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High frequency somatic embryogenesis and regeneration in different genotypes of *Sorghum bicolor* (L.) Moench from immature inflorescence explants

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Abstract This study describes a protocol for the induction of high frequency somatic embryogenesis directly from immature inflorescence explants in three sorghum genotypes (SPV-462, SPV-839, and M35-1). The effect of various growth regulators on somatic embryogenesis was investigated. High frequency somatic embrogenesis was obtained on Murashige and Skoog (MS) medium supplemented with 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), and addition of 0.5 mg l^{-1} kinetin (KN) in the medium further improved the formation of somatic embryos per explant in all genotypes. The presence of 1.5 mg l^{-1} 6-benzylaminopurine plus 1.0 mg l^{-1} KN in MS medium was most efficient for maturation and germination of somatic embryos. The genotype SPV-462 performed better than SPV-839 and M35-1 in terms of induction and germination of somatic embryos. Organogenesis also occurred in callus of all genotypes at the frequency of 20–25%. Regenerated plants from somatic embryos were successfully acclimatized in soil in the greenhouse where plants were grown to maturity, flowered, and set seeds. Regenerated plants appeared normal like that of the seed-raised plants.

Keywords Embryogenesis · Regeneration · Somatic embryos · Sorghum · Tissue culture

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Introduction

Successful application of plant biotechnology for crop improvement requires the development of efficient plant regeneration system from cultured cells or tissues. Sorghum is the most popular food, fodder, and fuel crop of arid and semiarid zones of the world, central and north Indian zones. Alternative uses of sorghum include production of starch, sugars, and alcohol. Sorghum ranks third among the major food crops of India with productivity of 65,000,000 metric tons (http://faostat.fao.org/faostat/form?collection=Production.Crops.Primary&Domain=Production&servlet=1&hasbulk=&version=ext&language=EN) from 9,200,000 ha, which is the highest in the world. Sorghum is known for its drought tolerance and has been considered a wonderful crop from plant physiological point of view. No cereal crop or even sugarcane can outperform sorghum in its productivity and capability to accumulate high levels of sugar in the stalk. These unique attributes of sorghum are considered highly favorable for its eventual emergence as a bio-energy crop.

Among the cereals, sorghum cultivation is known to be affected by several diseases and is prone to a variety of insect pests (Nwanze et al. 1995). Conventional breeding techniques have not yielded agronomically desirable genotypes basically because of the nonavailability of resistant sources in the germplasm (Nwanze et al. 1995). Genetic engineering provides an alternate strategy for the genetic improvement of sorghum by introducing new genes of economic importance. A prerequisite for genetic transformation of a crop system is the availability of an efficient protocol for plant regeneration *in vitro*. There have been several reports on callus induction and plant regeneration from immature inflorescence (Cai and Butler 1990; Gupta et al. 2006), shoot segments (Brar et al. 1979), shoot tips

Table 1. Effect of various synthetic auxins on direct somatic embryogenesis derived from immature inflorescence explants in 3 genotypes of sorghum

Plant growth regulator (mg l^{-1})	Embryogenesis frequency (%)			Number of embryos/explant		
	SPV-839	SPV-462	M35-1	SPV-839	SPV-462	M35-1
2,4-D						
1.0	70	75	65	18.2±0.09 bc	22.6±0.04 b	16.5±0.04 c
2.0	85	90	70	22.4±0.02 a	25.2±0.00 a	20.1±0.12 a
3.0	76	85	70	14.6±0.07 cd	20.1±0.04 bc	18.3±0.09 b
4.0	60	70	55	19.1±0.10 b	12.9±0.02 ef	10.4±0.00 f
NAA						
1.0	65	70	60	12.3±0.07 de	15.4±0.01 d	9.8±0.00 f
2.0	70	75	65	15.6±0.04 c	18.7±0.02 c	14.5±0.02 d
3.0	76	80	68	13.4±0.05 d	16.0±0.01 d	12.2±0.06 e
4.0	65	70	60	7.8±0.04 fg	10.0±0.10 f	6.5±0.05 h
2,4,5-T						
1.0	30	40	25	8.1±0.08 f	10.2±0.00 f	6.2±0.02 h
2.0	35	36	30	6.7±0.01 g	7.9±0.04 g	5.4±0.08 hi
3.0	35	30	25	4.8±0.10 hi	6.2±0.02 h	4.2±0.04 i
4.0	25	25	22	3.1±0.03 i	3.8±0.03 j	3.1±0.06 ij
Picloram						
1.0	40	48	36	5.6±0.05 h	7.1±0.01 g	5.3±0.07 hi
2.0	48	55	40	11.2±0.03 e	12.0±0.03 ef	8.5±0.07 g
3.0	40	50	38	8.4±0.04 f	9.0±0.02 fg	6.1±0.00 h
4.0	25	35	20	2.7±0.00 j	4.8±0.03 i	2.4±0.01 j
Dicamba						
1.0	50	60	42	7.8±0.05 fg	11.6±0.06 f	7.5±0.02 gh
2.0	58	65	50	11.2±0.04 e	14.5±0.05 e	9.4±0.03 f
3.0	50	55	50	7.0±0.02 fg	9.4±0.03 fg	6.4±0.01 h
4.0	40	48	40	4.0±0.00 hi	4.8±0.00 i	3.3±0.04 ij

Each value represents mean±SE of 3 replicates, each with 25 cultures. Same *letters* in a *column* are not significantly different at $P=0.05$ levels.

(Bhaskaran and Smith 1989; Maheswari et al. 2006), and from mature embryos of sorghum (McKinnon et al. 1986). However, the rate of plant regeneration is not sufficiently high in sorghum and practical difficulties still exist in establishing and propagating regenerable cultures for long periods. There are few reports on somatic embryogenesis using different explants, but conversion of somatic embryos into plantlets has remained inefficient and limited because of low frequency, genotype specificity, and occurrence of callus phase before embryogenesis (Maheswari et al. 2006). Moreover, conversion of somatic embryos into plants is the most prolonged phase in culture (Gupta et al. 2006).

To the best of our knowledge, there are no reports on direct somatic embryogenesis and subsequent plant regeneration from immature inflorescence without the intervention of callus from important genotypes of *Sorghum bicolor* (SPV-839, SPV-462, and M35-1). The present study describes a high frequency and reproducible protocol for plant regeneration via somatic embryogenesis from immature inflorescence explants in three important cultivars of sorghum.

Materials and Methods

Seeds of three *Sorghum bicolor* genotypes, SPV-462, SPV-839, and M35-1, were collected from the National Research Center for Sorghum, Hyderabad, India. Immature inflorescences were obtained from the boot leaves after 50–60 d of sowing. The boots were collected and 2–7 cm long young panicles were separated after surface sterilization with 0.1% $HgCl_2$ and rinsed for three to four times in sterile distilled water. The panicles were then chopped into small pieces (1–2 cm) aseptically and cultured on MS medium (Murashige and Skoog 1962) supplemented with various concentrations of auxins and cytokinins.

For direct somatic embryo induction, the explants were cultured on MS medium containing 25 g l^{-1} sucrose with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), napthaleneacetic acid (NAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram and dicamba singly or in combination with 6-benzylaminopurine (BAP) and kinetin (KN) (Tables 1 and 2). The pH of the medium was adjusted to 5.6–5.8 before adding 1% agar (Hi-media). All the cultures were maintained at 25±2°C under the 16 h

Table 2. Effect of various auxin and cytokinin combinations on somatic embryogenesis derived from immature inflorescence explants in 3 genotypes of sorghum

Plant growth regulator (mg l^{-1})			Embryogenesis frequency (%)			Number of embryos/explant		
			SPV-839	SPV-462	M35-1	SPV-839	SPV-462	M35-1
2,4-D	BAP	KN						
2.0	0.5	0.0	85	85	70	25.2±0.02 c	28.0±0.03 c	24.3±0.04 bc
2.0	1.0	0.0	85	85	73	27.6±0.01 b	30.2±0.04 b	25.2±0.05 b
2.0	0.0	0.5	85	95	78	32.2±0.04 a	35.3±0.01 a	28.2±0.02 a
2.0	0.0	1.0	80	90	75	26.1±0.04 bc	30.3±0.03 b	24.5±0.04 bc
NAA	BAP	KN						
2.0	0.5	0.0	60	65	55	10.0±0.02 f	12.3±0.01 h	8.3±0.04 h
2.0	1.0	0.0	56	60	52	12.7±0.05 e	14.2±0.04 g	10.3±0.02 g
2.0	0.0	0.5	70	75	66	14.3±0.02 d	18.4±0.06 e	12.4±0.04 e
2.0	0.0	1.0	66	70	64	11.7±0.06 ef	15.7±0.04 fg	11.1±0.00 fg
2,4,5-T	BAP	KN						
1.0	0.5	0.0	37	40	32	12.2±0.06 e	14.4±0.04 g	8.6±0.04 h
1.0	1.0	0.0	30	34	26	9.4±0.05 fg	12.1±0.04 h	7.0±0.03 i
1.0	0.0	0.5	35	38	31	7.0±0.04 gh	10.4±0.06 i	6.4±0.01 j
1.0	0.0	1.0	28	32	24	5.7±0.06 i	9.1±0.04 ij	4.3±0.04 l
Picloram	BAP	KN						
2.0	0.5	0.0	62	65	58	10.1±0.05 f	12.1±0.04 h	9.2±0.04 gh
2.0	1.0	0.0	57	60	55	8.1±0.04 g	10.6±0.04 i	7.4±0.00i
2.0	0.0	0.5	51	56	46	7.4±0.07 gh	8.4±0.02 j	5.4±0.01 k
2.0	0.0	1.0	48	50	44	5.1±0.05 i	6.4±0.04 k	4.3±0.05 l
Dicamba	BAP	KN						
3.0	0.5	0.0	55	60	52	15.7±0.08 cd	18.0±0.09 e	12.5±0.6 e
3.0	1.0	0.0	50	55	46	13.3±0.04 de	14.0±0.02 g	10.4±0.07 g
3.0	0.0	0.5	60	65	55	17.1±0.01 cd	20.7±0.01 d	14.6±0.06 d
3.0	0.0	1.0	54	60	48	14.2±0.04 d	16.4±0.03 f	12.0±0.04 e

Each value represents mean±SE of 3 replicates, each with 25 cultures. Same *letters* in a *column* are not significantly different at P=0.05 levels.

light and 8 h dark cycle with the light intensity of 30 μE m^{-2} S^{-1} provided by cool white fluorescent lamps. Somatic embryos obtained were later transferred on to the germination medium supplemented with BAP, KN singly or in combination (Table 3). Regenerated plantlets were washed with tap water to remove adhered agar, then transferred into pots containing the mixture of sterilized soil and sand (3:1). The cups were covered with a plastic sheet to maintain high humidity in the greenhouse, under diffused daylight. The plants were fertilized by adding Hoagland nutrient solutions at 2-d intervals. The plastic sheet was removed after 12 d of transfer.

The ontogeny of embryogenesis and shoot organogenesis was carried out after fixing the embryogenic, non-embryogenic and organ-forming callus from immature inflorescences in acetic acid and ethanol (3:1). For histological analysis, the tissues fixed in acetic acid and ethanol were placed in small “capsules”, which were transferred to an automatic processor (Shandon Processor) for dehydration using different solutions (80, 90, and 95% isopropanol for 1 h each and finally with 100% isopropanol thrice, each for 1 h). These dehydrated tissues were embedded in paraffin wax and sectioned. These sections (0.1–0.4 μm) were transferred onto the microscopic slides and stained with xylene, hematoxylene, and eosin using an autostainer. The sections were covered with glass slides and microphotography.

The experimental design was random and factorial with auxins and cytokinins as independent variables. Data pertaining to number of embryos formed, its maturation and conversion were subjected to analysis of variance (ANOVA). Mean value was calculated using Duncan’s New Multiple Range test. Thirty cultures were raised for each treatment, and all the experiments were repeated thrice.

Results and Discussion

Establishment of cultures. Direct somatic embryos were induced from immature inflorescences cultured on MS basal medium supplemented with various concentrations of auxin (2,4-D, NAA, 2,4-5-T, picloram and dicamba) alone or in combination with cytokinin (BAP and KN) as shown in Tables 1 and 2. The induction of embryogenic callus along with somatic embryos was observed (Figs. 1 and 2) at all the concentrations of auxin alone or in combination with

Table 3. Effect of different cytokinins on germination of somatic embryos derived from immature inflorescence explants in 3 genotypes of sorghum

Plant growth regulator (mg l^{-1})	Number of embryos cultured[a]			Number of embryos germinated[a]			Germination frequency (%)[a]		
	SPV 839	SPV 462	M35-1	SPV 839	SPV 462	M35-1	SPV 839	SPV 462	M35-1
BAP									
0.5	45	43	42	23.2±0.92 de	22.2±0.65 g	12.3±0.65 f	51.5	51.6	29.2
1.0	51	45	45	28.5±0.75 c	30.5±0.43 d	20.3±0.42 d	55.8	67.7	45.1
1.5	42	51	44	32.5±0.54 a	42.4±0.34 a	35.1±0.55 ac	77.3	83.1	79.7
2.0	41	53	43	28.4±0.32 c	35.1±0.12 c	26.3±0.21	69.2	66.2	61.2
KN									
0.5	45	41	41	22.1±0.24 e	20.3±0.21 gh	10.1±0.72 g	49.1	49.5	24.6
1.0	40	45	42	21.5±0.35 e	25.1±0.23 f	16.1±0.42 e	53.7	55.7	38.3
1.5	40	40	45	24.1±0.20 d	27.2±0.30 e	20.2±0.44 d	60.2	68.0	44.8
2.0	30	40	45	16.6±0.55 f	24.4±0.33 fg	18.6±0.35 de	55.3	61.0	41.3
BAP+KN									
1.5+0.5	40	43	42	22.4±0.52 de	28.4±0.21 de	18.4±0.71 de	56.0	66.0	43.8
1.5+1.0	35	46	41	30.4±0.31 b	38.3±0.34 b	28.4±0.25 b	86.8	83.2	69.2
1.5+1.5	35	45	45	25.3±0.41 d	30.6±0.35 d	28.4±0.34 b	72.2	68.0	63.1
1.5+2.0	35	45	45	22.4±0.24 de	29.4±0.61 d	19.3±0.22 d	64.0	65.3	42.8

[a] Each value represents mean±SE of 3 replicates, each with 25 cultures. Same *letters* in a *column* are not significantly different at $P=0.05$ levels.

cytokinins tested for all the three sorghum genotypes (Table 1). Pro-embryos were formed around the explants within 6–8 d of culture. After 10–25 d of culture, pro-embryos (Fig. 1*a*) developed into globular (Fig. 1*b*) shaped embryos. The highest frequency was recorded in SPV-462, SPV-839, and M35-1 was 90, 85, and 70% respectively, on MS medium supplemented with 2 mg l^{-1} 2,4-D (Table 1). The number of somatic embryos formed per explant was 25.2, 22.4, and 20.1 respectively for SPV-462, SPV-839, and M35-1 grown on the same medium. The frequency of embryogenesis was highest in 2,4-D followed by NAA, dicamba, picloram, and 2,4,5-T. Similar effect of 2,4-D was also reported in four grain and two sweet sorghum varieties (Rao et al. 1990), in *Cajanus cajan* (George and Eapen 1994) and *Arachis hypogaea* (Barna and Wakhlu 1993; Venkatachalam et al. 1997). Induction of embryogenic callus by 2,4-D is common in many cereals such as rice (Kavi Kishor and Reddy 1986a), fox-tail millet (Rao et al. 1988) and minor millets (Kavi Kishor et al. 1992). Auxins such as picloram, dicamba, and 2,4,5-T have not been attempted in sorghum for callus initiation. Increasing concentrations of all the five auxins tested decreased the frequency of somatic embryogenesis and the number of embryos differentiated irrespective of the variety (Table 1). Among the three varieties tested, SPV-462 produced more embryos per explant in 2,4-D, and the least responsive variety was M35-1 (Table 1). Genotype dependent embryogenesis was also reported earlier in *A. hypogaea* (Ozias et al. 1992) and *C. cajan* (Venu et al. 1999).

Effect of auxin–cytokinin interactions on somatic embryogenesis. The effect of auxin and cytokinin combinations on somatic embryogenesis from immature inflorescence explants was tested in three sorghum genotypes by culturing the explants on the medium containing 0.5 and 1.0 mg l^{-1} BAP or KN along with auxins 2,4-D, NAA, and 2.0 mg l^{-1} picloram, 1.0 mg l^{-1} 2,4,5-T, and 3.0 mg l^{-1} dicamba. In SPV-462, 2,4-D, dicamba, NAA, 2,4,5-T, and picloram in combination with 0.5 mg l^{-1} KN produced 35, 21, 18, 10, and 8 embryos, whereas in combination with 1.0 mg l^{-1} BAP produced only 30, 14, 14, 12, and 11 embryos respectively (Table 2). Furthermore, a high frequency and maximum number of globular embryos were induced directly from immature inflorescence in all three genotypes of sorghum grown on MS medium supplemented with 2.0 mg l^{-1} of 2,4-D and 0.5 mg l^{-1} of KN (without BAP). The frequency of somatic embryogenesis was 95, 85, and 78%, and the mean number of embryos formed per explant was 35, 32, and 28 in SPV-462, SPV-839, and M35-1 respectively (Table 2). Among the three varieties, the response was low in M35-1. When KN was replaced by BAP in the same medium, the frequency and the number of embryos formed per explant slightly declined (85, 85, and 70%) in all the varieties. The same trend was observed in the presence of NAA and dicamba but not in the presence of 2,4,5-T and picloram. In general, the response was better in 2,4-D, followed by NAA, dicamba, picloram, and 2,4,5-T (Table 2) for direct somatic embryogenesis from immature inflorescence. Our results were in line with the findings of a previous study (George and Eapen 1998;

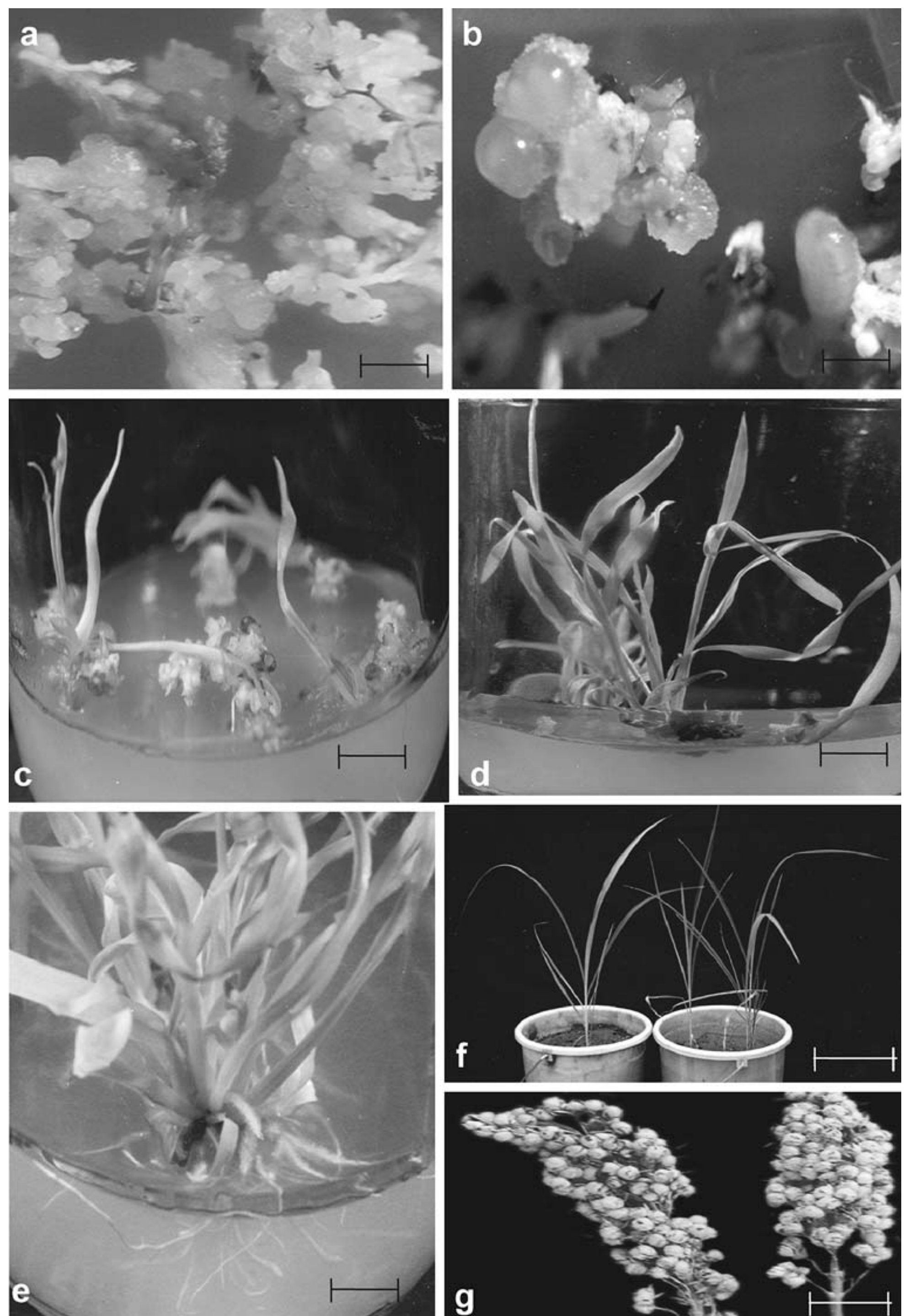

Figure 1. Various stages of callus induction and regeneration from immature inflorescence explants of *S. bicolor*. (*a*) Somatic embryogenesis from immature inflorescence (*bar*=1.0 mm). (*b*) Different shapes of somatic embryos (bar=1.5 mm). (*c*) Shoot regeneration (*bar*=1.0 cm). (*d*) Shoot maturation (*bar*=1.0 cm). (*e*) Rooting of shoots (*bar*=1.0 cm). (*f*) Hardened plants growing in pots (*bar*=1.5 cm). (*g*) Panicles after maturity (*bar*=2.0 cm).

Seetharama et al. 2000), but somatic embryos from callus phase derived from immature inflorescence and shoot tips can lead to somaclonal variation (Sarvesh et al. 1994; Chengalrayan et al. 2001), while direct embryogenesis can avoid this problem. Occasionally, abnormal embryos (1–2%) were formed in the present study, but these did not mature on all media tested (data not shown).

Germination of somatic embryo. For germination, globular-shaped embryos were transferred to MS medium supplemented with various concentrations of BAP (0.5–2.0 mg l^{-1}) or KN (0.5–2.0 mg l^{-1}) alone and in combination with 1.5 mg l^{-1} BAP plus 0.5–2.0 mg l^{-1} KN. The germination frequency of somatic embryos derived from immature inflorescences is presented in

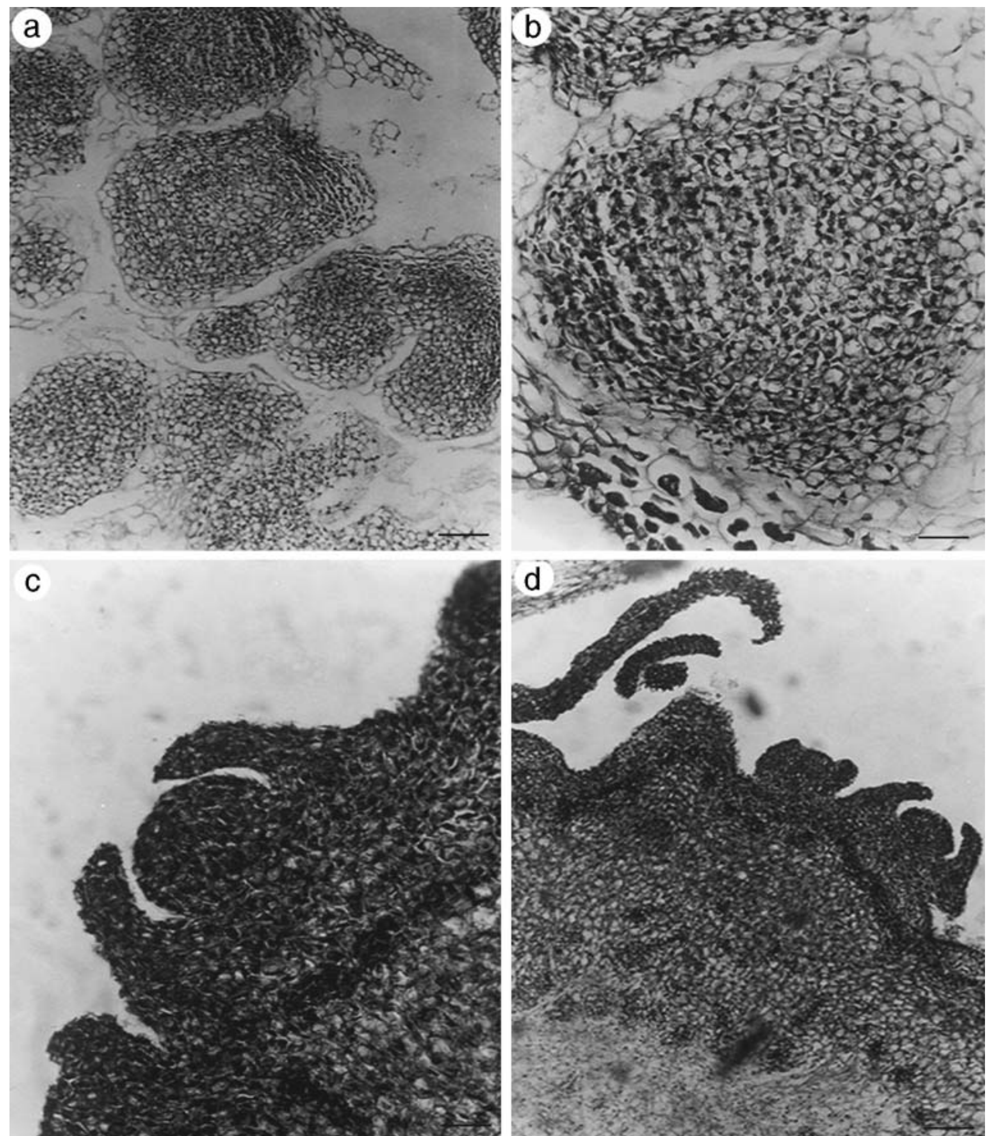

Figure 2. Histological studies showing embryogenic masses and shoot organogenesis from immature inflorescence-derived calli of *S. bicolor*. (*a*) Different shapes of embryos. (*b*) Enlarged view of the globular shaped embryo. (*c*) Shoot apex. (*d*) Multiple shoot apices (*bar*= 0.5 mm).

Table 3. Increasing concentrations of both BAP and KN alone up to 1.5 mg l^{-1} resulted in an increase in the germination frequency in all the three varieties. The germination response was 84.4, 80.2, and 77.2% for SPV-462, SPV-839, and M35-1, respectively, in MS medium supplemented with 1.5 mg l^{-1} BAP (Table 3). The replacement of BAP by KN at the same concentration resulted in the germination frequency of 67.4, 60.1, and 44.4% for SPV-462, SPV-839, and M35-1 respectively. The germination frequency declined with further increase of cytokinin concentration in the medium. The highest frequency of embryo germination was obtained when 1.5 mg l^{-1} BAP plus 1.0 mg l^{-1} KN were added into the medium with a frequency of 95.1, 85.1, and 70.4% for SPV-462, SPV-839, and M35-1 respectively (Table 3). Further increase in KN in the same medium containing 1.5 mg l^{-1} BAP decreased the frequency in all varieties. The embryo germination frequency was least in M35-1 (Table 3) at all cytokinin concentrations tested. It was observed that embryo germination was not only genotype dependent but was also affected by the cytokinin concentration present in the medium. BAP has been shown to affect several developmental stages and maturation of somatic embryos in *C. cajan* (Patel et al. 1994) and *A. hypogaea* (Venkatachalam et al. 1997, 1999; Chengalrayan et al. 2001).

Embryos grown on the medium containing 1.5 mg l^{-1} BAP and 1.0 mg l^{-1} KN turned green in color, which subsequently developed into whole plantlets (Fig. 1*c*–*d*). Germination of embryos with leafy structures was observed on the same medium. Thus, both BAP and KN seem to be required for maturation and germination of somatic embryos.

Complete plantlets were developed from fully mature embryos grown on MS basal medium within 2 wk. *In vitro* rooting of shoots was achieved using NAA or IBA or a combination of both. NAA and IBA are known to induce rhizogenesis in many cereals such as rice, maize, and triticale (Kavi Kishor and Reddy 1986b; Reddy et al. 1991). Subsequently, all plants regenerated via somatic embryogenesis were transferred to a greenhouse. Most plants (70–90%) survived during acclimatization. A total of 150 regenerated plants grown in the greenhouse produced normal flowers and set seeds.

Plant regeneration via either direct shoot organogenesis or somatic embryogenesis from explants is a prerequisite for genetic transformation. The present research focused on regeneration via somatic embryogenesis from immature inflorescence explants of sorghum genotypes. Callus initiation along with direct somatic embryogenesis from immature inflorescences was observed in all three sorghum varieties. Among the auxins tested, 2,4-D was most efficient for the induction of somatic embryogenesis in terms of frequency and the number of embryos formed per explant (Table 1). Callus formation from immature sorghum embryos has also been reported previously (Gamborg et al. 1977; Brar et al. 1979). Kavi Kishor and Reddy (1986a) studied the effect of 2,4-D on callus induction from mature seeds of rice who showed that callus initiation and subsequent regeneration were genotype-dependent. Rao et al. (1990) also reported genotypic differences in callus initiation from mature embryos in grain sorghum. In wheat, 2,4-D alone or in combination with cytokinins has been used for callus initiation (Mathias et al. 1986; Lazer et al. 1988), while it is a general practice to incorporate only 2,4-D during callus induction in cereals. Immature inflorescences were used as an alternative source to immature embryos to obtain totipotent cultures in maize (Suprasanna et al. 1986), but the developmental stage of inflorescence, as in the case of immature embryos, is critical. Immature inflorescences collected at the uninucleate stage of pollen development formed callus in maize and also in our study. Embryos were also isolated from embryogenic maize calli suspended in liquid medium devoid of hormones (Reddy et al. 1991).

In the present study, direct somatic embryogenesis was observed in all three sorghum varieties in the presence of 2,4-D, NAA, 2,4,5-T, picloram and dicamba (1–4 mg l^{-1}). However, the frequency of embryogenesis was highest in 2,4-D. Among the plant growth regulators used, 2,4-D was most commonly employed for the induction of somatic embryogenesis (Tomes 1985). Although 2,4-D analogs were also used, they exerted a differential effect on growth of maize calli (Suprasanna et al. 1989) which was also observed in our study. 2,4-D is also commonly used for embryogenic callus induction in sorghum (Bhaskaran and Smith 1990). Mythili et al. (1999) reported initiation of embryogenic suspensions in the presence of 2,4-D from wild sorghum. The embryogenic culture developed into mature somatic embryos after it was transferred to BAP and KN containing medium. The concentration of auxin has a profound influence on somatic embryo differentiation in *Pennisetum* (Vasil and Vasil 1982) and sorghum in this study.

In this study, a combination of 2.0 mg l^{-1} of 2,4-D plus 0.5 mg l^{-1} of KN (without BAP) resulted in the highest frequency of somatic embryogenesis from immature inflorescences in all three sorghum varieties. Murty et al. (1990a, b) compared the frequency of somatic embryo differentiation from the callus derived from scutella, shoot portions of young seedlings, and immature inflorescences. They observed that immature inflorescence was most suitable for inducing somatic embryogenesis and subsequent plant regeneration. The number of embryos formed per explant was dependent on the genotype and the type and concentration of plant growth regulators used in the medium. Contrary to the results in this study, Harshavardhan et al. (2002) reported direct somatic embryogenesis following a two-step culture procedure consisting of induction of multiple shoots and formation of somatic embryos. An amazing number of 700–1,000 somatic embryos per explant were reported on MS medium containing 17.72 μM BAP and 2.69 μM NAA in three sorghum varieties (BT × 623, 296B and M35-1). An attempt to reproduce these results using other sorghum varieties were not successful. We found that the combination of BAP and KN was most efficient for somatic embryo germination (Table 3), while 2,4-D and KN combination was best for the initiation of somatic embryogenesis. In general, the presence of BAP or KN promote germination of somatic embryos in other plant systems.

This protocol of direct somatic embryogenesis described in this study can be used for large-scale production of transgenic sorghum using gene gun or *Agrobacterium*-mediated genetic transformation and also for the production of synthetic seeds of this species.

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