Plant tissue culture studies in *Sorghum bicolor*: immature embryo explants as the source material

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Abstract

Sorghum is a wonder crop from physiological point of view. It is the most important cereal crop, after rice and wheat. The number of reports describing the use of transgenic *Sorghum* for basic studies in Biotechnology is still limited when compared to other crops. In one hand, the success of the transformation techniques is mainly dependent upon the availability of optimal protocols for highly efficient tissue culture techniques. On the other hand, regeneration in *Sorghum* is difficult. Hence, in this study an efficient and reproducible method for *in vitro* development of embryogenic callus and regeneration in *Sorghum bicolor* was developed. Immature embryo explants of *Sorghum bicolor* were cultured on MS nutrient medium supplemented with different concentrations and combinations of auxins and cytokinins. Embryogenic callus was initiated on MS medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l Kinetin (KN). Addition of KN to MS medium containing 2,4-D resulted in a significant enhancement on embryogenesis of the embryos. Amino acids also supported an improved frequency of embryogenesis. Therefore induction of a high frequency of somatic embryogenesis in immature embryos on MS medium is possible. Development of embryogenic callus, induction of somatic embryo and subsequent shoot regeneration was proficient at a concentration of 2 mg/l BAP. The regenerated shoots readily rooted on half strength MS medium supplemented with 1 mg/l naphthalene acetic acid (NAA). The regenerated plantlets were successfully transferred to soil and subsequently plants produced seeds. There was no difference between the acclimatized plants in comparison with *in vivo* plants in the respect of morphological characters.

Keywords: *Sorghum bicolor*; Immature embryo; 2, 4-D; Kinetin; Embryogenic callus; Regeneration.

Abbreviations

2, 4-D: 2, 4-dichlorophenoxyacetic acid
BAP: 6-benzylaminopurine.
E.C: Embryogenic callus
IAA: Indole-3-acetic acid.
KN: Kinetin

MS: Murashige and Skoog's medium (1962)
NAA: α-napthaleneacetic acid.
P.G.R: Plant growth regulators
TDZ: Thidiazuron.
ZN: Zeatin

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the major cereal in the world after rice, wheat, maize and barley. It is the third important staple food grain after rice and wheat for

millions of poor and most food insecure people in the semiarid tropics (Reddy, 2007). *Sorghum* is a rich source of various phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols and policosanols. These phytochemicals have the potential to significantly impact human health. *Sorghum* fractions possess high antioxidant activity *in vitro* relative to other cereals or fruits (Awika and Rooney, 2004). *Sorghums* containing tannins are widely reported to reduce caloric availability and hence weight gain in animals. This property is potentially useful in helping reduce obesity in humans and *Sorghum* phytochemicals also promote cardiovascular health in animals (Awika and Rooney, 2004). *Sorghum* and millet have anti-carcinogenic properties (Dyke and Rooney, 2006). A recent study showed that black and tannin *Sorghum* bran reduced colon carcinogenesis in rats (Turner et al., 2006).

Because of these widespread uses, improvement of *Sorghum* for agronomic and quality traits such as pest resistance, stress tolerance, grain quality and yield is considered essential. Previously, improvement of *Sorghum* has been carried out mostly using traditional plant breeding methods, but this method is not to be highly realistic. Advances in biotechnology and genetic transformation are used commonly to assist traditional breeding for crop improvement. But, genetic transformation of *Sorghum* for commercial use was much less successful because of the lack of effective protocols for plant tissue culture. In *Sorghum bicolor*, the capability to produce somatic embryos and their differentiation into green plantlets in the course of somatic embryogenesis was found to be influenced by quite a lot of factors, such as the genotype, organ/explant from which it is derived, the physiological state of the explant, its size and the plant growth regulators used in the medium, orientations of the explant on the medium, its density and the period of incubation may also affect shoot bud differentiation.

Reports in *Sorghum* (Zhu et al., 1998; Manjula et al., 2000; Maqbool et al,. 2001; Harshavardhan et al., 2002; Visarada et al., 2003; Gao et al., 2005; Kishore et al., 2006; Maheswari et al., 2006) indicated that *Sorghum* has been considered as one of the difficult plant species for genetic manipulation through tissue culture approach. Hence in the present study an attempt was made to fulfill this lacuna. In this study we achieved 100% callus induction and efficient plant regeneration i.e. 22 plantlets per culture.

Materials and methods

Plant material

In the preliminary experiment it was found that six grain *Sorghum* varieties IS 3566, SPV 475, CSV 13, CSV 15, CSV 112 and IS 348 were efficient for callus induction and high frequency of regeneration (Pola, 2005). Hence, further experiments were carried out with these six varieties only.

For callus initiation, healthy explants of immature embryo were used as the source materials. Field grown immature seeds (florets) of *Sorghum* containing immature embryos were harvested from main spikes of the caryopses of 14-17 days after anthesis. The explants were washed thoroughly under running tap water, followed by treating with 70% alcohol for 5 minutes and subsequently washed three times with sterile double distilled water. The immature seeds harvested for use as immature embryo were kept moist at 4°C until embryos were excised.

Surface sterilization of the explant

Immature embryos ranging in size from 0.5 to 2.0 mm in length, were removed from main spikes, and washed with running tap water. Then the surface sterilization was done with 70 % (v/v) ethanol for one minute and 15 min in a 2.5 % (m/v) sodium hypochlorite solution and followed by rinsing with 0.1% $HgCl_2$ for two minutes, before being meticulously rinsed with sterile distill water. Approximately 50 immature embryos (0.5-2.0 mm size) were aseptically removed from the spikelets using a dissecting microscope and placed on the medium with their axes and scutellum.

Preparation of media for callus induction

The media contained Murashige and Skoog basal mineral nutrients plus sucrose (30g/l). For callus initiation the basal MS media were supplemented with different auxins and cytokinins viz., 2,4-D, 2,4,5-T, IBA, IAA, NAA, KN, and ZN in different concentrations and combinations from 0.2 to 3.0 mg/l was used for all experiments.

The pH of media were adjusted to 5.8 using 1N HCl and 1N NaOH. Agar (0.8%) was used as the gelling agent and the media were heated to boiling for proper mixing. Then, the media were dispended into appropriate culture vessels i.e., culture tubes, Petri dishes and Baby jars (Borosil, India). The culture vessels were closed with non absorbent cotton, Para film and the Baby jar bottles were capped with polypropylene closures. The sterilization of media and glassware was carried on in an autoclave at 120ºC and 15 lb/in^2 pressure for 15 minutes.

Culture maintenance

Cultures were maintained in the darkness for callus initiation and proliferation at 25±2ºC and subculturing was carried out at an interval of 21 days. At the end of first subculture, the cultures containing smooth, shiny globular structures were defined as embryogenic calli and those having unorganized, creamy or yellow coloured structures were recorded as non embryogenic calli. For shoot regeneration, embryogenic calli were detached from the explants and cut into small pieces and transferred to the regeneration medium containing 0.5- 3.0 mg/l BAP/ ZN/KN or TDZ.

Root induction

Regenerated shoots were separated from the cultures individually and used for root induction. The media used for root induction was half strength MS media supplemented with various NAA concentrations 0.2, 0.5, 1.0 and 1.5 mg/l.

Establishment of regenerants in the green house

The regenerated plantlets were washed gently with double distill water for removing all traces of medium from the roots, and then transferred to small plastic cups containing sterile sand. The plastic cups were covered with sealed plastic vinyl bags to keep full humidity at 25±2ºC in light conditions (photon flux density at 25 μ mol m^{-2} s^{-1}, 16 h). As the plants grew vigorous, the bags were poked with chopsticks to allow air into the bags

until the plants self-supported. The polythene bags were removed after fifteen to twenty days. The plantlets were later transferred to larger pots containing sterile sand and soil (1:1 ratio) and kept under shade in the green house for another two weeks before transferring to field. Fully established regenerants were later established in the field for further growth.

Results

Callus induction

Bulging of the explants was observed from the third day after inoculation (Figure 1A). Callus initiation was observed on the surface of the explants started on 6th day after inoculation (Figure 1B). After one week of culturing, in all the explants the exudation of a dark brown and purple colour pigment was observed leading to the brown colorization of the medium around their bases and these cultures underwent necrosis. To control these phenolic secretions, activated charcoal (1%) was used in culture media. Frequent subculturing to fresh media with same composition was also tested to overcome the problem.

Calli formed from all the explants, usually appear in two types based on their colour and quality i.e., friability or compactness. These two types were similar to the embryogenic and non embryogenic types of calli described earlier in *Sorghum* by Cai and Butler (1990). Of these two calli, the embryogenic callus appeared white, comparatively more compact and morphogenic in nature. At the same time the non embryogenic calli were unorganized, friable, soft, loosely packed and pale yellow or dull creamy in colour. Generally embryogenic calli showing globular structures are visible on 16th day after inoculation. By increasing the number of subcultures, changes in callus morphology was observed in both embryogenic and non embryogenic calli. The embryogenic calli becomes compact, opaque, white and more nodulated in nature. Interestingly, after becoming yellowish, the embryogenic calli showed proliferation. These structures were gradually converted to cup or club shaped structures, in the callus maturity period. In contrast, subculturing the non embryogenic callus resulted in turning to brown in colour and becoming more unorganized.

Formation of globular compact or loose friable calli was observed in all the six varieties, irrespective of their auxin concentration. Callus induction frequencies were ranged from 40% to 84%. In this study, callus induction frequency was highest in the variety IS3566 i.e., 84% on MS+2 mg/l 2, 4-D, followed by SPV475 and low in CSV112 and IS348 (Table 1). Addition of 0.5mg/l KN in the callus induction medium significantly increased the embryogenic callus frequency at its maximum level (Table 2). While using both 2, 4-D and KN at the concentration of 2mg/l+0.5mg/l, the frequency of callus induction was maximum (100%). At this combination, almost 100% of the explants produced white compact calli. In all the varieties a significant increase in embryogenic callus frequency was observed. In addition, these embryogenic calli were mostly compact and globular on the medium containing KN.

Six different concentrations of 2, 4-D, 2, 4, 5-T, IBA, IAA, NAA, KN and ZN of viz., 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l have been tested for their effect on callus formation. Though callus initiation was observed in all the concentrations, its callus induction frequency and its quantity varied from concentration to concentration. The effect of

Figure 1. Plant regeneration in immature embryo explants of *Sorghum*. (A) Bulging of the explant on 2, 4-D+KN medium. (B) Embryogenic callus initiation. (C-D) Somatic embryo development. (E-H) Shoot development. (I) Root development in rooting media. (J) Potted regenerated plantlets growing in a greenhouse.

different plant growth hormones and their concentrations on callus induction were summarized in the Table 1. When high concentration of strong auxin like 2, 4-D was used in combination with lower concentrations of cytokinins in the callus induction medium resulted in high frequency of callus induction. The callus induction was inadequate when a cytokinin like KN was used alone; however a combination of KN with other auxins resulted in efficient callus induction (Table 2).

The area or quantity of the callus formed was genotype dependent in all the auxin concentrations used. To analyze these differences in the callusing ability of the different accessions, data were recorded three weeks after inoculation. Total area (embryogenic callus+ non embryogenic callus) and embryogenic callus area were studied. Of the six varieties used in this study, genotypic differences were observed with respect to total callus area. The total area of the callus was highest in the variety CSV 13 and minimum in CSV 112 (Figure 2).
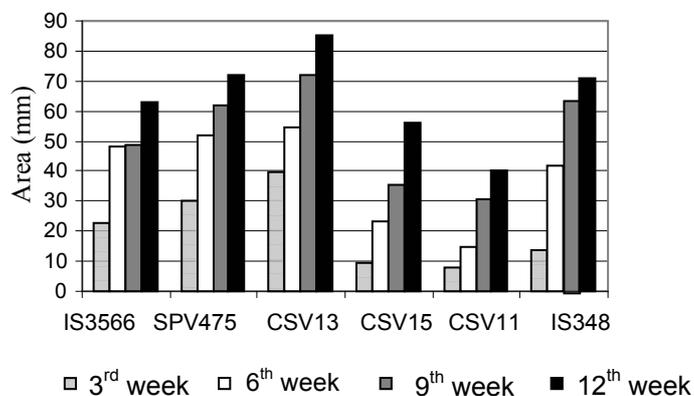
Figure 2. Growth rate of total callus area in different varieties.

Area of embryogenic callus in IS 348 was minimum and maximum in IS 3566. Though total callus area is highest in CSV 13, but the embryogenic callus area was highest in IS 3566 only. The average embryogenic callus area in different varieties at 3, 6, 9 and 12th week intervals was shown in the Figure 3.

For the study of growth rate of the callus, data on the increase in fresh and dry weights were gathered at 3, 6, 9 and 12 week intervals as mentioned under materials and methods. In general, Maximum growth rate, in terms of fresh and dry weights were observed in IS 3566. The general pattern of total callus and embryogenic callus growth rate in all the accessions was an increase in the weight of the callus with increasing no. of subcultures (Figure 4 and 5); however the quantity of increase was different in different accessions. The dry weights of the calli showed nearly comparable differences in all the subcultures i.e., 3, 6, 9 and 12th week intervals. There is correlation between callus fresh and dry weight growth and plant regeneration. Faster growth varieties gave more regeneration. In IS 3566 the callus dry weights were higher than remaining varieties (Figure 5) at the same time the regeneration is also higher than remaining varieties. Lower growth rate and low regeneration was observed in IS 348.

Initial incubation of explants under dark conditions increases fresh and dry weights, when compared to the explants incubated under light. The callus cultures incubated under light underwent necrosis due to the phenolic exudation.

Plant regeneration

At the end of 3rd week, the embryogenic calli and Non embryogenic calli were cut into small pieces and transferred to regeneration medium containing different combinations and concentrations of BAP, KN, ZN, TDZ, GA_3 viz., 1.0, 1.5, 2.0, 2.5 and 3 mg/l. The non embryogenic callus underwent necrosis after transferring onto regeneration medium. Embryoid formation and regeneration were observed in the embryogenic calli only.

Embryogenic calli after transferring to regeneration medium, the calli developed into embryoids and germinated within nine days after culture transfer (Figure 1 C and D). Embryoids at various stages of development could be seen by gently dissecting and teasing

the regenerating callus. The embryogenic callus was found to consist of small cells which were generally globular in shape. After 12-16 days clusters of rounded or oval structures were observed with their basal ends embedded in the callus mass, these structures further turned into green coloured shoot buds (Figure 1. E) and later developed into shoots in the presence of light (Figure 1, F and G).

Table 1. Effect of P.G.R's on Callus induction in immature embryo culture.

Concentration of PGR							No. of Explants responded/25 Explants	
2,4-D	2,4,5-T	IBA	IAA	NAA	KN	ZN	With E.C	E. C. Frequency (%)
1.0							12	48
1.5							16	64
2.0							21	84
2.5							20	80
3.0							16	64
	1.0						10	40
	1.5						14	56
	2.0						18	72
	2.5						16	64
	3.0						14	56
		1.0					10	40
		1.5					14	56
		2.0					18	72
		2.5					16	64
		3.0					14	56
			1.0				10	40
			1.5				14	56
			2.0				18	72
			2.5				14	56
			3.0				10	40
				1.0			10	40
				1.5			14	56
				2.0			18	72
				2.5			14	56
				3.0			12	48
					0.2		14	56
					0.5		18	72
					1.0		16	64
					1.5		16	64
					2.0		14	56
						0.2	12	48
						0.5	16	64
						1.0	14	56
						1.5	14	56

Table 2. Comparative effect of 2, 4-D and KN and their combination in Callus induction frequency from immature embryo.

| Variety	PGR concentration mg/L	Total explants inoculated	Explants with. E calli	E calli frequency %
IS 3566	2,4-D 2 mg/L	25	21	84
	KN 0.5 mg/L	25	18	72
	2,4-D 2 mg/L + KN 0.5 mg/L	25	25	100
SPV 475	2,4-D 2mg/L	25	16	64
	KN 0.5 mg/L	25	16	64
	2,4-D 2 mg/L + KN 0.5 mg/L	25	24	96
CSV 13	2,4-D 2 mg/L	25	16	64
	KN 0.5 mg/L	25	14	56
	2,4-D 2mg/ L+ KN 0.5 mg/L	25	22	88
CSV 15	2,4-D 2 mg/L	25	12	48
	KN 0.5 mg/L	25	13	52
	2,4-D 2 mg/L + KN 0.5 mg/L	25	16	64
CSV 112	2,4-D 2 mg/L	25	10	40
	KN 0.5 mg/L	25	13	52
	2,4-D 2mg/L + KN 0.5 mg/L	25	16	64
IS 348	2,4-D 2 mg/L	25	10	40
	KN 0.5 mg/L	25	10	40
	2,4-D 2 mg/L + KN 0.5 mg/L	25	12	48
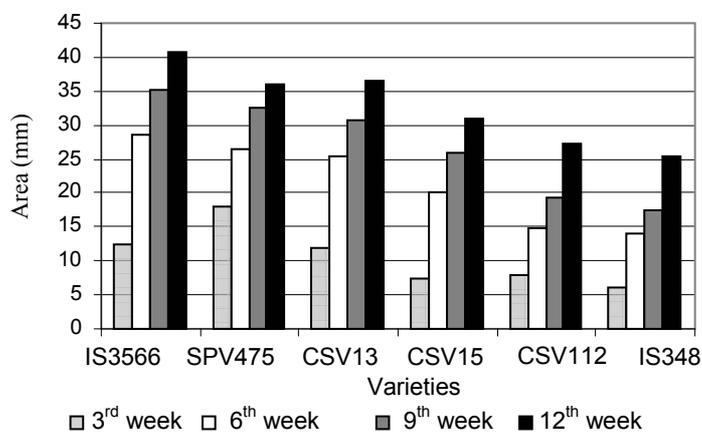
Figure 3. Growth rate of embryogenic callus area in different varieties.
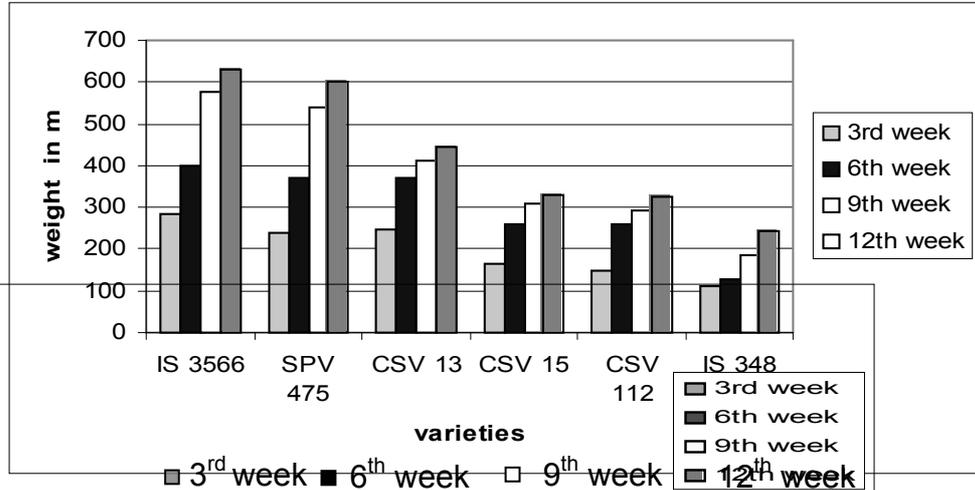
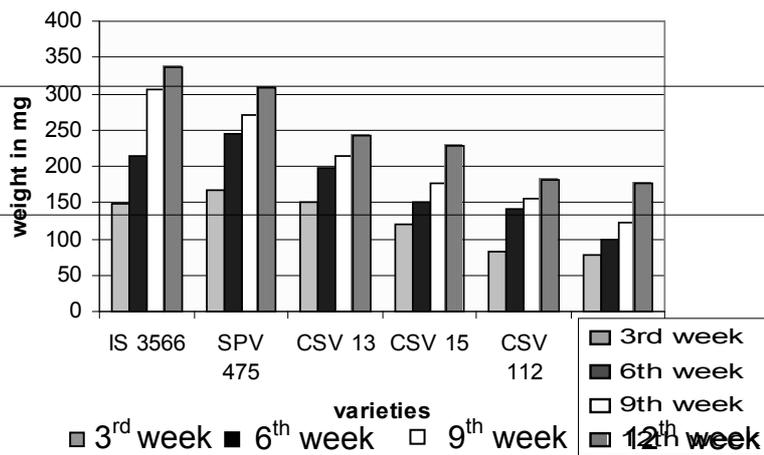Figure 4. Growth rate of fresh callus weight in different varieties

Figure 5. Callus dry weights in different varieties.

Different concentrations of cytokinins were tested for their effects on regeneration. High frequency of regeneration was observed on the regeneration medium with 2.0 mg/l BAP (Table 3). In general, regeneration on media containing higher concentrations of hormones was less prominent in appearance and turned yellow without further development. When TDZ, a phenyl urea compound used in the medium to observe its effect on shoot regeneration, it inhibited callus formation, and germination occurred in those embryos without any callus formation/ changing the medium. In all the varieties tested in this study, high shoot induction was observed in the variety IS 3566 *i.e.*, 22.85 shoots per culture and

low in IS 348 i.e., 8 shoots per culture (Figure 6). The shoot growth is initially slow during the 3rd week, latter increased growth was observed during the subculture period. In all the varieties, shoot growth was highest in the variety IS 3566. Shoots after attaining a length of 2-4 cms in height were transferred to a rooting medium containing half strength MS medium with 1mg/l NAA and 2% sucrose. Initially different concentrations of 0.5mg/l – 1.5mg/l of NAA were used for root induction; in those concentrations 1mg/l NAA gave good response. Root number was proportional to the shoot no. in all the varieties. Highest number of roots was observed in the genotype IS 3566 i.e., 40.62 (Figure 7). After root initiation cultures were transferred onto GA_3 medium for further shoot elongation and development (Figure 1H).

Well established plantlets with prominent shoot and root system (Figure 1I) was transferred to acclimatization chambers in green house. Plantlets were removed from the culture medium and after washing the roots gently under running tap water, plantlets were transferred to plastic pots containing. The potted plantlets were covered with porous polyethylene sheets for maintaining high humidity and were maintained under the culture room conditions. The plantlets kept under shade in a net house for further growth and development. There was no difference among the acclimatized plants as good as to *in vivo* plants with respect to morphological, growth characters (Figure 1J).

Table 3. Effect of different PGR concentrations on regeneration.

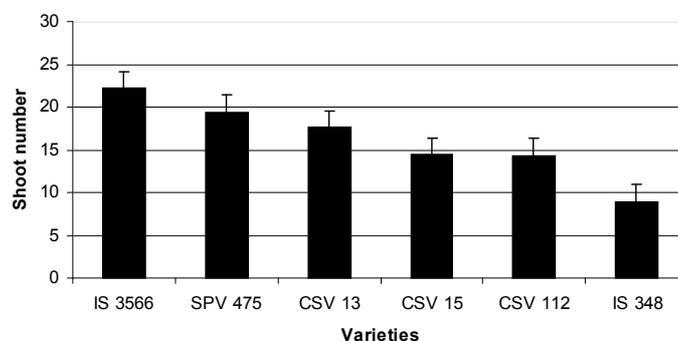| Concentration of PGR					Shoot number/ Treatment
BAP	KN	ZN	TDZ	GA$_3$	
1.0					14.2
1.5					18.6
2.0					22.82
2.5					20.65
3.0					18.42
	1.0				12.8
	1.5				16.4
	2.0				18.6
	2.5				18.66
	3.0				16.24
		1.0			12.71
		1.5			16.87
		2.0			18.13
		2.5			18.32
		3.0			16.78
			1.0		12.28
			1.5		16.39
			2.0		18.46
			2.5		16.14
			3.0		14.91
				1.0	10.82
				1.5	14.53
				2.0	18.98
				2.5	16.53
				3.0	14.39

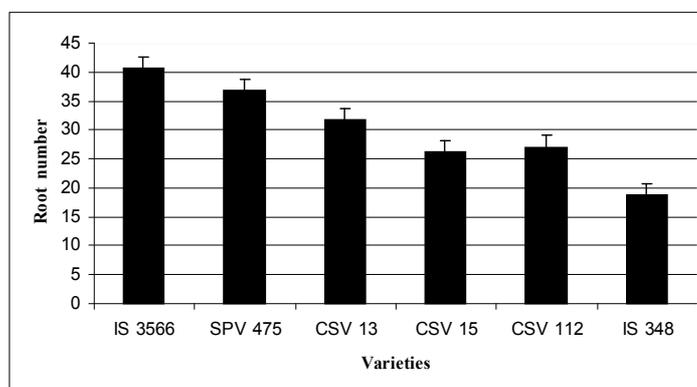Figure 6. Average number of shoots in different varieties from immature embryo.

Figure 7. Average number of roots in different varieties from regenerated shoots**.**

Discussion

Among the six varieties studied, the most suitable variety to produce maximum embryogenic callus was that of IS3566. Variety IS3566 showed higher values in terms of frequency of embryogenic callus, quantity of embryogenic callus, growth rate, regeneration frequency, number of regenerated plantlets per explant and number of roots per culture. While IS 348 showed lower values for most of the characters. Such genotypic differences were also observed by Hagio (1994), Rao et al., (2000), Gupta et al., (2006) and Jogeswar et al., (2007) in *Sorghum.*

In the present study, the use of immature embryo tissue enabled high embryogenic callus induction frequency, as well as the regeneration frequency, while mature explants failed to show such efficient response. In fact, this was the most critical factor for obtaining large number of somatic embryos from immature embryo. Bhojwani and Razdan (1996) reported that this ability is especially true for cereals. Rathus et al., (2004) reported that, the physiological stage of the source material (explant) used for callus initiation was found to be critical. Gupta et al., (2004) also reported that immature embryos size influenced callus formation and plant regeneration in *Sorghum*. Serhantova et al., (2004) found that small size immature embryo had 100% callus formation and a high efficiency of plant

regeneration than those with large size embryos, which corresponds with the results of our study.

Low level of 2, 4-D has been the most commonly used callus inducing hormone in the cereals, Hagio (1994) Manjula et al., (2000) and Bi et al., (2007) also reported that Cereals in general require 2, 4-D to initiate the callus cultures and its higher concentrations have been found to be less effective in the formation of embryogenic callus (Lu et al., 1983). A similar trend was observed in the present study, i.e., 2 mg/l 2, 4-D was optimum concentration to obtain high frequency of embryogenic calli.

Our results revealed that a combination of auxins with cytokinins boosted the embryogenic callus formation. Previous reports by Maheswari et al., (2006) Gupta et al., (2006), Pola and Mani (2006) also observed that, auxin and cytokinin combination will improve the embryogenic callus induction. Gupta et al., (2006) suggest that, to overcome the genotypic limitations of plant regeneration in *Sorghum,* the callus induction medium must be supplemented with strong cytokinin like Kinetin with 2,4-D. Whereas, Haliloglu (2006) reported that composition of cytokinin in callus induction medium could not increase the regeneration frequency in *Triticum aestivum.*

A distinct feature of callusing in cereals and grasses is the formation of two different types of calli viz., embryogenic and non embryogenic calli which differ markedly in their regenerative potential. A clear-cut difference between the two callus types could be made morphologically by the end of 12 -16 days after explant inoculation. The possibilities of such early distinction have some benefit for their separation quite at an early stage before the embryogenic type is out grown by the non embryogenic type.

Results on the growth rate of the embryogenic callus as reflected by the increase in fresh and dry weights upto 12 weeks indicated significant difference among the genotypes and auxin concentrations. Higher concentrations of auxins in the callus induction medium appear to be unfavorable for the faster growth rate of embryogenic callus. The culture response was greatly influenced by the genotype in all types of explants. Genotype effects on callusing ability from *Sorghum* were reported previously by Cai and Butler (1990).

A variety of plant growth hormones like those of BAP, KN, ZN and TDZ also been tried in several cereals and grass systems with varying effects (Arti et al., 1994; Kuruvinashetti et al., 1998; Murthy et al., 1990; Ouf et al., 1996; Shan et al., 2000). The regeneration in the immature embryo explants of the present study was also found to be influenced by the presence of BAP, ZN and TDZ .Whereas, Manjula et al., (2000) reported shoot initiation on hormone free medium. Rathus et al., (2004) observed the addition of various concentrations of cytokinins to the callus induction medium generally had a deleterious effect on both callus induction and subsequent regeneration. Whereas in our study the addition of cytokinins to the callus induction medium will result better embryogenic callus frequency and subsequent regeneration.

Treatment of callus with proline and asparagine in the regeneration medium, greatly improved the regenerative capacity of relatively low responding genotype like IS348. A number of amino acids like tryptophan, proline and serine have been reported to foster the development of somatic embryos in diverse taxa like *Oryza sativa* (Siriwardana and Nabors, 1883; Chowdhury et al., 1993) and *Zea mays* (Vasil and Vasil 1986). In *Sorghum*, Rao et al., (1995) reported that, a combination of proline and Serine provide appropriate nitrogenous compounds, to the growth medium and thus stimulate embryogenesis as well as regeneration. Vikrant and Rashid (2002) reported that, addition of amino acids and $AgNo_3$ to regeneration medium resulted in significant enhancement of embryogenesis on immature embryo cultures of minor millet.

Visarada et al., (2003) reported efficient regeneration with 0.5 mg BAP+IBA by using immature embryo as source material. Prathibha et al., (2001) reported that 2mg/l BAP+0.5 mg/l 2, 4-D were found to be suitable to obtain maximum percentage of regeneration in *Sorghum*. Rathus et al., (2004) observed that, the combination of 1mg/l IAA and 1mg/l ZN gave the best results for shoot regeneration.

Gupta et al., (2006) reported the correlation between the shoot induction frequency and the shoot number in *Sorghum* tissue culture and he also reported that, such studies are missing in *Sorghum* tissue culture. In our present study, a significantly positive correlation between embryogenic callus induction frequency and number of shoots was observed. These observations recommends that choice of genotypes with high embryogenic callus induction frequency would also result in selection of genotypes with more number of shoots and so the need of counting of shoots in a large selection programme may be obviated.

In our study among the different levels of NAA tried for rooting, 1.0 mg l^{-1} was found to give the best response. George and Eapen (1989), Rao and Kishore (1989) and Pola and Mani (2006) also obtained good rooting on MS medium supplemented with NAA.

Gao et al., (2005) reported that, *Sorghum has* been considered to be one of the most difficult plant species to manipulate through tissue culture. Shrawat and Lörz (2006) reported that, most of the cereal crops were difficult to genetically engineer, mainly as a result of their inherent limitations associated with the resistance to *Agrobacterium* infection and their recalcitrance to *in vitro* regeneration. Gupta et al., (2006) reported that, the genotypic limitations which is one of the major constraints in tissue culture of *Sorghum*. Whereas, in our study we have achieved efficient callus induction and enhanced regeneration from immature embryo explants of *Sorghum*. By means of appropriate culture conditions like selection of genotype, explant size, culture medium, P.G.R type and concentration and other environmental factors like temperature and light intensity were improved the embryogenic callus induction and regeneration frequency. Thus when all the five characters which are connected with the somatic embryogenesis process viz., frequency of embryogenic calli, quantity of embryogenic callus, growth rate, percentage of regeneration and number of regenerated plantlets per explant were considered together, IS 3566 showed higher response while IS 348 showed lower response for most of the characters studied in this experiment.

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