RESEARCH PAPER | Asian Journal of Bio Science, Volume **9** | Issue 1 | April, 2014 | 43-52
Received : 23.11.2013; **Revised :** 03.03.2014; **Accepted :** 13.03.2014

Somatic organogenesis and plant regeneration in castor (*Ricinus communis* L.)

V.K. ZALAVADIYA[1], D.R. MEHTA[1], **R.M. JAVIA[2]**, S.M. PADHIYAR[1] **AND** R.B. MADARIYA[1]

[1]Department of Biotechnology, Junagadh Agricultural University, JUNAGADH (GUJARAT) INDIA
[2]Department of Plant Breeding, Krishi Vigyan Kendra (J.A.U.), Nana-Kandhasar (Chotila), SURENDRANAGAR (GUJARAT) INDIA
Email : rmjavia@gmail.com

An *in vitro* propagation system developed for castor-bean (*Ricinus communis* L.) through hypocotyl derived callus cultures. Seeds were surface sterilized with 5 per cent bavistin for 30 min followed by 0.01 per cent $HgCl_2$ for 4 min to obtain *in vitro* seedlings germinated with growth regulator free MS medium. The impacts of different concentrations of auxins and cytokinins were evaluated for callus induction, shoot proliferation and root induction. Hypocotyls were excised from 10-12 days old *in vitro* seedlings and were cultured on Murashige and Skoog's (MS) medium supplemented with different concentration of BA, KIN and 2IP. White compact, nodular organogenic callus was obtained on the MS medium fortified with B_5 vitamins and 1.0 mg/l BA (80.84%) or 2.0 mg/l BA(80.17%). Shoot induction from the callus cultures was achieved on MS medium with 0.5 mg/l KIN + 0.25mg/l BAP (75.00%). Use of 0.2 mg/l GA_3 in combination with 0.5 mg/l KIN and 0.25 mg/l BAP induced maximum number of shoots per explants (7.00) as well as shoot length (6.49cm). For root induction, *in vitro* shoots were transferred to rooting media containing IAA, IBA and $AgNO_3$ singly or in combinations but root induction was not achieved even after 30 days of culture.

Key words : Ricinus communis, Auxins, Cytokinins, Callus cultures, Hypocotyl explants

How to cite this paper : Zalavadiya, V.K., Mehta, D.R., Javia, R.M., Padhiyar, S.M. and Madariya, R.B. (2014). Somatic organogenesis and plant regeneration in castor (*Ricinus communis* L.). *Asian J. Bio. Sci.*, **9** (1) : 43-52.

INTRODUCTION

Castor-bean (*Ricinus communis* L.) is one of the medicinally important non-edible oilseed crops widely cultivated in tropical, sub-tropical and temperate regions of the world for its essential oil and ricin (Atsmon, 1989) and belongs to the Euphorbiaceae family. The seeds contain approximately 60 per cent oil and are the only commercial source of ricinoleic acid that is used as industrial lubricants, paints, coatings, and plastics (Caupin, 1997). India is the largest producer of castor oil, representing 60 per cent of the global production followed by China and Brazil (FAO, 2006).

Globally, the most important aspect of castor breeding is to incorporate resistance against insect and pathogens while attaining high seed-oil content. The losses caused by biotic stresses and the cost of their control that most often limit more profitable production in castor. Reliable sources of resistance to the major insect pests are rather limited in the available germplasm of this monotypic genus (Sujatha and Sailaja, 2005).

Genetic engineering appears as one of the necessary tools for the improvement of cultivars of this species to lower the toxicity of seed meal and to confer resistance to biotic stresses. Despite the research efforts over the past three decades in castor tissue culture, no facile protocol of regeneration has been developed so far (Sarvesh *et al.*, 1992; Sujatha, 1996). Transformation of meristematic cell is perhaps an alternative through which transgenics from recalcitrant species like castor can be produced.To improve the transformation efficiency in castor, there is an urgent need to develop protocol for plant regeneration through adventitious shoot formation by testing various explants *viz.*, hypocotyls, epicotyls, endosperms, embryonic root, immature leaf, cotyledons etc.

The success of using such approach largely depends on an efficient *in vitro* regeneration system, which is rapid, reproducible and applicable to a broad range of genotypes. However, castor is extremely recalcitrant to *in vitro* regeneration (Ahn *et al.*, 2007). The previous reports on *in vitro* shoot multiplication of castor (Reddy *et al.*, 1987; Sarvesh

et al., 1992) using vegetative tissues as explants was either inefficient or difficult to reproduce (Ahn *et al.,* 2007). While shoot induction from embryonic tips and shoot apex involved pre-existing meristem and inefficient in adventitious shoot formation (Molina and Schobert, 1995; Sujatha and Reddy, 1998). Therefore, it is necessary to develop an effective regeneration protocol by a range of different techniques which would widen the possibilities of developing transgenic lines and/or somaclonal variants. Therefore, the objective of this work was planned to standardize a high frequency plant regeneration protocol in castor using various explants.

RESEARCH METHODOLOGY

***In vitro* seed germination :**

The castor-bean (*Ricinus communis* L.) seeds of two genotypes *viz.,* GC-3 (open pollinated variety) and JP-65 (pistillate line) collected from Main Oilseed Research Station, Junagadh Agricultural University, Junagadh, Gujarat state, India, and was surface sterilized with 5 per cent bavistin for 30 min followed by (0.01 and 0.1%) $HgCl_2$ for 4 min. The sterilized seeds were inoculated on germination medium containing of Murashige and Skoog's (1962) MS salts, 30 g/l sucrose and 0.8 g/l agar. The pH of the medium was adjusted to 5.8, using 0.1 M NaOH before autoclaving at 121^0 C for 15 min. The cultures were maintained in the light at 25 ± 2^0C and about 50 per cent relative humidity. Uniform light (40 to 50 $\mu E\ m^{-2}s^{-1}$) was provided by cool white fluorescent tubes over a light/dark cycle of 16/8 hours photoperiod with 2000-3000 a photon flux density.

Organogenic callus induction and maintenance :

Cotyledons, hypocotyls, epicotyls, immature leaves and embryos were used as initial explants and placed horizontally incallus initiation medium which contained MS salts, B_5 vitamins and varying concentrations of BA (1.0 to 3.0 mg/l), kinetin (KIN; 1.0 to 3.0 mg/l) and 6-(γ, γ, dimethyl allyl amino)-purine (2IP; 1.0 to 3.0 mg/l). After 3 weeks of culture, callus formation was observed from the cut end of the explants. The organogenic nature of the callus was identified by the presence of white colour with compact texture. The organogenic portions were isolated and sub-cultured in the same medium. The selected organogenic callus was weekly sub-cultured for another two weeks for the induction of matured white compact organogenic callus. For callus induction, maximum of 50 explants were tested and these experiments were repeated for three times with three replicates.

Adventitious shoot induction :

Six week-old organogenic callus (100 mg) was transferred to 200 cm^3 narrow bottles containing 25 cm^3 of shoot initiation medium. The cultures were sub-cultured weekly for2 months for the initiation of shoots. During each subculture, removal of dead, dark brown cells was necessary. Otherwise, the whole callus tissues become necrotic and become dead. The plant growth regulators such as BA (1.0 mg/l), KIN (1.0 mg/l) and 2IP (1.0 or 3.0 mg/l) in combination with NAA (0.6, 1.0, 1.4 mg/l) were tested for proliferation of shoots along with the medium consisting of MS salts, sucrose 30 g/l, and 0.8 per cent agar (pH 5.8). Ten such culture bottles were inoculated for each regeneration treatment. The cultures were incubated in a culture room under light for shoot regeneration.

Multiple shoots proliferation :

The regenerated shoots were separated from the callus masses. The shoots of approximately 1 to 2 cm of height were then transferred into best medium from organogenesis and shoot induction stage and different concentration of 1.0 mg/l BA + 0.6 mg/l NAA; 1.0 mg/l KIN + 0.6 mg/l NAA; 1.0 mg/l 2IP + 0.6 mg/l NAA; 3.0 mg/l 2IP + 1.4 mg/l NAA; 0.5 mg/l KIN + 0.25 mg/l BAP incombination with different concentrations of GA_3 (0.2, 0.3, 0.4 mg/l). A set of ten shoots per treatment was tested and each treatment was minimum three times sub-cultured on best medium for multiple shooting at 3-4 weeks interval.

Root induction in regenerated shoots :

The regenerated shoots were separated either from the callus masses or from multiple shoots. Old roots, if any, and remnants of callus were removed. The shoots of approximately 3 to 5 cm of height were then transferred to different MS medium with different concentrations of IAA (1.0, 2.0 mg/l), IBA (0.5, 1.0, 1.5 mg/l) and $AgNO_3$ (0.4, 0.6, 0.8 mg/l) alone or in combinations as rooting media.

Statistical analysis :

As all the experiments were conducted under the defined and controlled condition of the Plant Tissue Culture Laboratory, the data were analyzed by using factorial CRD as per stage according to Steel and Torrie (1960).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

***In vitro* seed germination :**

Even after three decades of research and development in plant tissue culture, microbial contamination by yeasts, fungi, bacteria and viruses are still the major problem that hampered the establishment of truly aseptic plants and their successful regeneration. Castor seeds from the field grown plants are generally laden with large numbers of spores of fungi and bacteria and hence to obtain the optimum sterilization for proper aseptic culture establishment and *in vitro*

germination, the castor seeds were partially de-coated and surface sterilized in 5 per cent bavistin for 30 min followed by 4-5 times rinses in sterile distilled water and 0.1 per cent and 0.01 per cent (w/v) mercuric chloride for 4 min followed by 4-5 times rinses in sterile deionized water. The surface sterilized seeds were then cultured on basal MS medium for 10 days.

Perusal of Table 1 showed that treatment S_2 (5% Bavistin for 30 min. and 0.01 % $HgCl_2$ for 4 min) was better surface disinfection for *in vitro* seed germination than treatment S_1 since S_2 showed less contamination (19%) and higher establishment (58%) as compared to treatment of S_1. Further all experimentation for *in vitro* seed germination was carried out with the same S_2 treatment. Surface sterilization with mercuric chloride ($HgCl_2$) and commercial bleach solution has also been reported by Ahn *et al.* (2007) and Alam *et al.* (2010) as an effective sterilant to reduce the risk of contamination in the cultures.

Organogenic callus induction :

The cotyledons, hypocotyls, epicotyls, immature leaves and embryos were collected from 10 to 12-d-old seedlings. Cotyledon, epicotyls, immature leaves and embryos showed very poor callus induction and hence, discarded from the further experiment, while compact and nodular calli were induced after 15 days of culture in case of hypocotyls as explants in both genotypes.

Callus response :

There were significant differences between two genotypes for callus induction. GC-3 variety (47.14%) had significantly higher per cent callus induction (Table 2) than that of JP-65 (45.05%). Different medium combinations did not affect appreciably the type of callus produced. However, the frequency of production of morphogenic type of callus varied with the levels of different PGR. The highest per cent callus induction (Fig. 1a) was observed in MS medium supplemented with 1.0 mg/l BA (80.84%) which was statistically at par with MS medium containing 2.0 mg/l BA (80.17%). In general, MS medium supplemented with any combination of BA gave higher per cent callus induction as compared to other PGR. Out of three plant growth regulators, BA is a potent cytokinin for callus induction and play a critical role in conferring morphogenic competence in tissue cultures of oilseed crops. Ganeshkumari *et al.* (2008) also reported highest callus induction in cotyledons of castor when cultured on MS medium supplemented with 2.0 mg/l BA (25.3%). Mean square due to genotypes × medium interaction was also significant for callus response and revealed that maximum per cent callus induction (96.11%) was recorded in MS medium supplemented with 1.0 mg/l BA in JP-65 (Table 2) which was statistically superior over rest of medium combinations. MS medium containing 2.0 mg/l BA in GC-3 variety also gave 92.11 per cent callus induction followed by MS + 3.0 mg/l BA in GC-3 (88.67%).

Table 1 : Effect of surface sterilization agent on establishment of castor seeds

No.	Surface sterilizing agent	No. of seeds inoculated	No. of seeds *in vitro* germinated (%)	No. of contaminated seeds (%)	No. of non-survival seeds (%)
S_1	5% Bavistin + 0.1 % $HgCl_2$	200	78 (39%)	64 (32%)	58 (29%)
S_2	5% Bavistin + 0.01 % $HgCl_2$	200	116 (58%)	38 (19%)	46 (23%)

Table 2 : Effect of different PGRs on organogenic callus induction from hypocotyl explants of castor

Treatment No.	mg/l	% callus responce		Days to callus initiation		Callus fresh weight (mg)		Callus dry weight (mg)	
		GC-3	JP-65	GC-3	JP-65	GC-3	JP-65	GC-3	JP-65
1.	1.0 BA	65.57	96.11	27.33	17.67	259.33	240.00	70.33	50.33
2.	2.0 BA	92.11	68.23	19.67	24.67	246.67	211.67	52.67	28.67
3.	3.0 BA	88.67	67.47	24.00	26.33	110.00	90.00	19.67	20.67
4.	1.0 KIN	66.23	28.23	29.00	29.00	220.00	92.33	39.67	21.67
5.	2.0 KIN	22.00	21.00	34.33	34.33	85.00	67.67	14.33	14.67
6.	2.0 KIN	14.33	22.33	36.33	33.33	65.00	78.67	9.67	11.67
7.	1.0 2IP	32.33	12.33	30.33	36.00	105.00	44.67	18.67	5.33
8.	2.0 2IP	23.67	14.67	34.00	33.33	77.67	54.33	14.00	9.33
9.	3.0 2IP	19.33	75.11	34.67	26.33	54.33	190.00	9.33	28.67
Mean of genotypes		47.14	45.05	29.96	29.00	135.89	118.81	27.59	21.22
		S.Em.	CD (P=0.05)	S.Em.	CD (P=0.05)	S.Em.	CD (P=0.05)	S.Em.	CD (P=0.05)
Genotypes		0.75	2.17	0.33	0.95	1.35	3.88	0.25	0.73
Medium combination		0.92	2.65	0.41	1.16	1.65	4.75	0.31	0.89
Genotypes × Medium combination		1.85	5.31	0.81	2.33	3.31	9.49	0.62	1.78

Days to callus initiation :

The visible callus in hypocotyls started appearing within 17 to 37 days in culture (Table 2). The callus initiation commenced from the cut end of the explants and later part or whole explants were covered with the callus. The comparison of mean for days to callus initiation in two genotypes revealed that minimum number of days to callus initiation was recorded in JP-65 (29.00 days) which was significantly lower than GC-3 (29.96 days). Pooled over both genotypes (Fig. 1b), MS medium supplemented with 2.0 mg/l BA recorded minimum number of days to callus initiation (22.17 days) which was statistically at par with MS + 1.0 mg/l BA (22.50 days). The maximum number of days to callus initiation was recorded in MS + 3.0 mg/l KIN (34.83 days) which was statistically at par with MS medium containing 2.0 mg/l KIN (34.33 days). The effect of genotypes × medium interaction as presented in Table 2 revealed significantly the least numbers of days to callus initiation (17.67 days) by the callus of JP-65 on MS medium containing 1.0 mg/l BA followed by MS medium supplemented with 2.0 mg/l BA in case of GC-3 (19.67 days). The maximum numbers of days to callus initiation was recorded by the callus initiated on MS + 3.0 mg/l KIN in GC-3 (36.33 days) and MS + 1.0 mg/l 2IP in JP-65 (36.00 days). During present experiment in our plant tissue culture laboratory, it was proved that phenolic excretion and oxidation was severe problem during callus induction and callus mediated regeneration and this problem was solved by the addition of some additives along with plant growth regulators as reported by Ganeshan and Jayabalan (2005).

Nature of callus :

In present study, newly initiated callus cultures from both genotypes were a mixture of two types *i.e.*, morphogenic and non-morphogenic. Morphogenic callus was nodular, hard and slow growing, while that of non-morphogenic was watery, loose and fast growing. Such mixed existence of two types of callus is a common phenomenon in castor (Ganeshkumari *et al.*, 2008; Naz *et al.*, 2011) and in other oilseed crops (Soomro and Memon, 2007; Rahman and Bari, 2013). Genotypic differences were also observed for callus colour. The callus induced from GC-3 was white, while JP-65 induced pale yellow coloured callus (Fig. 2a). Both genotypes did not differ with respect to callus morphology. The morphogenic callus of both genotypes was nodular, hard and slow growing. They reported two types of callus, one was green, compact, nodular and morphogenic and other yellow, friable and non- morphogenic which was similar to the callus described by Ganeshkumari *et al.*(2008). Rajore and Batra (2007) reported two types of callus *i.e.*, one was green, compact, and fast growing and other was yellowish pale green, watery. Soomro and Memon (2007) observed soft, very friable, compact, globular and lush green coloured callus. Compact and yellowish coloured calli was observed by Li *et al.* (2007) on MS medium containing on 1.5 mg/l BA and 0.05 mg/l IBA.

Callus fresh and dry weight :

GC-3 produced higher callus fresh weight (135.89 mg) and callus dry weight (27.59 mg) than that of JP-65 (118.81mg and 21.22 mg, respectively) (Table 2). Averaged over both

Table 3 : Effect of different concentration of plant growth regulators (PGRs) on shoot induction from callus cultures of castor

Treatment No.	Mg/l	Days to shoot initiation		Response (%)	
		GC-3	JP-65	GC-3	JP-65
1.	1.0 BA + 0.6NAA	32.67	29.33	54.33	61.33
2.	1.0 BA + 1.0NAA	34.33	34.33	52.00	53.00
3.	1.0 BA + 1.4NAA	36.67	38.00	47.33	42.33
4.	1.0 KIN + 0.6NAA	30.33	30.33	60.33	56.33
5.	1.0 KIN+ 1.0NAA	34.67	32.33	54.33	53.33
6.	1.0 KIN + 1.4 NAA	37.67	35.67	37.00	36.67
7.	1.0 (2IP) + 0.6NAA	27.00	NR	62.67	NR
8.	3.0 (2IP) + 0.6NAA	NR	30.00	NR	62.67
9.	1.0 (2IP) + 1.0NAA	31.00	NR	55.33	NR
10.	3.0 (2IP) + 1.0NAA	NR	32.33	NR	59.67
11.	1.0 (2IP) + 1.4NAA	32.00	NR	49.00	NR
12.	3.0 (2IP) + 1.4NAA	NR	35.67	NR	45.67
13.	0.5 KIN + 0.25BAP	20.33	19.33	72.33	77.67
Mean of genotypes		31.67	31.73	54.47	54.87
		S.Em.	CD (P=0.05)	S.Em.	CD (P=0.05)
Genotypes		0.27	NS	0.39	NS
Medium combinations		0.33	0.94	0.47	1.36
Genotypes × Medium combinations		0.65	1.87	0.94	2.71

genotypes, the MS media supplemented with 1.0 mg/l BA (Fig. 1 c&d) yielded significantly maximum callus fresh weight (249.70 mg) and dry weight (60.3mg) followed by media containing 2.0 mg/l BA (229.20 mg and 40.7 mg, respectively). The results of genotypes × medium interactions given in Table 2 revealed that significantly the highest callus fresh weight (259.33 mg) and dry weight (70.33 mg) was produced by GC-3 on MS + 1.0 mg/l BA followed by GC-3 on MS + 2.0 mg/l BA (246.67 mg and 52.67 mg) and that by JP-65 on MS + 1.0 mg/l BA (240.00 mg and 50.33 mg, respectively).

Callus maintenance :

The most suitable medium for maintenance of callus was selected on the basis of callus morphology and the frequency of morphogenic type of callus produced by the induction medium. For long term maintenance of callus and subsequent plant regeneration from it, friable, prolific and morphogenic type of callus is very important. Due to morphogenic nature of calli derived from hypocotyls of both genotypes, they were used for maintenance and multiplication to carry out further studies on regeneration of shoots.

After recording the observations from callus induction, the calli induced from hypocotyl explants of both the genotypes on MS medium containing different levels of BA, KIN and 2IP were sub-cultured on respective medium combinations. The subcultures were repeated for atleast two times at an interval of 35 days. The members of Euphorbiaceae are known to produce different types of callus which differed in their morphology and shoot regeneration capacity (Rajore and Batra, 2007; Ganeshkumari *et al.*, 2008; Naz *et al.*, 2011). It was observed that medium containing lower level of BA produced higher frequency of morphogenic type of callus. In the context, of nine induction medium tested, either MS + 1.0 mg/l BA or MS + 2.0 mg/l BA as the most suitable maintenance medium as it gave higher frequency of morphogenic type of callus with hypocotyl explants of both the genotypes. The morphogenic callus of GC-3 was green, nodular, hard and slow growing and JP-65 was pale yellow, nodular, hard and slow growing (Fig. 2 b). The colour and morphology of callus obtained in present study were similar to the morphogenic type of callus described by several workers (Li *et al.*, 2007; Rajore and Batra, 2007; Soomro and Memon, 2007; Ganeshkumari *et al.*, 2008; Naz *et al.*, 2011). During each sub-culturing, only the morphogenic type of callus was selected for maintenance.

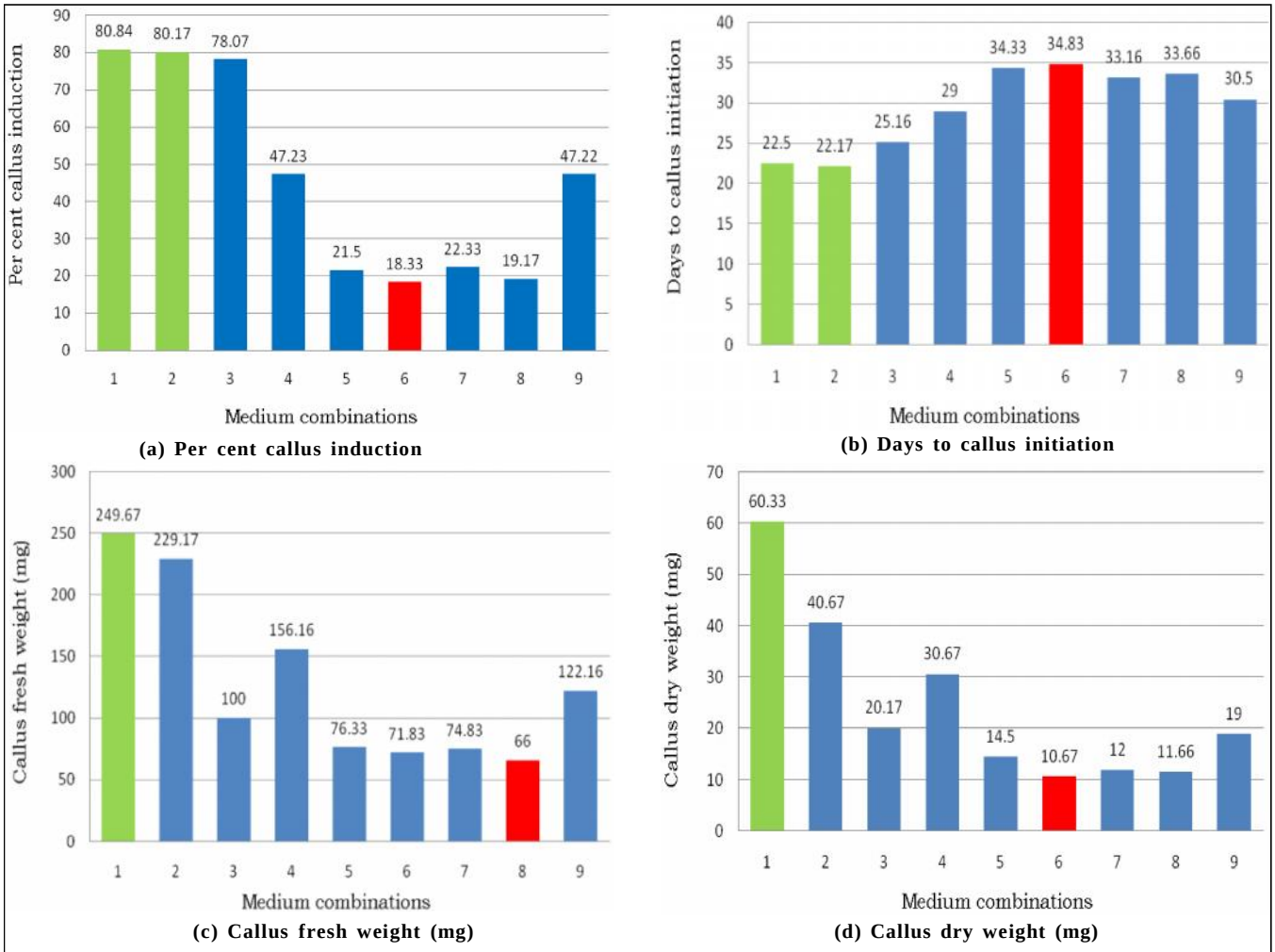
Thus, the actively growing calli from hypocotyls were selectively sub-cultured and maintained routinely after every five weeks on the same medium composition (MS + 1.0 mg/l BA or 2.0 mg/l BA) without any appreciable changes in morphology.

Adventitious shoot induction from callus cultures :

During shoot proliferation, all the treatments of BA + NAA, KIN + NAA, 2IP + NAA and KIN + BAP showed shoots

Table 4 : Effect of different PGRs on multiple shoot proliferation from callus cultures of castor

Treatment no.	Mg/l	Number of shoots per callus piece		Shoot length (cm)	
		GC-3	JP-65	GC-3	JP-65
1.	1.0 BA + 0.6 NAA + 0.2 GA_3	4.67	4.00	4.67	4.67
2.	1.0 BA + 0.6 NAA + 0.3 GA_3	2.73	2.67	2.67	3.00
3.	1.0 BA + 0.6 NAA + 0.4 GA_3	1.00	1.00	1.67	2.67
4.	1.0 KIN +0.6 NAA + 0.2 GA_3	3.67	3.00	6.00	3.67
5.	1.0 KIN + 0.6 NAA + 0.3 GA_3	2.33	1.67	3.33	2.00
6.	1.0 KIN + 0.6 NAA + 0.4 GA_3	1.67	1.00	1.67	1.67
7.	1.0 2IP + 0.6 NAA + 0.2 GA_3	5.33	NR	5.67	NR
8.	3.0 2IP + 1.4 NAA + 0.2 GA_3	NR	1.67	NR	1.67
9.	1.0 2IP + 0.6 NAA + 0.3 GA_3	3.67	NR	4.00	NR
10.	3.0 2IP + 1.4 NAA + 0.3 GA_3	NR	2.67	NR	2.67
11.	1.0 2IP + 0.6 NAA + 0.4 GA_3	2.00	NR	2.67	NR
12.	3.0 2IP + 1.4 NAA + 0.4 GA_3	NR	5.00	NR	5.00
13.	0.5 KIN + 0.25 BAP + 0.2 GA_3	6.67	7.33	5.97	7.00
14.	0.5 KIN + 0.25 BAP + 0.3 GA_3	6.00	5.67	3.00	5.67
15.	0.5 KIN + 0.25 BAP + 0.4 GA_3	2.33	3.33	1.67	3.33
Mean of genotypes		3.51	3.25	3.58	3.47
		S.Em.	C.D. (P=0.05)	S.Em.	C.D. (P=0.05)
Genotypes		0.06	0.16	0.06	NS
Medium combinations		0.07	0.20	0.07	0.20
Genotypes × Medium combinations		0.14	0.39	0.14	0.41



(a) Per cent callus induction **(b) Days to callus initiation**

(c) Callus fresh weight (mg) **(d) Callus dry weight (mg)**

Fig. 1 : Mean effect of different medium combinations on

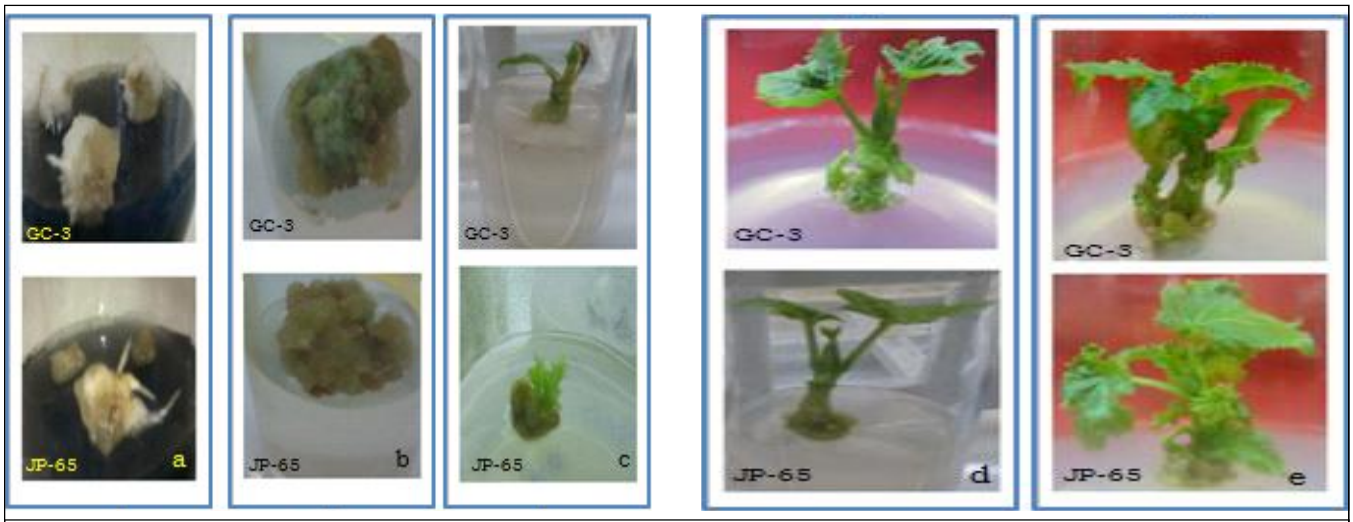


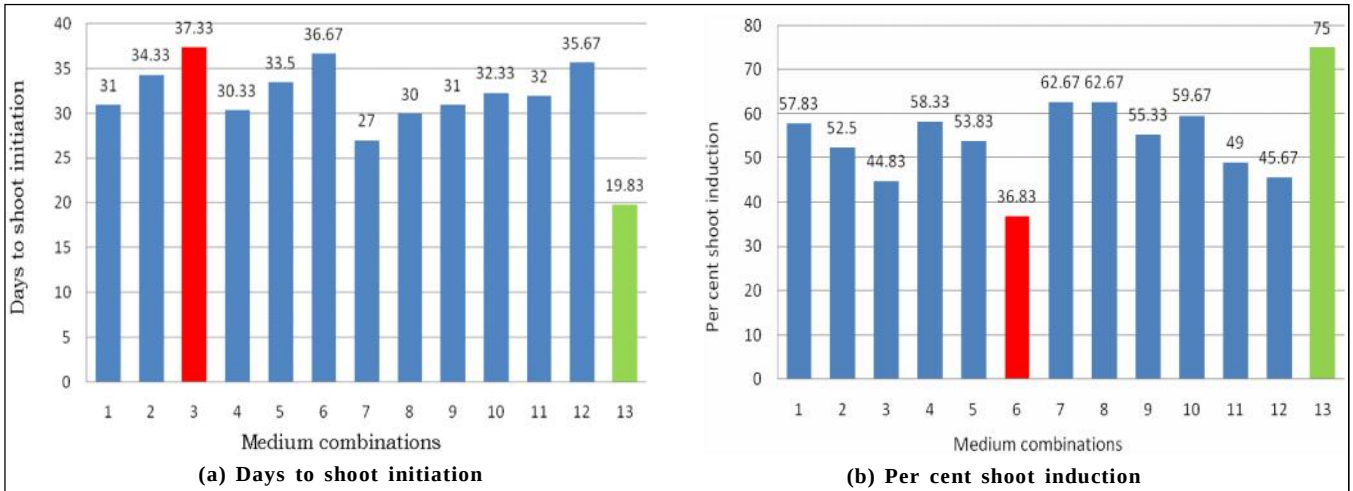
Fig. 2 : (a) Three weeks old white compact nodular organogenic callus obtained from hypocotyl explants; (b) Four-week old green compact nodular organogenic callus; (c) Initiation of shoot from callus cultures after 3 weeks of culture; (d) Five weeks old induced shoot; (e) Three-week-old proliferated multiple shoots

induction from the obtained callus cultures. The comparison of mean of main effects and the interaction effect for days to shoot initiation and per cent shooting is presented in Table 3. Pooled over different media, the callus of GC-3 took less number of days to shoot initiation (31.67 days) and was statistically at par with the days taken by JP-65 (31.73 days). Ganeshkumari *et al.* (2008) reported that cotyledon explants had higher in regeneration potential than other explants. Similar responses were also observed in *Jatropha* by Nogueira *et al.* (2011). Both results were contradictory to present finding those days to shoot initiation was observed only on callus grown on hypocotyl explants. In present study, the days taken for shoot initiation were highly influenced by the culture medium combinations. Pooled over genotypes, MS medium supplemented with 0.5 mg/l KIN + 0.25 mg/l BAP recorded significantly less number of days to shoot initiation (19.83 days) followed by treatment of MS + 1.0 mg/l 2IP +0.6 mg/l NAA (27.00 days) (Fig. 3a). The effect of genotypes × medium interaction (Table 3) revealed significantly the least numbers of days to shoot initiation by the callus of JP-65 (19.33 days) and of GC-3 (20.33 days) on MS medium containing 0.5 mg/l KIN + 0.25 mg/l BAP. The maximum numbers of days to shoot initiation was recorded by the callus of JP-65 (38.00 days) on MS + 1.0 mg/l BA + 1.4 mg/l NAA as well as by the callus of GC-3 (37.67 days) on MS + 1.0 mg/l KIN + 1.4 mg/l NAA.

Pooled over different media, the comparison of mean for per cent shooting revealed that the callus of JP-65 produced numerically higher per cent of shoot induction (54.87%) which was statistically at par with per cent shooting by GC-3 (54.47%). In present study, per cent shoot induction was observed only in hypocotyls explants of both genotypes. However, some workers observed that higher per cent frequency of shoot induction was found in leaf segments (Krishna *et al.*, 2010).In present study, the per cent shoot induction was highly influenced by the culture medium combinations. The maximum per cent shoot induction (75%) was also found on MS medium supplemented with 0.5 mg/l KIN and 0.25 mg/l BAP (Fig. 2 c,d) which was followed by the results obtained on MS + 1.0 mg/l 2ip +0.6 mg/l NAA in GC-3 (62.67%) and MS + 3.0 mg/l 2ip + 0.6 mg/l NAA in JP-65 (62.67%) (Fig. 3 b). The interaction effect of genotypes × medium combinations presented in Table 3 revealed significantly highest per cent shoot induction by the callus of JP-65 (77.67%) on MS medium containing 0.5 mg/l KIN + 0.25 mg/l BAP which was followed by the callus of GC-3 (72.33%) on the same medium combinations. The minimum per cent shooting was recorded by the callus of GC-3 (37.00%) and JP-65 (36.67%) on MS + 1.0 mg/l KIN + 1.4 mg/l NAA.Overall, during shoot proliferation, all the treatments of BA + NAA, KIN + NAA, 2IP + NAA, KIN + BAP showed shoot proliferation from the obtained callus cultures. After 20 to 30 days, maximum per cent shoot initiation (75%) within minimum number of days (~20 days) was recorded from the callus in the media fortified with MS + 0.5 mg/l KIN + 0.25 mg/l BAP.

Multiple shoot proliferation :

During multiple shoot proliferation, all the treatments of BA + NAA + GA_3, KIN + NAA + GA_3, 2IP + NAA + GA_3 and KIN + BAP + GA_3 showed multiple shoot proliferation from callus culture.The comparison of mean of main effects and interaction effect for number of shoot per callus piece and shoot length are presented in Table 4. Averaged over media, the callus of GC-3 produced significantly higher number of shoots per callus piece (3.51) as compared to the callus of JP-65 (3.25). Different medium combinations played a significant role in producing number of shoots from a piece of callus. Pooled over genotypes, MS medium containing 0.5 mg/l KIN + 0.25 mg/l BAP + 0.2 mg/l GA_3 (Fig. 4 a) regenerated significantly the highest number of shoots (7.0) (Fig. 2 e) which was followed by the results obtained on MS + 0.5 mg/l KIN +



(a) Days to shoot initiation **(b) Per cent shoot induction**

Fig. 3 : Mean effect of different medium combination on

0.25 mg/l BAP + 0.3 mg/l GA_3 (5.83) (Table 4). BAP in combination with GA_3 was also reported by Ganeshkumari *et al.*, 2008; Saharan *et al.*, 2011 for getting more numbers of shoots. The genotypes × medium interaction effect (Table 4) revealed that the callus of JP-65 regenerated maximum number of shoots per callus piece (7.33) on MS containing 0.25 mg/l BAP + 0.5 mg/l KIN + 0.2 mg/l GA_3 which was followed by the number of shoots produced by GC-3 on MS + 0.5 mg/l KIN + 0.25 mg/l BAP + 0.2 mg/l GA_3 (6.67). In general, higher number of shoot per callus piece was recorded on MS containing 0.5 mg/l KIN + 0.25 mg/l BAP + 0.2 mg/l GA_3 or 0.3 mg/l GA_3 in both genotypes (GC-3 and JP-65) of castor.

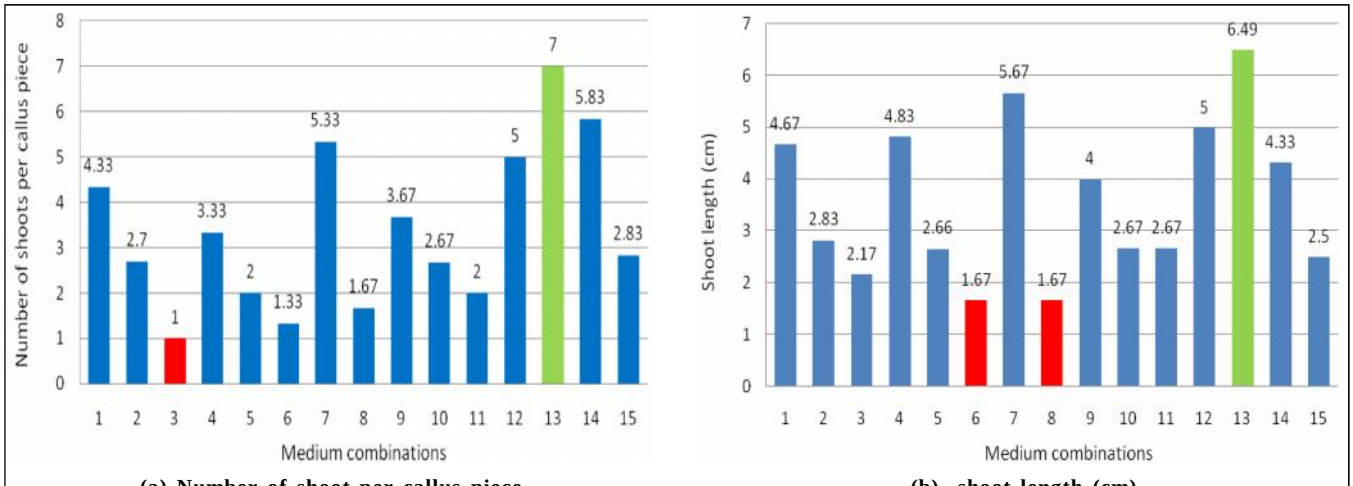
The comparison of mean of shoot length averaged over media revealed that the callus of GC-3 numerically produced higher length of shoot (3.58 cm) which was, however, statistically at par with the callus of JP-6 (3.47 cm). High response of regeneration from cotyledon explants in terms of highest length of shoots in castor was reported by Ganeshkumari *et al.* (2008) and Alam *et al.* (2010). Different medium combinations played a significant role in producing higher shoot length. Pooled over both genotypes, MS medium containing 0.5 mg/l KIN + 0.25 mg/l BAP + 0.2 mg/l GA_3 regenerated significantly the highest length of shoots (6.49 cm) which was followed by the results obtained on MS + 1.0 mg/l 2IP + 0.6 mg/l NAA + 0.2 mg/l GA_3 (5.67 cm) (Fig. 4b). MS medium containing 1.0 mg/l KIN + 0.6 mg/l NAA + 0.4 mg/l GA_3 gave the minimum shoot length of 1.67 cm. Several workers used BAP either alone or in combination with GA_3 for getting maximum length of shoots (Ganeshkumari *et al.*, 2008; Alam *et al.*, 2010; Raja *et al.*, 2011; Saharan *et al.*, 2011). The significant genotypes × medium interaction effect (Table 4) revealed that the callus of JP-65 regenerated maximum length of shoots (7.00 cm) on MS containing 0.5 mg/l KIN + 0.25 mg/l BAP + 0.2 mg/l GA_3 which was followed by the length of shoots produced by GC-3 on MS + 1.0 mg/l KIN + 0.6 mg/l NAA + 0.2 mg/l GA_3 (6.00 cm) and GC-3 on MS + 0.5 mg/l KIN + 0.25 mg/l BAP + 0.2 mg/l GA_3 (5.97 cm). In the present investigation, use of BAP in combination with GA_3 favors multiple shoot proliferation from the callus cultures of castor-bean.

Root induction :

Induction of rooting is an important step for *in vitro* plant propagation. The classical root induction method uses high auxin concentration, however, the roots are often stunted and malformed (Rao and Purohit, 2006). In present study, different medium combinations were tried for root induction *viz.*, (0.5, 1.0, 1.5 mg/l) IBA, (1.0 2.0 mg/l) IAA and (0.4, 0.6, 0.8) $AgNO_3$ singly or in combinations. However, root induction was not achieved even after 30 days of culture. Root induction in castor bean has been difficult compared to other plants (Ganeshkumari *et al.*, 2008). Hence, along with different auxin (IBA and IAA), or $AgNO_3$, combinations of IBA + $AgNO_3$ and IAA + $AgNO_3$ were also tried. $AgNO_3$ can influence root emergence and growth and can improve rooting efficiency (Bais *et al.*, 2000). However, Ganeshkumari *et al.* (2008) showed best response root induction from elongated shoots. Sujatha and Reddy (1998) obtained highest rooting with least callusing using 1.0 mg/l IBA, while Ahn *et al.* (2007) observed two different rooting pattern using IBA and NAA and percentage of rooting was inversely correlated to shoot development.

Conclusion :

An efficient and simple protocol for *in vitro* adventitious shoot multiplication from callus cultures of hypocotyl explants has been described. The protocol was optimized by manipulation of different plant growth regulators for enhanced multiplication. This partial protocol could be used for induction of somaclonal variation induction and production of transgenic plants through *Agrobacterium* and biolistic methods after standardization of *in vitro* rooting in castor.



(a) Number of shoot per callus piece **(b) shoot length (cm)**

Fig. 4 : Mean effect of medium combination on

LITERATURE CITED

Ahn, Y.J., Vang, L., McKeon, T.A. and Chen, G.Q. (2007). High-frequency plant regeneration through adventitious shoot formation in castor (*Ricinus communis* L.). *In Vitro Cell Dev. Biol.-Plant,* **43** (1) : 9-15.

Alam, I., Sharmin, S.A., Mondal, S.C., Alam, M.J., Khalekuzzaman, M., Anisuzzaman, M. and Alam, M.F. (2010). *In vitro* micropropogation through cotyledonary node culture of castor bean (*Ricinus communis* L.). *Australian J. Crop Sci.,* **4**(2) : 81-84.

Atsmon, D. (1989). *Castor : Oil crops of the World : Their breeding and utilization*, In: Robbelen G., Downey RK., Ashri A. (eds), McGraw-Hill, New York, pp. 438-447.

Bais, H.P., Sudha, G.S. and Ravishankar, G.A. (2000). Putrescine and silver nitrate influences shoot multiplication, *in vitro* flowering and endogenous titers of polyamines in *Chicorium intybus* L. cv. Lucknow Local., *Plant Growth Regul.,* **19** (2) : 238-248.

Caupin, H.J. (1997). Products from castor oil: Past, present, and future. In: Gunstone F.D. and Padley F.B. (eds.) Lipid Technologies and Applications., Marcel Dekker, New York, pp. 787-795.

Ganesan, M. and Jayabalan, N. (2005). Carbon source dependent somatic embryogenesis and plant regeneration in cotton, *Gossypium hirsutum* L. cv. SVPR2 through suspension cultures. *Indian J. Exp. Biol.,* **43** (10) : 921-925.

Ganeshkumari, K., Ganesan, M. and Jayabalan, N. (2008). Somatic organogenesis and plant regeneration in *Ricinus communis*., *Biol. Plantarum,* **52** (1) : 17-25.

Krishna, G., Reddy, S.P., Bhattacharya, P.S., Rambabu, P., Ramteke, P.W. and Masih, H. (2010). Shoot organogenesis and plantlet regeneration from *in vitro* raised leaf segments of *Jatropha curcas* L., *Trends Bio. Sci.,* **3**(1) : 45-48.

Li, M., Li, H., Jiang, H., Pan, X. and Wu, G. (2007). Establishment of an *Agro-bacteriuim*- mediated cotyledon disc transformation method for *Jatropha curcas. Plant Cell, Tissue & Organ Cult.,* **39** : 83–87.

Molina, S.M. and Schobert, C. (1995). Micropropagation of *Ricinus communis*. *J. Plant Physiol.,* **147** : 270–272.

Naz, S., Tabassum, F., Javad, S., Ilyas, S., Aslam, F., Munir, N. and Ali, A. (2011). Micropropagation and callogenesis of a recalcitrant species *Ricinus Communis. Pak. J. Bot*., **43**(5) : 2419-2422.

Nogueira, A.R.C., Soares, A.A., Ibrahim, A.B. and Campos, F.A.P. (2011). Analysis of organogenic competence of cotyledons of *Jatropha curcas* and their *in vitro* histological behavior. *African J. Biotech*., **10**(54) : 11249-11258.

Rahman, M.A. and Bari, M.A. (2013). Antibacterial activity of cell suspension cultures of castor (*Ricinus communis* L. cv. Roktima). *European J. Medicinal Plants,* **3**(1) : 65-77.

Raja, A., Blessy, J.A., Sathiya, R. and Jayabalan, N. (2011). *In vitro* regeneration of *Phyllanthus maderaspatensis* L. – A traditional medicinal plant. *Indian J. Natural Sci.,* **1**(4) : 217-224.

Rajore, S. and Batra, A. (2007). An alternative source for regenerable organogenic callus induction in *Jatropha carcus* L., *Indian J. Biotech.,* **6** (4) : 545-548.

Rao, M.S. and Purohit, S.D. (2006). *In vitro* shoot bud differentiation and plantlet regeneration in *Celastrus paniculatus* Wild. *Biol. Plant*., **50** (4): 501-506.

Reddy, K.R.K., Rao, G.P. and Bahadur, B. (1987). *In vitro* morphogenesis from seedling explants and calluscultures of castor (*Ricinus communis* L.). *Phytomorphology,* **37** : 337–340.

Saharan, V., Patel, B., Shah, M.A., Ranwah, B.R., Chabra, K., Shrimali, D. and Shukla, K.B. (2011). Adventitious plant regeneration and transient *gus* gene expression in *Jatropha curcas* (L.). *J. Cell & Tissue Res.,* **11**(2) : 2803-2808.

Sarvesh, A., Ram Rao, D.M. and Reddy, T.P. (1992). Callus initiation and plantlet regeneration from epicotyls and cotyledonary explants of castor (*Ricinus communis* L.). *Adv. Plant Sci.,* **5** : 124–128.

Soomro, R. and Memon, R.A. (2007). Establishment of callus and suspension culture in *Jatropha curcas. Pak. J. Bot.,* **39**(7): 2431-2441.

Steel, R.G.D. and Torrie, J.H. (1960). *Principle and procedure of statistics*. McGraw Hill Company Inc., New York, pp. 99-128.

Sujatha, M. (1996). Genetic and tissue culture studies in castor (*Ricinus communis* L.) and related genera. Dessertation, Osmania University, Hydrabad (A.P.) INDIA.

Sujatha, M. and Reddy, T.P. (1998). Differential cytokinin effects on the stimulation of *in vitro* shoot proliferation from meristematic explants of castor (*Ricinus communis* L.). *Plant Cell Rep.,* **17** : 561–566.

Sujatha, M. and Sailaja, M. (2005). Stable genetic transformation of castor (*Ricinus communis* L.) via *Agrobacterium tumefaciens*-mediated gene transfer using embryo axes from mature seeds. *Plant Cell Rep.*, **23** : 803-810.

■ WEBLIOGRAPHY

FAO, (2006). Online, *http://faostat.fao.org.*