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Effect of hormones, explants and genotypes in in vitro culturing of sorghum

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Abstract

In vitro culturing of sorghum is very much influenced by genotypes, explants, media composition and hormones. Among the genotypes tested CO 25 was found to show high frequency of callus induction and regeneration. Regeneration efficiency of embryogenic calli from CO 25 was found to be greater (88-98%) than that of calli derived from other explants (Immature inflorescence, Mature embryo and Shoot tips) of different genotypes (CO 26, TNS 586 and CO-S-28). Out of the different media tried for callus induction and regeneration of sorghum, I_6 medium + kinetin 0.5 mg l^{-1} + 2,4-D 2 mg l^{-1} showed high frequency of embryogenic calli from immature embryo of CO25. Regeneration medium I_6 + Kinetin (1.0) + NAA (0.5) + casein hydrolysate at levels of 250 mg l^{-1} and 500 mg l^{-1} was found to best medium composition for regeneration. An efficient protocol has been developed from suitable explant and genotype for callus induction, regeneration of sorghum and thus to produce transgenic plants for biotic and abiotic resistance.

Keywords: *In vitro* culture, Sorghum, explants, genotypes, hormones

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the important grain and fodder crops in many parts of the world. It is well adapted to a wide range of soil types and environmental conditions, especially drought. It ranks as the sixth most planted crop in the world, behind wheat, rice, maize, soybean and barley. The nutrient composition of sorghum is comparable to rice and wheat. Mineral matter, crude fibre, calcium, phosphorous and protein content are specifically higher in sorghum than in rice. Despite continuous improvements in plant breeding, dramatic yield losses caused by fungal infections still occur. The spread of epidemic disease is at present combated by breeding for resistance, by variation of the culture practice and by fungicide treatments. Besides these traditional methods, genetic engineering may provide an additional tool to control fungal diseases. Many methods available for transformation ultimately end with tissue culture techniques emphasizing a need for a well-established regeneration system for a particular genotype of the crop selected for transformation. If the manipulated genotypes show low frequencies of callus induction and regeneration, finally it will not lead to fruitful results. Though there are a number of reports available for embryogenesis of cereals, it varies from crop to crop, genotype to genotype, locality and environmental factors. Sorghum has been categorized as one of the most difficult plant species to manipulate for tissue culture and transformation (Zhu *et al*., 1998).

In sorghum, as with most grasses, primarily very young tissue spatially close to the meristematic state (seedling, immature inflorescence, shoot apices and immature embryo) have proven responsive in *in vitro* culture (Jogewar *et al*., 2007, Smith and Bhaskaran, 1986, Zhong *et al*., 1995, Tomes, 1985). Difficulties arise also due to phenol production by grasses and sorghum (Oberthur *et al*., 1983). Retarded growth and necrosis of the calli formed have been observed in sorghum cultures producing black and purple pigments (Brettell *et al*., 1980; Davis and Kidd, 1980). Despite these problems, the status and achievements of sorghum tissue culture seem to be modest. And also, efficient regeneration protocols have yet to be evolved for sorghum genotypes that are used by Tamil Nadu farmers.

Previous studies on sorghum tissue culture revealed that callus has also been induced in sorghum from cultured unemerged inflorescences (Boyes and Vasil, 1984., Brettell et al., 1980) and immature leaves (Wernicke and Brettell, 1980). Elhag and Butler (1992) have reported about the effect of genotype, explant age and medium composition on callus production and plant regeneration from immature embryos of sorghum. Ma et al. (1983, 1984) regenerated plantlets from embryos excised 9 to 18 days after pollination and found that the response was genotype-dependent and the frequency of regeneration was too low.

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Mature and immature embryos were cultured on MS medium with additions of 2, 4-D and BA at 1.0 and 0.5 mg l^{-1} and average percentage of mature embryos producing the regenerating calli ranged from 2.8 to 87.9 and regenerants were obtained after two years of subculture. Friable callus was obtained when immature embryos of cultivars were cultured on modified B_5 medium (Chourey and Sharpe, 1985). Seetharama *et al*., 2000, also discussed sorghum plant regeneration system through somatic embryogenesis from shoot tip explants. Based on this background, the present work is contemplated in sorghum to standardize tissue culture protocols for different genotypes and explants with different hormone levels in order to get a well-established regeneration system that is further useful in developing transgenic crops.

Materials and methods

Genotypes and explants

Genotypes like CO 25, CO 26, TNS586 and CO-S-28 were used as the source for getting explants like immature embryos, matured Seeds, Shoot tip and immature inflorescence (Fig.1). All the sorghum cultivars were obtained from Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore, India

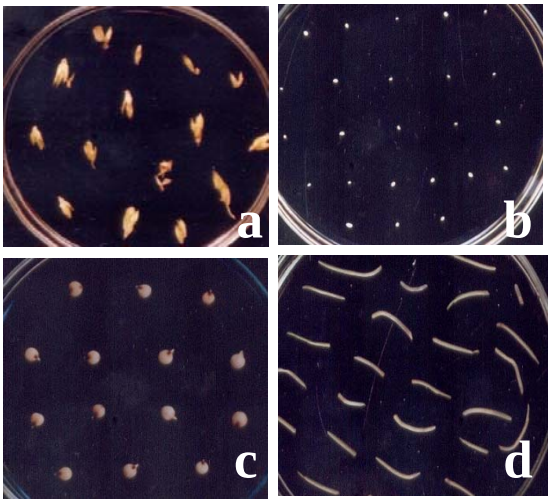

Fig 1. Explants used in in vitro culturing of sorghum, a. Immature inflorescence, b. Immature embryos, c. Matured seeds, d. Shoot tips

Explant preparation

Shoots with immature inflorescences were collected from 60 to 70 day old plants. After all the outer leaves were removed, the shoots were surface sterilized with 70 per cent ethanol for 30 seconds. Then the immature inflorescences were dissected and those less than 2cm long were selected and cut into 3mm segments. The inflorescence segments were cultured on callus induction medium. The immature panicles were harvested at 9 – 12 days after pollination. At the time of harvest, the immature embryos should be at the size of 1-1.5 mm in diameter. The kernels were removed by hand. The immature embryos were removed aseptically and cultured on callus induction medium, with the scutellum side touching the medium. Matured seeds were collected from the panicle and dried to remove excess moisture to prevent fungal contamination. The sterilized seeds were soaked for 20-24h in liquid nutrient medium to allow the medium to imbibe into the embryo and to facilitate embryo excision. Then the seeds were blot dried and placed in callus induction medium with the scutellar orientation on the medium. To obtain shoot tips, the matured seeds were sterilized and placed in MS-basal medium and kept in dark for 2-3 days and allowed for germination. After 3 days the shoot tip position were excised and placed in the callus induction medium and incubated at 28°C in dark condition for callusing.

Surface sterilization steps

First washing was done with 70 per cent ethanol for 5 min. followed by rinsing twice with sterile double distilled water. Second washing was done again with 4 per cent sodium hypochlorite for 15 min. followed by rinsing twice with sterile double distilled water. Final washing with 0.1 per cent mercuric chloride for 5 min. and further washed 4 to 5 times with sterile double distilled water.

Preparation of medium

The media chosen for callus induction and plant regeneration as given in Table 1 and 2 were prepared according to Murashige and

Table 1. Media used for somatic embryogenesis and plant regeneration

Ingredients	Media mg l^{-1}				
	MMS	**CIM**	**SM**	**SRM**	**RRM (0.5 x conc.)**
Macronutrients					
$(NH_4)_2SO_4$	1650	1650	1650	1650	1650
KNO_3	1900	1900	1900	1900	1900
$CaCl_2$. $2H_2O$	440	440	440	440	440
$MgSO_4$. $7H_2O$	370	370	370	370	370
KH_2PO_4	170	170	170	170	170
Micronutrients					
$MnSO_4.4H_2O$	22.3	22.3	22.3	22.3	22.3
$ZnSO_4$. $7H_2O$	8.6	8.6	8.6	8.6	8.6
H_3BO_3	6.2	6.2	6.2	6.2	6.2
KI	6.2	6.2	6.2	6.2	6.2
Iron Stock					
$FeSO_4$. $7H_2O$	29.8	29.8	29.8	29.8	29.8
Na_2 EDTA	37.2	37.2	37.2	37.2	37.2
Trace elements					
Na . MoO_42H_2O	0.25	0.25	0.25	0.25	0.25
$CuSO_4$, $5H_2O$	0.025	0.02	0.025	0.025	0.025
$CoCl_2$ $6H_2O$	0.025	0.02	0.025	0.025	0.025
Vitamins					
Glycine	2.000	2.000	2.000	2.000	2.000
Nicotinic acid	0.500	0.50	0.500	0.500	0.500
Pyridoxine hydrochloride	0.500	0.50	0.500	0.500	0.500
Thiamine hydrochloride	1.000	1.00	1.000	1.000	1.000
Myo-inositol	100	100	100	100	100
Asparagine	150	-	-	-	-
Growth regulators					
2-4Dichlorophenoxy acetic acid (2,4-D)	2	-	-	-	-
Kinetin	-	0.5	0.5	-	-
Indole acetic Acid (IAA)	-	-	1.0	-	-
Napthalene acetic acid (NAA)	-	-	-	-	0.5
Indole-3 butyric acid (IBA)	-	-	-	-	0.5
Sucrose	30	30	20	20	-
pH	5.2	5.2	5.2	5.2	5.2
Phytagel	2.5	2.5	2.5	2.5	2.5

MMS: Modified MS, CIM: Calli induction medium, SM: Subculture medium, SRM: Shoot regeneration medium, RRM: Root regeneration medium

Skoog (1962) with a few modifications and the modified basal medium is referred I_6.

Maintenance

Different explants were cultured for callus induction on I_6 medium and incubated in darkness at 26°C for callus induction, subcultured every 2 weeks and maintained at 26°C.

Regeneration

After 3 weeks, calli obtained were transferred to shoot regeneration medium, subcultured every 4 weeks and grown at 26°C under a 16h photoperiod under cool white fluorescent light and at a constant temperature of 26°C. Followed by culturing the plantlets on rooting medium (MS salts 0.5X concentration, α-NAA 0.5 µg ml^{-1}, IAA 0.5 µg ml^{-1}, sucrose 20% and phytagel 0.25%). The plants with well-established root system were transferred to the vermiculate soil, covered for 5 days with plastic covers to prevent desiccation, grown in tissue culture room and transferred to green house 15 days later.

Statistical Analysis

The experimental data were analysed statistically by three way analysis of variance (ANOVA) using SigmaStat V3.1 software (Systat Software, Inc., USA) to determine the variation between and within the treatments, explants and genotypes with respect to percentage of callus induction and regeneration.

Results

In vitro culture of Sorghum explants and plant regeneration

Different genotypes *viz.*,CO25, TNS 586, CO26, CO-S-28 and explants like immature inflorescence, immature embryos, matured seeds and shoot tips were selected for *in vitro* culture studies.

Effect of different phytohormone levels on callus induction and plant regeneration

Sorghum genotypes, explants and different phytohormone levels tried were given in Table 1. In all the sorghum genotypes, callus induction was found to be high with immature embryo explants in treatments T_7 and T_8 (Fig. 3 and 4).

Explant age and nature of the calli induced

Nature of the embryogenic calli is highly dependent on explant age, size and medium composition. Nature of the embryogenic calli induced, duration for callus induction and regeneration were presented in Table 2.

Immature inflorescence

Immature inflorescences were collected from plants before the emergence of the inflorescence from the boot leaf stage. The immature inflorescences were dissected and those less than 2 cm long were selected and cut into 3mm segments. Embryogenic callus formed after 25-35 days of culture. The phenolic exudation resulted in the browning and necrosis of callus. The callus induction is very much dependent on age of immature inflorescence. Nodular creamy embryogenic calli formed was noticed only after 3-4 weeks. Due to the phenolic exudation in CO 26 and CO-S-28, brown friable / slimy calli were induced .

Immature embryos

Embryos obtained 2-3 weeks after pollination and cultured with the scutellum-side down on I_6 medium supplemented with asparagine, germinated within 3 days without callus induction. Embryos obtained 7-11 days after pollination neither germinated nor formed callus. This study indicated that immature embryos of 0.8-1.4mm in size obtained 11-15 days after pollination produced high percentage of callus induction, which was again found to be genotype-dependent. But all the genotypes CO25, CO26, CO-S-28 and TNS 586 were found to produce calli.

The callus induction was observed from the scutellar region on CO 25 showed highest percentage of callus induction (84-85%) within 7-10 days culturing. The following morphological variations exhibited by the developing calli were used to identify embryogenic calli from non-embryogenic calli.

a. Embryogenic calli could be distinguished by their globular shape, compactness, friability and creamy colour.
b. Non embryogenic calli were translucent, slimy and yellowish to brown in colour.

Mature embryos / seeds

Fully matured, healthy seeds showed callus induction after 8-10 days of culturing on MS medium supplemented with 2,4-D. The healthy bold seeds showed better proliferation whereas, shrunken seeds neither germinated nor formed callus. Callus induction was again found to be genotype and medium dependent.

In matured seeds, the callus induction and proliferation was first observed in the scutellar region on 10 and 15 days after culturing. At

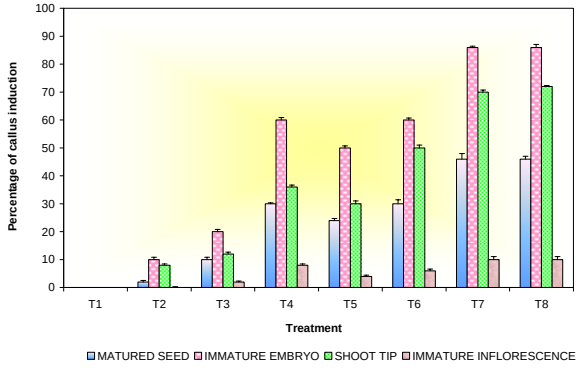

Fig 3. Effect of hormonal levels on callus induction in Sorghum genotype C025

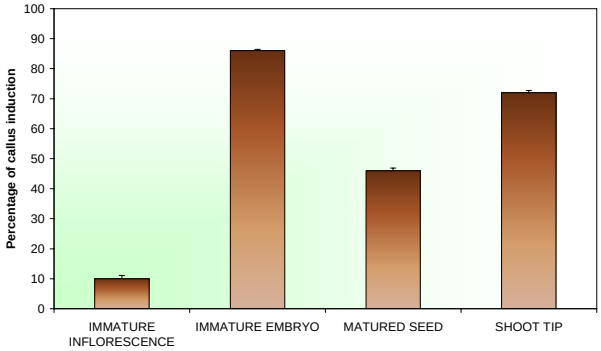

Fig 4. Callus induction percentage of different explants in Sorghum genotype CO25

Table 2. Callus induction (%) in different hormonal levels (50 explants / treatment)*

I_6 + PGR (mg/l)	Callus induction (%) ± SE															
	Matured seed				Immature embryo				Shoot tip				Immature inflorescence			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
T1 - Control	No response															
T2 - 2,4 – D (1.0)	2 ± 0.316	0	0	2±0.316	10±0.894	12±0.707	12±0.316	8±0.707	8±0.547	10±0.447	10±0.894	10±0.707	0	2±0.316	2±0.547	0
T3 - 2,4 – D (1.5)	10±0.547	4±0.547	4±0.316	8±0.632	20±0.894	28±0.316	22±0.707	20±0.894	12±0.447	14±0.447	16±2.376	14±2.033	2±0.316	4±0.316	4±0.632	0
T4 - 2,4 – D (2.0)	30±0.894	14±0.316	14±0.894	12±0.707	60±0.836	62±0.632	56±0.707	56±0.707	36±0.707	40±1.095	40±0.707	38±0.632	8±0.316	10±0.447	10±0.547	4±0.542
T5 - 2,4 – D (2.5)	24±0.316	8±0.447	10±0.447	12±0.547	50±0.894	54±0.316	48±0.894	48±0.547	30±0.707	32±0.894	32±0.948	32±1.414	4±0.447	4±0.316	4±0.547	2±0.632
T6 - 2,4 – D (1.0) + Kinetin (0.5)	30±0.707	12±0.547	14±0.447	12±0.316	60±0.707	56±0.547	50±0.707	54±0.547	50±1.000	66±0.707	56±1.225	56±0.894	6±0.447	12±0.447	14±0.894	6±0.316
T7 - 2,4 – D (1.5) + Kinetin (0.5)	46±1.414	20±0.894	26±0.547	20±0.547	86±0.707	76±0.894	60±0.547	62±0.707	70±1.000	84±0.547	60±0.447	60±00.707	10±0.632	20±0.632	22±0.948	10±0.632
T8 - 2,4 – D (2.0) + Kinetin (0.5)	46±0.894	26±0.316	28±0.447	20±0.707	86±0.447	78±0.948	62±0.547	64±0.707	72±0.707	84±0.707	62±1.049	60±0.948	10±1.095	22±0.707	24±0.707	10±0.316

* Values are expressed as mean of callus induction percentage ± SE, n=5 in each treatment.

Table 3. Regeneration efficiency of explants in different hormonal levels (50 explants / treatment)**

I_6 + PGR (mg/l)	Regeneration (%) ± SE															
	Matured seed				Immature embryo				Shoot tip				Immature inflorescence			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	C	d
T1 - Control	No response															
T2 - I_6 + NAA (0.5)	20±0.707	18±0.707	18±0.894	10±0.447	50±0.894	48±0.547	44±0.707	42±0.547	30±0.707	26±0.707	20±1.000	14±0.836	2±0.447	0	0	0
T3 - I_6 + NAA (1.0)	32±0.707	26±0.707	20±0.547	18±0.836	56±0.894	52±0.836	46±0.707	46±0.447	42±1.304	28±0.316	28±0.894	26±0.894	4±4.503	2±4.236	2±2.302	2±2.860
T4 - I_6 + NAA (0.5) + Kinetin (0.5)	40±0.894	30±0.707	22±0.894	18±0.447	68±0.707	58±0.894	56±0.707	52±0.316	50±0.707	42±0.836	36±0.707	30±1.342	10±0.707	4±0.632	2±0.316	2±0.542
T5 - I_6 + NAA (0.5) + Kinetin (1.0)	44±0.707	38±0.894	36±0.707	30±1.414	70±0.836	70±0.707	68±1.000	60±0.948	62±0.547	56±0.547	44±0.707	42±0.836	12±0.707	8±1.000	4±0.316	4±0.707
T6 - I_6 + NAA (0.5) + Kinetin (1.0) + Casein hydrolysate (250 mg/l)	52±0.547	50±0.707	48±0.707	36±0.707	86±0.836	80±1.140	74±0.316	72±0.836	76±0.707	60±1.000	60±1.304	58±1.304	20±1.414	14±1.095	8±0.316	4±0.447
T7 - I_6 + NAA (0.5) + Kinetin (1.0) + Casein hydrolysate (500 mg/l)	60±1.414	54±0.632	50±1.140	42±1.673	98±0.547	94±0.707	88±0.707	84±0.707	88±0.948	76±1.095	72±1.225	68±2.025	30±1.225	20±1.140	14±1.000	10±0.707

*The percentage calculated based on the no. of embryogenic calli inoculated on shoot induction medium, ** Values are expressed as mean of callus induction percentage ± SE, n=5 in each treatment

a - CO 25
b - TNS 586
c - CO 26
d - CO-S-28

the end of the induction period, a friable, creamy calli formed was noticed.

Shoot tips

Shoot tips of 2-3 mm in size obtained from germinated seeds produced high percentage (62-84%) of callus induction. This was again found to be dependent on genotype and size of the explants. The shoot tips less than 2-3 mm size does not proliferate, instead it turned brown and undergone necrosis. The shoot tips of size more than 2-3 mm in size produced non embryogenic calli which were translucent; slimy and yellowish in colour.

The cultured shoot tip grown on I_6 medium turned to pale brown colour and the proliferation was observed at the edges of the shoot tips. After 8-10 days of culturing, 1-2 cm of nodular, whitish, compact embryogenic calli was induced.

Plant regeneration

The experiments conducted to study the effect of hormonal levels on regeneration indicated the treatments T_6 (I_6 + NAA (0.5) + kinetin (1.0) + casein hydrolysate (250 mg/l) and T_7 (I_6 + NAA 0.5 mg/l + kinetin (1.0) + casein hydrolysate (500 mg/l) showed high percentage of shoot regeneration as indicated in Table 3, Fig 7. Duration of plant regeneration from sorghum explants were tabulated in Table 4.

Table 4. Duration of plant regeneration from sorghum explants and genotypes

Genotype	Explant	Duration for regeneration of shoots (in days)
CO 25	Immature inflorescence	70
	Immature embryos	60
	Matured embryos	67
	Shoot tip	65
TNS 586	Immature inflorescence	71
	Immature embryo	60
	Matured embryo	66
	Shoot tip	64
CO 26	Immature inflorescence	72
	Immature embryo	60
	Matured embryo	68
	Shoot tip	65
CO-S-28	Immature inflorescence	75
	Immature embryo	60
	Matured embryo	62
	Shoot tip	70

With respect to shoot regeneration, in all the genotypes immature embryo derived embryogenic calli showed higher percentage of regeneration followed by calli derived from shoot tip. Since the performance was the same with all the genotypes tried except TNS 586 results of CO 25 alone shown (Fig. 6). Callus induction and plant regeneration percentage of sorghum genotypes tried using immature embryo as explants were shown in Fig.5 and 8. Though shoot induction was observed in all the genotypes tried for tissue culture, the whole plants obtained from CO26 and CO-S-28 were less in number (Table 5).

The same type of results were obtained with rooting medium (MS medium (0.5x)+IBA (0.5mg/l)+NAA (0.5 mg/l). No change in phytohormone levels were attempted with respect to rooting medium. Callus induction, Shoot induction and elongation in sorghum genotype CO 25 was shown in Fig 2.

Effect of genotype on callus induction and regeneration

Earlier experiments conducted to study the suitable explant, phytohormone levels etc. revealed that callus induction and regeneration differs from genotype to genotype as indicated in Tables 2 and 3. Among the 4 genotypes selected, CO25 performed

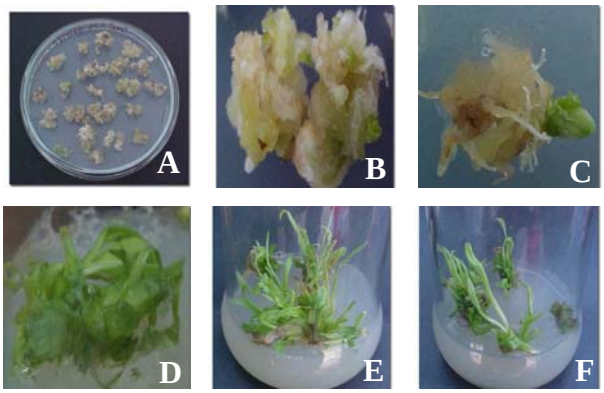

Fig 2. Callus induction and regeneration of immature embryos of Sorghum (CO 25), (A. Calli in regeneration medium., B. Shoot bud initiation., C. Shoot initiation., D. Multiple shoot induction., E. Elongation of multiple shoots., F. Regenerated plantlets)

best with callus induction and regeneration followed by TNS586 using immature embryos as explants.

Table 5. Duration and Nature of the callus induced in different Sorghum genotypes and explants

Geno-type	Explant	Nature of callus induced	Days
CO 25	Immature inflorescence	Nodular yellowish calli	25-35
	Immature embryos	Nodular calli	6-10
	Matured embryos	Nodular whitish calli	10-15
	Shoot tip	Nodular whitish calli	7-10
TNS 586	Immature inflorescence	Creamy calli	23-32
	Immature embryo	Nodular calli	5-7
	Matured embryo	Creamy friable calli	11-14
	Shoot tip	Nodular creamy calli	8-10
CO26	Immature inflorescence	Brown friable calli	24-33
	Immature embryo	Nodular creamy calli	8-10
	Matured embryo	Creamy friable calli	7-10
	Shoot tip	Compact, creamy calli	8-10
CO-S-28	Immature inflorescence	Brown slimy calli	25-33
	Immature embryo	Nodular whitish calli	5-6
	Matured embryo	Creamy nodular calli	8-11
	Shoot tip	Compact creamy calli	8-10

With respect to shoot tip, TNS586 showed higher percentage of callus induction and regeneration followed by other genotypes. The statistical analysis results also showed that there is a significant difference between the treatments T2 to T8 for callus induction, T2 to T7 for regeneration, and also between the explants and genotypes used in this study (Table 6 & 7). Our results indicated the differential behaviour of sorghum genotypes with respect to explants, callus induction and regeneration.

Discussion

In vitro culture of sorghum plants

A routine and efficient tissue culture procedure for plant regeneration is a pre requisite for generating transgenic plants. Problems related to regeneration of plants from the target cells used

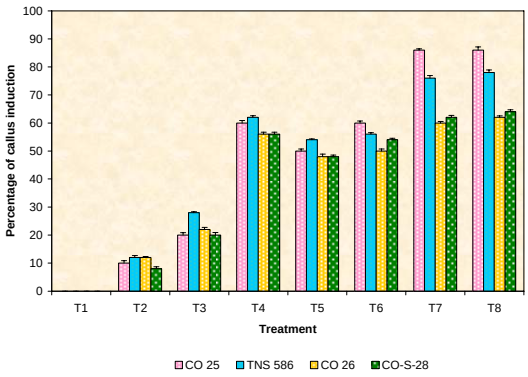

Figure 5

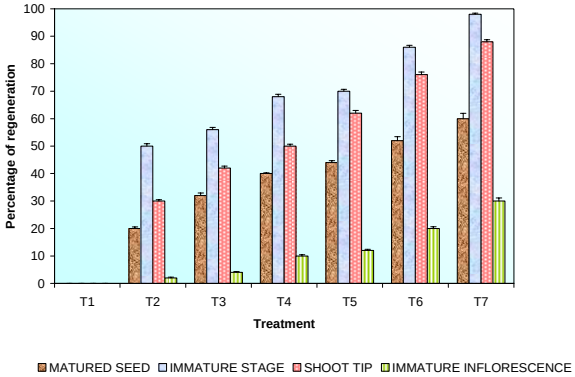

Figure 6

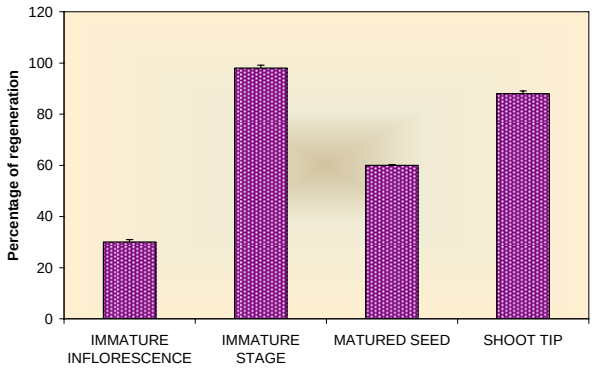

Figure 7

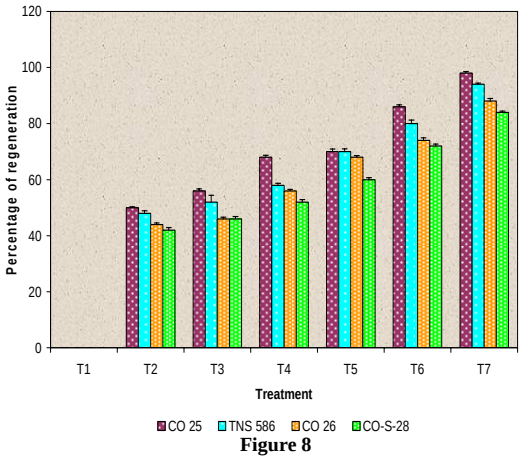

Figure 8

Fig 5. Callus induction (%) of immatured embryo, **Fig 6.** Effect of hormonal levels on regeneration in Sorghum genotype CO25, **Fig 7.** Regeneration percentage of different explants in Sorghum genotype CO25, **Fig 8.** Regeneration (%) of immatured embryo

Table 6. Three way ANOVA table for percentage of callus induction

Source of Variation	DF	SS	MS	F	P*
Treatments[a]	6	121357.1	20226.18	8619.984	<0.001
Explants[b]	3	159163.5	53054.49	22610.74	<0.001
Genotypes[c]	3	3598.048	1199.349	511.138	<0.001
Treatments x Explants	18	35542.61	1974.589	841.53	<0.001
Treatments x Genotypes	18	3297.039	183.169	78.063	<0.001
Explants x Genotypes	9	7023.716	780.413	332.596	<0.001
Treatments x Explants x Genotypes	54	4870.546	90.195	38.439	<0.001
Residual	448	1051.2	2.346		
Total	559	335903.7	600.901		

a : Treatments - T2, T3, T4, T5, T6, T7 and T8, b : Explants - Mature seeds, Immature embryos, Shoot tips, Immature inflorescence, c : Genotypes - CO25, TNS586, CO26,COS28

Table 7. Three way ANOVA table for percentage of regeneration

Source of Variation	DF	SS	MS	F	P*
Treatments[a]	5	82495	16499	4679.185	<0.001
Explants[b]	3	212629.2	70876.39	20100.84	<0.001
Genotypes[c]	3	12427.5	4142.5	1174.83	<0.001
Treatments x Explants	15	10878.33	725.222	205.676	<0.001
Treatments x Genotypes	15	910	60.667	17.205	<0.001
Explants x Genotypes	9	982.5	109.167	30.96	<0.001
Treatments x Explants x Genotypes	45	1290	28.667	8.13	<0.001
Residual	384	1354	3.526		
Total	479	322966.5	674.252		

a : Treatments - T2, T3, T4, T5, T6 and T7, b : Explants - Mature seeds, Immature embryos, Shoot tips, Immature inflorescence, c : Genotypes - CO25, TNS586, CO26,COS28

for gene transfer are major limiting factors in the generation of transgenics. Most of the reports on sorghum transformation pertain to genotypes which are inherently more amenable for genetic manipulations. Efficient regeneration protocols have yet to be evolved for sorghum genotypes that are used by Tamil Nadu farmers. Here, we discuss the results of the experiments conducted to select genotypes with high frequency of callus induction and regeneration.

Effect of genotype, explant and media on the frequency of callus induction and regeneration

Our study using four genotypes *viz.*, CO 25, CO 26, TNS 586 and CO-S-28 revealed that the frequency of callus induction and plant regeneration was genotype and explant dependent. The callus induction percentage ranged from 62.0 per cent to 86.0 per cent for immatured embryos of CO 25 followed by TNS 586. Variability in

the regeneration was also observed in earlier research of our laboratory (Gnanam, 1987; Kumaravadivel and Rangasamy, 1994;). Kresovich *et al*. (1987) reported that the frequency of regeneration ranged from 26 per cent to 69 per cent across the genotypes in sorghum. Previous work on sorghum tissue culture using immature inflorescence, immature embryos, mature embryos, shoot tips and anthers as explants (Bhaskaran and Smith, 1988; Cai and Butler, 1990; Devi and Sticklen, 2001; Kumaravadivel and Rangaswamy, 1994; Thomas *et al*., 1977) also revealed that regeneration is genotype-dependent.

Not only in sorghum but also in cereals such as maize (Green and Philips, 1975), oats (Cummings *et al.,* 1976), barley (Dale and Deambrogio, 1979), wheat (Sears and Deckard, 1982), pearl millet (Devi *et al.,* 2000 , Srivastav and Kothari 2002) and rice (Chung, 1982) a relationship between genotypes and *in vitro* response has been reported.

However, the relative influence of the genotypes on tissue response has been a matter of controversy. Vasil and Vasil (1986) suggested that the differential response of the genotypes may be due to differential expression which in turn depends upon the spatial and temporal distribution, their physiological and developmental stages.

Explant

It is not just genotype but also the nature and developmental stage of explants greatly influence and particularly play a very critical role in development in *in vitro* culture (Vasil, 1987). We were able to regenerate plants from matured seeds. Immature embryos of 11-15 days age and 0.8 – 1.4 mm size yielded more embryogenic calli which are in agreement with the results of Ma *et al*. (1987). Our results also indicated the influence of age of immature embryos on plant regeneration in sorghum as observed in other cereals such as in barley (Dale and Deambrogio, 1979), oats (Cummings *et al*., 1976) and wheat (Sears and Deckard, 1982). Calli were obtained from immature sorghum embryos that were 0.8 to 1.4 mm in length. Barley embryos that were 0.5 mm to 2.0 mm (Dale and Deambrogio, 1979) and 0.7 to 1.77 mm (Hanzel *et al*., 1985) in length produced rapidly growing calli with high frequency of plant regeneration. Optimum sizes for oat (Cummings *et al*., 1976) and wheat (Sears and Deckard, 1982) have also been reported.

Shoot tip of size 0.5, 0.8 mm in length yielded more embryogenic calli which are agreement with the results of Mythili *et al*. (1999). Immature inflorescence of size 3 mm showed embryogenic calli which are agreement with the observations of Casas (1997).

Medium composition and plant regeneration

Increasing the level of kinetin to a concentration of 0.5 mg l^{-1} in the I_6 medium than in MS medium increased the frequency of embryogenic calli. The interaction between genotype and medium components is complex (Cai and Butler, 1990) and our results are in agreement with them. Our experiments also suggest that the medium supplemented with asparagine (150 mg l^{-1}) might have assisting embryogenic calli proliferation. The regenerated plants from the above calli looked very healthy with many tillages and well established root systems suggesting that I_6 basal medium with asparagine (150 mg l^{-1}), sucrose (30 g l^{-1}), 2,4-D (2 mg l^{-1}) and kinetin (0.5 mg l^{-1}) may be used for all genotypes. With respect to shoot induction, the medium (I_6 + kinetin 1.0 mg l^{-1} + NAA 0.5 mg l^{-1} and casein hydrolysate at levels of 250 mg l^{-1} or 500 mg l^{-1}) were found highly suitable. Our results are in agreement with the reports on sorghum tissue culture by Elhag and Butler. (1992).

Conclusion

The present study yielded a suitable explant and genotype for callus induction, regeneration of sorghum and which could be the valuable tool to produce transgenic plants for biotic and abiotic stress tolerance.

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