	370	2500	-	194.8 ab	38.6a	21.02
MSAP	370	2500	A[3] + P[4]	441.6 ef	129.2 ab	28.59
M2	1130	4500	-	111.6a	38.2 a	26.24
M2A	1130	4500	A	374.6 de	139.0 b	37.74
M2P	1130	4500	P	199.4 ab	80.6 ab	40.65
M2AP	1130	4500	A + P	501.2 f	260.8 c	51.85
M21AP	371	4495	A + P	323.2 cd	99.4 ab	32.26
M22AP	2250	8160	A + P	127.4 a	64.8 ab	48.54
M23AP	1125	2476	A + P	272.8 bcd	117.2 ab	44.07

1. Means of five replications. Means with different letters are significantly different (5% level), according to Duncan's Multiple Range Test.
2. MS = Murashige and Skoog medium;
3. A = aspargine (1g L^{-1}); and
4. P = proline (2 g L^{-1})

References

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Androgenic Response of Cultured Anthers and Microspores of Sorghum

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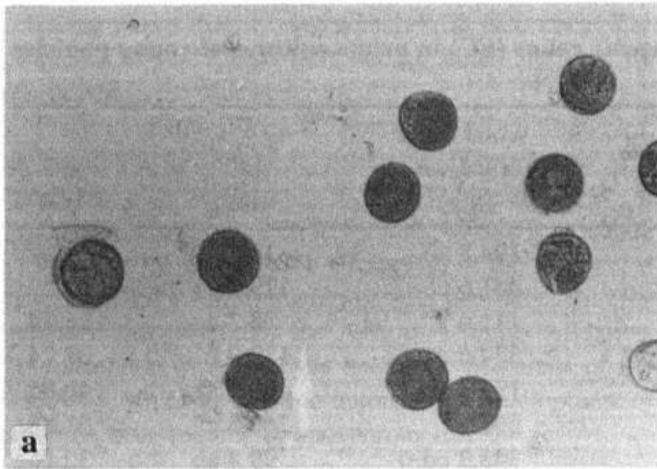
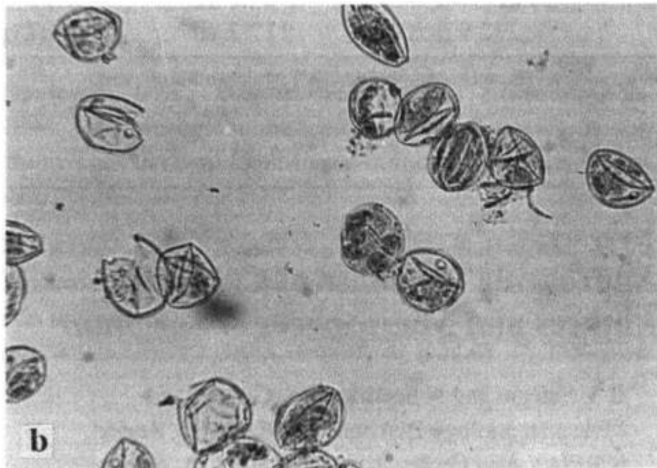
Introduction

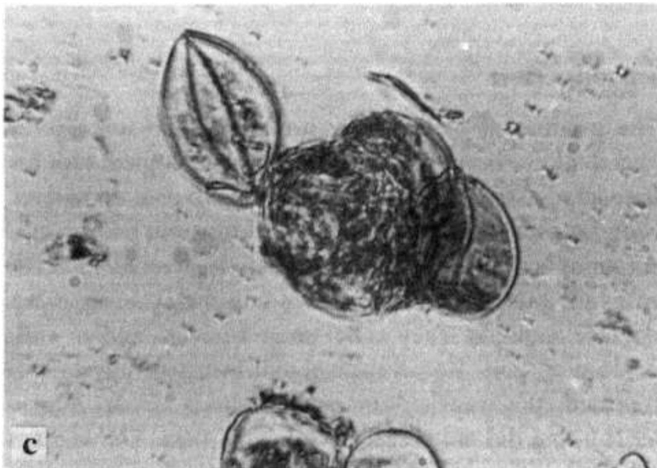
The production of haploids through anther and microspore culture is an intermediary biotechnological tool for breeders, geneticists, and map makers. To date there have been four serious attempts to produce haploid plants from sorghum anthers cultured in vitro. However, in most cases the plants regenerated from culturing anthers were not true haploids. They either arose from the anther wall, or were albinos. Kumarvadivel and Rangaswamy (1994) reported the regeneration of haploids from hybrid CSH 5, but did not present cytological data. The authors were unable to reproduce their results. Therefore, various factors that could affect androgenic response in sorghum have been studied.

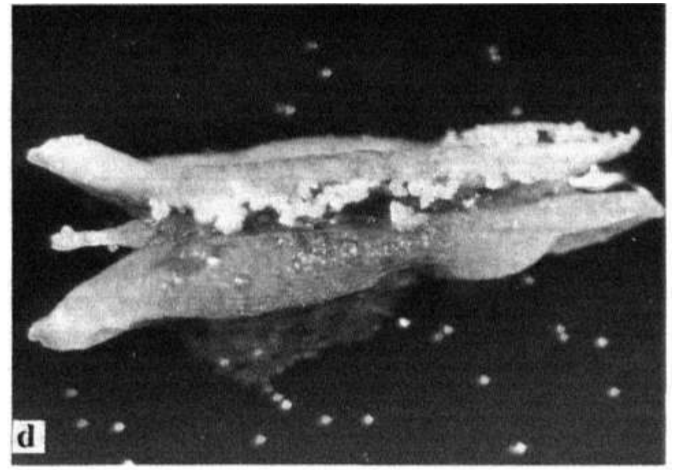
Materials and methods

Seeds of three *Sorghum bicolor* (L.) Moench hybrids CSH 5 (2077 A x CS 3541), CSH 9 (296 B x CS 3541), CSH 12R (296 B x M 148 138) were sown in pots and grown in









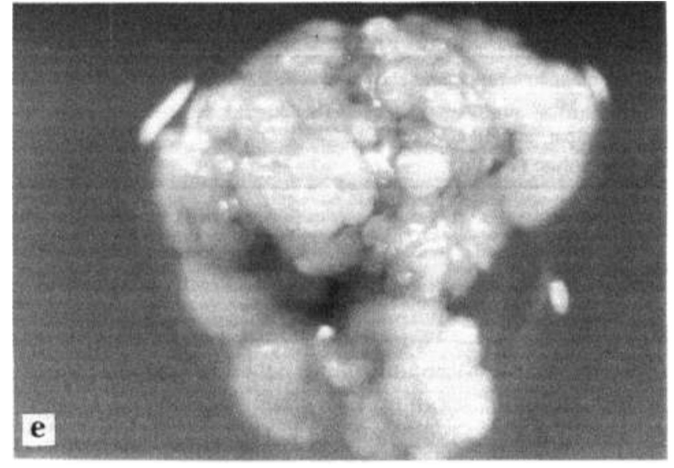


Figure 1. Haploid cultures of sorghum.
a. Microspores at uninucleate stage.
b. Dividing microspores after 15 days of culture on MS medium.
c. Multicellular divisions leading to formation of pro-embryoids.
d. Calli growing from within the anthers on MS medium.
e. Irregular masses of calli after 30 days of culture on MS medium.

a greenhouse. Their panicles enclosed in their leaf sheaths were harvested, and the developmental stage of the microspores in the anthers assessed by staining with 1% acetocarmine. Only the panicles with mid- to late-uninucleate stage microspores (Fig. la) were retained. The panicles were removed from the leaf sheaths and surface-sterilized with 0.1% mercuric chloride for 3 min. Anthers with uninucleate microspores were dissected out of 10 spikelets and plated on MS, B5, and N6 media with 0.5 mg L^{-1} kinetin (KN), 2.0 mg L^{-1} NAA, and 2.0 mg L^{-1} 2,4-D and incubated at 26 °C. Microspore division and callus formation were observed as measures of androgenic response.

Results

Effect of pre-culture conditions

Of the three hybrids studied, the maximum callus induction frequency was observed in CSH 9 (60% of anthers plated) followed by CSH 5 (20-30%). CSH 12 R showed the lowest response (15-20%). The uninuclear vacuolated microspores were most responsive as inferred from the rate of cell division. Cultures of anthers with micro-

spores at earlier (small cells without vacuoles) or later (binucleate microspores) stages did not show any androgenic response. The highest response was obtained when material was incubated at 26°C. Pretreatment of panicles at low temperatures (4°C, 6°C, 8°C, and 10°C) showed no advantage. Unsuitable pretreatment could inhibit callus production.

Culture conditions

By the 12th day, the anthers plated on all the three media started turning black (more on B5 and N6 media than on MS medium). Acetocarmine squashes of anthers revealed degeneration of microspores in B5 and N6 media while they were healthy, and showed divisions on MS medium (Fig. lb). The vegetative cells of the microspores take part in the formation of multicellular microspores while the generative cells are quiescent. Microspores grown on medium with 0.8% agar showed more divisions (up to 60%) than those grown in the liquid medium (15-20%). As carbon source, sucrose was more effective in inducing divisions (50-60% of the microspores dividing) than maltose (15-20%). Increase in sucrose concentration from 3% to 6%, marginally increased the percentage of responding anthers, but a further rise in concentration to 10% or 12% decreased this percentage.

Comparison of auxins, and effect of kinetin

Two auxins (NAA, and 2,4-D) were used at 2.0 mg L^{-1}. Acetocarmine squashes of sorghum anthers observed after 15-20 days of culture revealed that 2,4-D or NAA (2.0 mg L^{-1}) alone could not induce divisions, but that the combined effect of 2,4-D and NAA at 2.0 mg L^{-1} could induce divisions. When 2,4-D and NAA at a concentration of 2.0 mg L^{-1} was used in combination with 0.05,0.1, 0.15, and 0.2 mg L^{-1} concentrations of kinetin (KN), the percentage response increased. A maximum number of multicellular microspores (pro-embryoids) was observed at 0.2 mg KN L^{-1} (Fig. lc). Microcalli were found growing from within the anthers after 20-30 days of culture (Fig. 1d). These microcalli were transferred to MS medium, and irregular masses of calli were observed after 30 days of culture (Fig. le). The addition of such supplements as coconut milk and charcoal had no effect on the division of microspores or induction of callus.

Conclusions, and suggestions for future work

The above results are encouraging, and justify additional research to produce sorghum haploids. Microspore cultures should be emphasized more than anther cultures because they may be less dependent on genotype, or on the cultural conditions under which the mother plant is grown.

Reference

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Food and Feed Quality

Use of Popped versus Malted Sorghum Flour in Supplementary Foods for Children

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The use of malted sorghum (*Sorghum bicolor* L. Moench) flour in supplementary foods for children has been well established (Gopaldas 1992). However, the long processing time required to prepare malted sorghum flour limits the use of the malting technique as a routine way to prepare supplementary foods at household level. The technique of popping is quick, relatively simple, and improves the digestibility of starch in grains. Even though popped sorghum is a traditional snack food in certain parts of India, popped sorghum flour has not yet been used to develop supplementary foods. The present study was undertaken to compare the sensory quality of supplementary foods based on popped sorghum flour with that of malted sorghum flour to find out if popped sorghum flour is suitable for use in supplementary foods for children.

Sorghum grains of genotype SPH 509 were obtained from the Department of Plant Breeding, College of Agriculture, G B Pant University of Agriculture and Technology, Pantnagar. Popped sorghum flour was prepared by popping the grains in common salt (Singh and Srivastava 1993) before milling. Malted sorghum flour was prepared by steeping the grains for 12 h and subsequently by sprouting them for 48 h by the traditional household method (Gopaldas 1992). Mixes were prepared by combining popped or malted sorghum flour, roasted legume