

the mutants showed higher pod yield compared to JL-24 at 90 days. However, when harvested at 110 days yields were similar (table 1). This indicated that all three mutants matured earlier and had similar yield potential as the parent. Calculated yield per day was also more in the mutants at 90 days. The new cultures are under multilocation testing.

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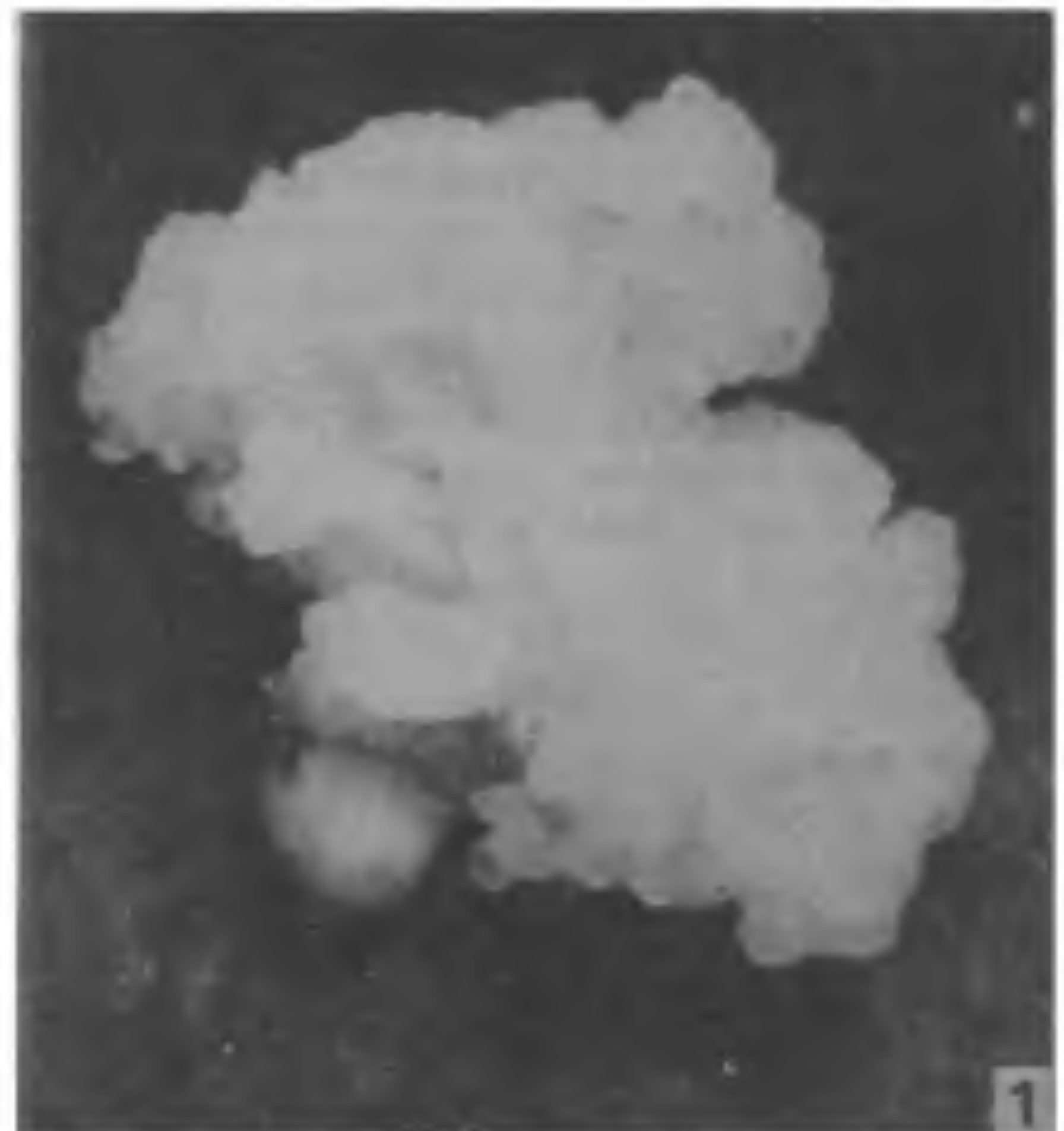
IN VITRO PLANT REGENERATION POTENTIAL FROM CALLUS CULTURES OF GRAIN SORGHUM

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A number of studies on sorghum tissue culture from immature explants have been conducted but without any consistency in embryogenic callus production or high per cent frequency of whole plant regeneration^{1,2}. To obtain immature explants, plants should be grown continuously in a greenhouse which is space- and labour-consuming. An attempt was therefore made in the present investigation to induce whole plant regeneration from mature seed callus of three cultivars of grain sorghum.

Seeds of *Sorghum bicolor* (L.) Moench cultivars IS 18417, IS 1054 and IS 18758 were sterilized with 0.1% mercuric chloride for 12 min and then thoroughly washed with sterile distilled water (3-4 times). One or two seeds were inoculated into Linsmaier and Skoog's (LS) medium³ containing either 2 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D), or 2 mg/l of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), or 2 mg/l 2,4-D+0.5 mg/l 2,4,5-T, or 2 mg/l 2,4-D+1 mg/l 2,4,5-T. Fifteen ml of medium was taken in each culture tube and autoclaved at 1.2 kg/cm² at 120°C for 15 min. The cultures were incubated under continuous fluorescent light (1000 lux at 25±2°C) for callus proliferation. After 15 days, the calli were transferred to Murashige and Skoog's (MS) medium⁴ supplemented with 2.5 mg/l

of 2,4-D. The cultures were maintained on this medium for 6 subcultures at 15-day intervals. For development of shoots and roots, callus (150±15 mg) was transferred into MS medium containing different



Figures 1 and 2. 1, Callus from seed of IS 1054 on LS medium containing 2 mg/l 2,4-D and 2% sucrose; 2, Regenerated plantlet from callus culture of IS 1054 on MS medium supplemented with 5 mg/l BAP, 1 mg/l 2,4-D and 3% sucrose.

Table 1 Comparison of callus regeneration potential of sorghum cultivars

Growth regulators (mg/l)	Sucrose (%)	Frequency of response (%)					
		IS 18417		IS 1054		IS 18758	
		Shoots	Roots	Shoots	Roots	Shoots	Roots
0.5 BAP+0.5 NAA	2	Nil	Nil	Nil	Nil	Nil	Nil
2 BAP+0.2 NAA	2	Nil	Nil	Nil	Nil	Nil	Nil
5 BAP+1 2, 4-D	3	80	84	76	29	42	69
10 BAP+1 2, 4-D+0.2 NAA	2.5	87	13	76	6	33	53

Data are average values from 20-30 replicates.

concentrations of 6-benzylaminopurine (BAP), naphthaleneacetic acid (NAA), 2,4-D and sucrose, and incubated at $25 \pm 2^\circ\text{C}$ under continuous light (2000 lux).

Callus initiated on LS medium was white, watery and friable (figure 1). Of the four media used, LS medium containing 2 mg/l 2,4-D, 0.5 mg/l 2,4,5-T and 2% sucrose was found to be better for callus initiation and growth (data not shown). At the beginning of the second passage on MS medium the callus of all three cultivars became creamy white, compact and nodular. Fortyfive-day-old calli were used for comparison of regeneration capacities of the three cultivars and the frequencies of response are given in table 1. While low concentrations of BAP (0.5 and 2 mg/l) along with NAA (0.5 and 0.2 mg/l) and 2% sucrose failed to induce shoots or roots in all the three cultivars, higher concentrations of BAP (5 and 10 mg/l) in combination with 1 mg/l 2,4-D and 3% sucrose or 1 mg/l 2,4-D, 0.2 mg/l NAA and 2.5% sucrose produced shoots as well as roots. Some calli, of all three cultivars, produced rooted shoots. A regenerated plantlet from a callus culture of IS 1054 is shown in figure 2. The frequency of shoot regeneration was maximum in the cultivar IS 18417, followed by IS 1054 and IS 18758. This variation in regeneration capacity could be attributed to genotypic differences. However, there was a decline in regeneration potential in all three cultivars after 5 subcultures (data not shown). Loss of totipotency in cereal tissue cultures was common^{5,6} and this could be because of polyploidization⁷. Regenerated shoots were transferred to MS medium supplemented with 1 mg/l NAA plus 2% sucrose for rooting. Roots developed from all the shoots in 15-20 days. Though reports of plant regeneration in sorghum are available^{8,9}, high frequency regeneration from mature caryopses is lacking. The present investigation has clearly demonstrated whole plant

regeneration at high frequency in known genotypes of grain sorghum.

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