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Research Article

HIGH FREQUENCY SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION OF BAUHINIA VARIEGATA, A MULTIPURPOSE TREE LEGUME

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ABSTRACT
High frequency somatic embryogenesis and plantlet regeneration was established using cotyledon and hypocotyl explant through direct and indirect ways in *Bauhinia variegata*, a recalcitrant tree legume. Explants cultured on Murashige and Skoog's basal medium supplemented with B5 vitamins in presence of either 2, 4-dichlorophenoxy acetic acid or picloram on induction medium, after 30 days, when sub-cultured on plant growth regulator-free medium, developed globular, translucent somatic embryos. Picloram (4 mg L^{-1}) in the induction medium gave best response in both types of explants. Explants were cultured on semi-solid Murashige and Skoog's basal medium containing different concentrations of either picloram or 2, 4-dichlorophenoxy acetic acid (1- 10 mg L^{-1}) in combination with benzylaminopurine (1mg L^{-1}) for the development of white friable callus. Calli were sub-cultured on plant growth regulator-free Murashige and Skoog's basal medium fortified with B5 vitamins for the induction of somatic embryos. The regeneration of somatic embryos was achieved on plant growth regulator-free medium but a low concentration of benzylaminopurine in the culture medium enhanced the percentage of regeneration. Germinated plantlets with well developed root and shoot, were acclimatized successfully, and transplanted to the experimental garden.

Key Words: *2, 4-Dichlorophenoxy Acetic Acid, Cotyledon, Hypocotyl, Picloram*

INTRODUCTION
Bauhinia variegata, commonly called orchid tree, grows in humid tropical countries, especially in the acid and degraded soils to which it can restore fertility owing to its natural nitrogen fixing ability (Acharya and Kafle, 2009). With the expanding biomass requirements due to population explosion, the demand for quality and quantity of firewood based products is continuously increasing leading to shrinking of forest cover with the species of *Bauhinia*. Besides, an infusion from the bark is used as an astringent, tonic, and for treating scrofula, skin disorders and ulcers. The decoction of the roots is used in dyspepsia and act as an antidote to snake poison (Frohne, 1999). Further phytochemical studies revealed the presence of several flavonoids (Gordon and David, 2001).The anti-inflammatory and antibacterial activity of all the extracts of *Bauhinia variegata* has also been reported (Rajkapoor *et al.,* 2006; Parekh and Chanda, 2007).

The conventional methods of propagation of *Bauhinia,* sexual as well as vegetative, are impeded with many problems that restrict their multiplication on a large scale. A fairly long time gap between pod formation and maturation hampers adequate supply of seeds in natural conditions (Jorge *et al.,* 2005). Micropropagation of this tree species offers a rapid means of producing clonal plant stock for afforestation, woody biomass production and conservation of elite germplasm (Mathur and Mukunthakumar, 1992). In order to meet the ever increasing demand of planting material, an *in vitro* regeneration protocol using seedling (Upreti and Dhar, 1996) and mature nodal explants in *Bauhinia vahlii* (Dhar and Upreti, 1999) was developed. Several investigations regarding developing suitable

methodology for rapid propagation of elite germplasm of *Bauhinia* species from seedling explants have been reported (Bhattacharya *et al.,* 2000).

Although the shoot tip and axillary bud culture are the most commonly practiced method for the large scale production of plantlets, somatic embryogenesis is the preferred technique, as it requires fewer steps with a concomitant reduction in labour, time and cost (Haissig *et al.,* 1987). Therefore somatic embryogenesis technique in the forest tree species will gradually replace the shoot tip culture method especially because it will be more amenable to automation. Biotechnological approaches of tree tissue culture lag behind those of herbaceous crop species mainly because tree tissues taken from mature individuals cannot be manipulated in a similar fashion to yield high frequencies of plantlets from any desired starting cell, cell aggregates or organs (Haissig *et al.,* 1987). It seems from the review of literature that somatic embryogenesis in *Bauhinia variegata* has not been done so far.

Therefore, the objective of the present investigation was to develop an efficient protocol of somatic embryogenesis through direct and indirect ways with high frequency regeneration of plantlets of this recalcitrant species.

MATERIALS AND METHODS

Preparation Of Explants

Seeds of *Bauhinia variegata* were obtained from the forests of Santiniketan, Birbhum, West Bengal. The seeds were surface sterilized by the method of Banerjee *et al.,* (2007). The surface sterilized seeds were allowed to germinate on the sterile wet cotton bed within conical flask. After 10-12 days of germination, cotyledon and hypocotyl were excised and used as explants for direct and indirect somatic embryogenesis.

Direct Somatic Embryogenesis

Excised cotyledons and hypocotyls were cultured on (induction medium) the semi solid (0.8% w/v agar) MS basal medium (Murashige and Skoog, 1962) supplemented with B5 vitamins (Gamborg *et al.,* 1968), and plant growth regulators 2, 4-D (1-15mg L^{-1}) and picloram (1-15 mg L^{-1}) individually for direct embryoid induction. The pH of the media was adjusted to 5.8 with NaOH and HCl prior to autoclaving at 121°C for 15 minutes with 1.06 Kg cm^{-2} pressure. The explants were incubated for 30 days at 25 ± 2°C under cool white fluorescent light at 40µ $Em^{-2}s^{-1}$ with 16 hour photoperiod. The explants were cultured on 20 ml medium in glass test tubes plugged with non-absorbent cotton plugs for 30 days and were sub-cultured for direct embryo induction on the same medium without PGR (secondary medium) for another 30 days.

Indirect Somatic Embryogenesis

Cotyledon and hypocotyl explants were used to initiate callus mediated somatic embryogenesis. Explants were cultured for the development of white friable callus on the MS basal medium semi solidified with agar (0.8% w/v) for 30 days. Callus inducing media were PGR-free as well as with different concentrations of either picloram or 2, 4-D (1- 10 mg L^{-1}) individually or in combination with BAP (1 mg L^{-1}). Calli were sub-cultured on semi solid PGR-free MS basal medium fortified with B5 vitamins (Gamborg *et al.,* 1968) for another 30 days for the induction of somatic embryos.

Regeneration of Plantlets

The induced embryoids of both direct and indirect ways were then subcultured on the MS medium fortified with B5 vitamins without PGR and allowed to reach maturation. Matured embryos were detached and transferred to embryoid regeneration medium for further growth and development. Embryoid regeneration medium was semi solid PGR-free MS basal medium supplemented with B5 vitamins as well as with different concentrations of BAP (0.1- 0.3 mg L^{-1}).

Transplantation of plantlets

Germinated embryos with well developed shoot and root were removed from the medium. The roots were gently washed under running tap water and the plantlets transferred to plastic pots containing autoclaved

soil. The potted plants were covered by plastic bags to provide a high relative humidity. The plantlets were maintained in a growth room under a 16 hr photoperiod at 25 ± 2^0 C, and the plants were watered every alternate day. Plantlets were gradually acclimatized to room temperature and humidity conditions by progressively opening the bag until plants were ready to be completely removed from the bags. After adaptation, the selected plants were transferred to earthen pots for further growth and development.

Statistical Analyses

Experimental data were analyzed by ANOVA. After obtaining a significant F- value ($\alpha = 0.05$), the treatment means were separated by DMRT. All statistical analyses were performed according to Little and Hills, (1978).

Histological Analysis

Tissues containing somatic embryos were fixed in formalin, acetic acid and alcohol, dehydrated in a tertiary butyl alcohol series and embedded in paraffin wax for histological studies. Paraffin embedded plant materials were sectioned on a microtome at 10 µ thicknesses, stained with saffranin and mounted on DPX.

RESULTS AND DISCUSSION

Direct induction of somatic embryogenesis was achieved on cotyledon and hypocotyl explants in presence of picloram and 2, 4-D individually. PGR-free MS medium with B5 vitamins was unable to bring any sort of morphogenesis directly from cotyledon and hypocotyl. Explants cultured in presence of either 2, 4-D or picloram on induction medium, after 30 days, when sub-cultured on PGR-free medium, developed globular somatic embryos (Fig. 1a). Histological observation made on the explants bearing globular masses showed the presence of somatic embryos of different stages (Fig. 1g).

Somatic embryos were developed from a meristematic zone of cells developing below the epidermis, which is in accordance with the observation made by Guz *et al.*, (1999) in myrtle. Picloram (4mg L^{-1}) in the induction medium gave best response in both types of explants (Table: 1). Picloram proved better than 2, 4-D in terms of the percentage of response and the mean number of embryoids per explants which is in conformity with the findings of Bhanumathi *et al.*, (2005). Picloram is a very potent phytohormone capable of inducing somatic embryogenesis in many plants (Eapen and George, 1990; Venkatesh *et al.*, 2009).

Cotyledon showed better response than hypocotyls and proved greater potentiality in the development of somatic embryo than hypocotyl which is in conformity with (Chee, 1990) in cucumber plants. This might be due to the presence of residual axillary meristem (Zambre *et al.*, 1998). Residual axillary meristem favours the formation of somatic embryo (Garcia *et al.*, 2010). However, different tissues can respond in different ways during the *in vitro* culture process (Jimenez, 2001; Banerjee *et al.*, 2007; Banerjee *et al.*, 2011) and the requirements for PGRs appear to be tissue specific (Venkatachalam *et al.*, 1999; Banerjee *et al.*, 2007; Banerjee *et al.*, 2011). Further, (Picciarelli *et al.*, 2005) and (Venkatesh *et al.*, 2009) deduced from their experiment that endogenous auxin in the cotyledon explants plays an important role in the induction of somatic embryos. Cotyledon derived from zygotic embryos reported to be suitable explants for somatic embryogenesis either directly or indirectly in various tree species like *Hevea brasiliensis* (Herve, 1993), *Azadirachta indica* (Srikhande *et al.*, 1993). Zygotic embryogenic tissues are amenable to somatic embryogenesis as they contain large amount of pre-embryogenic determined cells. However, globular somatic embryos subsequently developed into heart-shaped, late heart-shaped, torpedo-shaped embryos and these torpedo-shaped embryos further matured into cotyledonary-stage embryo (Fig. 1d) in the same medium.

PGR-free medium, as well as either auxin (Picloram or 2, 4-D) or cytokinin (BAP) individually was unable to induce callus in both cotyledon and hypocotyl. Embryogenic callus was induced on MS basal medium fortified with B5 vitamins and a constant amount of BAP (1 mg L^{-1}) with various concentrations (1-10 mg L^{-1}) of picloram or 2, 4-D (Fig. 1b). Creamy white friable callus became brown in higher

concentrations of picloram and 2, 4-D (10 mg L^{-1}). Somatic embryos were developed from the surface of callus after sub-culture on the same medium without PGR (Fig. 1c). Picloram at all concentrations (1-10 mg L^{-1}) in callus induction medium resulted in the development of somatic embryos (Table: 2).

Table 1: Effect of picloram and 2, 4-D on direct somatic embryogenesis of *Bauhinia variegata* from different explants cultured on MS medium fortified with B5 vitamins (response recorded after 30 days of incubation)

Concentrations of growth regulators in induction medium (mg L^{-1})	PGR-free secondary medium			
	Type of explant			
	Cotyledon		Hypocotyl	
	Explant response (%)	Mean no. of embryoids / explant	Explant response (%)	Mean no. of embryoids / explant
PIC				
1.0	78.44 ^{d}	32.41±2.28 ^{e}	0	0
2.0	86.25^{c}	66.72±4.36^{bc}	23.12^{c}	08.46±2.82^{c}
4.0	97.33 ^{a}	83.64±4.28 ^{a}	49.44^{a}	17.55±4.75^{a}
6.0	92.54 ^{b}	79.52±6.32 ^{b}	48.24^{a}	15.25±3.34^{a}
8.0	90.64 ^{bc}	61.44±4.29 ^{c}	38.44^{b}	12.24±4.21^{ab}
10.0	84.25 ^{cd}	46.23±5.30 ^{cd}	36.22^{b}	11.63±3.24^{b}
12.0	74.22 ^{de}	42.16 ±3.26 ^{d}	28.84^{c}	08.12±2.44^{c}
15.0	72.84^{e}	41.42±4.23^{e}	16.25^{d}	07.39±4.39^{d}
2, 4-D				
1.0	56.66^{d}	17.4 ± 0.18 ^{f}	0	0
2.0	62.25^{bc}	33.6 ± 0.18 ^{e}	0	0
4.0	67.33 ^{b}	56.3 ± 0.21 ^{b}	0	0
6.0	70.66 ^{a}	67.5 ± 0.22 ^{a}	0	0
8.0	65.25^{b}	52.1 ± 0.24 ^{b}	12.24^{d}	07.29±2.19^{d}
10.0	61.66 ^{c}	47.2 ± 0.20 ^{c}	24.66^{c}	10.21±3.16^{bc}
12.0	56.25^{d}	41.0 ± 0.18^{d}	32.44^{a}	14.34±3.39^{a}
15.0	51.33 ^{e}	30.7 ± 0.18 ^{e}	26.84^{b}	09.78±2.57^{c}

Values are mean ± SE of three repeated experiments
Mean values followed by the same letter are not significantly different at P=0.05 level according to DMRT

Friable callus generated on the medium with higher concentrations of auxins when transferred onto the auxin free medium, developed somatic embryos which is in conformity with the observation made in *Vigna mungo* by Muruganantham *et al.*, (2010). Embryogenic callus generated with lower levels of auxin (either picloram or 2, 4-D) when sub-cultured in PGR-free medium developed lesser number of somatic

embryos. The frequency and the number of embryo increased with the increase in concentration of picloram or 2, 4-D in the induction medium, although at very high concentration of auxin, the frequency of embryogenesis decreased significantly. Reduction in the number of embryos with the increase in the

Table 2: Effect of picloram and 2, 4-D level in induction medium on indirect somatic embryogenesis from cotyledon and hypocotyl explants of *Bauhinia variegata* after 30 days on PGR-free secondary medium

Concentrations of growth regulators in callus induction medium (mg L^{-1})	PGR-free secondary medium			
	Type of explants			
	Cotyledon		Hypocotyl	
	Explant response (%)	Mean no. of somatic embryos ± SE	Explant response (%)	Mean no. of somatic embryos ± SE
PIC				
1.0	100	75.16±3.12 bc	62	35.79±8.32 c
2.0	100	78.37±4.23 b	100	42.26±5.12 b
4.0	100	82.45±2.91 a	100	43.22±5.12 b
6.0	100	83.30±2.32 a	100	49.36±4.80 a
8.0	100	68.66±5.38 d	100	48.32±3.34 a
10.0	100	59.34±3.43 e	100	21.79±8.32 d
2, 4-D				
1.0	0	0	0	0
2.0	0	0	0	0
4.0	44.22[c]	21.36±2.41[d]	32.22[d]	12.10±2.19[c]
6.0	74.66[b]	35.42±2.69[c]	36.66[c]	14.35±2.49[bc]
8.0	78.44[a]	53.21±4.46[a]	48.24[a]	19.15±3.42[a]
10.0	62.22[b]	42.36±3.61[b]	44.48[b]	19.39±3.17[a]

Values are mean ± SE of three repeated experiments
Mean values followed by the same letter are not significantly different at P=0.05 level according to DMRT

Table 3: Effect of BAP on regeneration of plantlets from somatic embryos derived from direct and indirect somatic embryogenesis in *Bauhinia variegata* after 30 days on regeneration medium

Concentrations of BAP in regeneration medium (mg L^{-1})	Percentage of regenerated plantlets ± SE	
	From direct somatic embryos	From indirect somatic embryos
0.00	51.23±3.34bc	52.88±4.23bc
0.10	55.17±2.13b	58.01±3.71b
0.20	88.21±3.87a	81.66±2.34a
0.30	86.11±2.42a	84.33±3.45a
0.50	82.42±1.87a	80.27±1.17a

Values are mean ± SE of three repeated experiments
Mean values followed by the same letter are not significantly different at P=0.05 level according to DMRT

concentration of picloram or 2, 4-D was in conformity with the results obtained in a grain legume, groundnut (Baker and Wetzstein, 1994; Bhanumathi *et al.*, 2005) and also in other species like tea (Bano *et al.*, 1991) and papaya (Fitch and Mansherdt, 1990).

One of the striking features of somatic embryogenesis recorded in this woody species was that the embryogenic callus distinctly differed from non-embryogenic one and the former was often covered with translucent globular somatic embryos (Fig. 1c). Differentiation of the callus following such pattern was also recorded in *Sapindus mukorossi* (Sinha *et al.*, 2000).

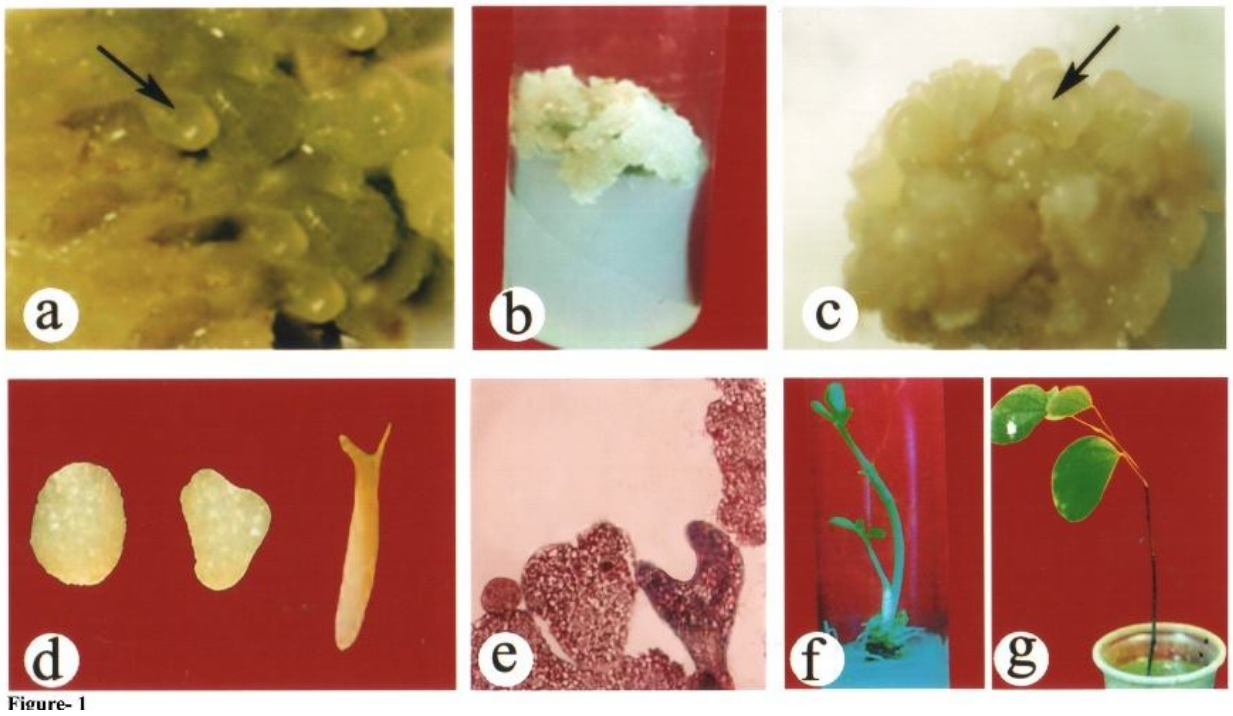

Figure- 1

Figure 1: Somatic embryogenesis of *Bauhinia variegate* (a)Somatic embryos developed directly on the surface of the cotyledon explants, (b)White, friable embryogenic callus, (c)Somatic embryos developed on the surface of embryogenic callus, (d)Somatic embryos of different developmental stages - globular, heart and torpedo-shaped, (e)Plantlet regenerated from Somatic embryo, (f)Plantlet transferred to plastic pot, (g)Microtome section through tissues containing somatic embryos

Somatic embryos, derived from direct and indirect embryogenesis, were regenerated into plantlets in the MS medium fortified with B5 vitamins and BAP (0.1- 0.3 mg L^{-1}) (Fig. 1e). The regeneration of somatic embryos derived both from direct and indirect method was achieved in PGR free medium but a low concentration of BAP (0.2mg L^{-1}) in the culture medium enhanced the percentage of regeneration (Table: 3). Although the somatic embryos posses both root and shoot meristems, simultaneous development of root and shoot was less frequent which corroborated with the findings of Venkatachalam *et al.*, (1999). The conversion of somatic embryos into plantlets depended on the type and concentration of auxin used in the somatic embryo induction medium (George and Eapen, 1993). However, BAP-mediated enhanced somatic embryo regeneratrion was also reported in leguminous plants (Chengalrayan *et al.*, 2001; Bhanumathi *et al.*, 2005; Ghanti *et al.*, 2010), and other species like *Eryngium foetidum* (Ignacimuthu *et al.*, 1999) and cotton (Ganesan and Jayabalan, 2004).

Regenerated plantlets with well developed root and shoot, were acclimatized successfully (Fig. 1f), and transplanted to the experimental garden. Transplanted plants showed 100% survival, appeared normal and continued to grow showing the development of new leaves.

In the present study, a protocol of high frequency of somatic embryogenesis and plantlet regeneration of *Bauhinia variegata* has been achieved. The technique described here for the propagation of this commercially important species could be effectively applied for rapid propagation as well as for conservation purposes. Somatic embryos could possibly be encapsulated to use as artificial seeds for mass propagation of elite germplasm during afforestation.

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REFERENCES

Acharya A and Kafle N (2009). Land degradation issues in Nepal and its management through agro forestry. *Journal of Agriculture and Environment* **10**(1) 115-123.

Baker CM and Wetzstein HY (1994). Influence of auxin type and concentration on peanut somatic embryogenesis. *Plant Cell Tissue and Organ Culture* **36**(3) 361-368.

Banerjee P, Maity S, Maity SS and Banerjee N (2007). Influence of genotype on *in vitro* multiplication potential of *Arachis hypogaea* L. *Acta Botanica Croatica* **66**(1) 15-23.

Banerjee P, Maity S and Banerjee N (2011). *In vitro* propagation of *Arachis hypogaea* L. var. AK 1224: Direct and indirect plant regeneration. *Plant Cell Biotechnology and Molecular Biology* **12**(1-4) 57-62.

Bano Z, Rajarathnam S and Mohanty BD (1991). Somatic embryogenesis in cotyledon cultures of tea (*Thea sinensis* L.). *Journal of Horticultural Sciences* **66**(4) 465- 470.

Bhanumathi P, Ganesan M and Jayabalan N (2005). A simple and improved protocol for direct and indirect somatic embryogenesis of peanut (*Arachis hypogaea* L.). *Journal of Agricultural Technology* **1**(2) 327-344.

Bhattacharya S, Dasgupta S and Chatterjee P (2000). In vitro regeneration of plantlets from seedling explants of different species of *Bauhinia* L. *Plant Cell Tissue and Organ Culture* **10** 103-109.

Chee PP (1990). High frequency of somatic embryogenesis and recover of fertile cucumber plants. *Horticultural Science* **25**(7) 792-793

Chengalrayan K, Hazra S and Meagher MG (2001). Histological analysis of somatic embryogenesis and organogenesis induced from mature zygotic embryo-derived leaflets of peanut (*Arachis hypogaea*). *Plant Science* **161** 415-421.

Dhar U and Upreti J (1999). *In vitro* regeneration of a mature leguminous liana (*Bauhinia vahlii* Wight & Arnott). *Plant Cell Reports* **18** 664-669.

Eapen S and George L (1990). Ontogeny of somatic embryos of *Vigna aconitifolia, Vigna mungo* and *Vigna radiata*. *Annals of Botany* **66** 219– 226.

Fitch MM and Manshardt RM (1990). Somatic embryogenesis and plant regeneration from immature zygotic embryos of papaya (*Carica papaya* L.). *Plant cell Reports* **9** 320-324.

Frohne D (1999). Plants intoxicants - Is there a risk for herbal preparation? *Journal of Phototherapy and Pharmaceutical Biology* **39** 8-17.

Gamborg OL, Miller RA and Ojima K (1968). Nutrient requirements of suspension cultures of soyabean root cells. *Experimental Cell Research* **50** 151-158

Ganesan M and Jayabalan N (2004). Evaluation of haemoglobin (erythrogen): for improved somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L. cv. SVPR 2). *Plant Cell Reports* **23** 181–187.

Garcia LR, Perez J, Kosky RG, Bermudez-Caraballoso I, Veitia N, Collado R, Padron Y, Torres D, Romero C and Reyes M (2010). Regeneración de plantas via embriogénesis somática en *Phaseolus acutifolius* A. Gray. *Biotechnologia Vegetal* **10**(3) 143-149.

George L and Eapen S (1993). Influence of genotype and explants source on somatic embryogenesis in peanut. *Oleaginelux* **48** 361-364.

Ghanti SK, Sujata KG, Srinath Rao M and Kavikishor PB (2010). Direct somatic embryogenesis and plant regeneration from immature explants of chickpea. *Biologia Plantarum* **54** (1) 121-125.

Gordon MC and David JN (2001). Natural product drug discovery in the next millennium. *Pharmaceutical Biology* **39** 8-17.

Guz SG, Lopes ML and Canhoto JM (1999). Somatic embryogenesis and plant regeneration in myrtle. *Plant Cell Tissue and Organ Culture* **57** 13-21.

Haissig BE, Nelson ND and Kidd GH (1987). Trends in the use of tissue culture in forest improvement. *Biotechnologia* **5** 52-59.

Herve E, Sotta B, Montoro P, Miginiac E and Carron MP (1993). Somatic embryogenesis in rubber from seedling explants. *Plant Science* **88** 91-96.

Ignacimuthu S, Arockiasamy S, Antonysamy M and Ravichandran P (1999). Plant regeneration through somatic embryogenesis from mature leaf explants of *Eryngium foietidum*, a condiment. *Plant Cell Tissue and Organ Culture* **56** 131-137.

Jimenez VM and Bangerth F (2001). Hormonal status of maize initial explants and of the embryogenic and non-embryogenic callus cultures derived from them as related to morphogenesis *in vitro*. *Plant Science* **160** 247 – 257.

Jorge I, Ali M and Rocha JO (2005). Selective seed abortion affects the performance of the offspring in *Bauhinia ungulate*. *Annals of Botany* **95** 1017-1023.

Little TM and Hills FJ (1978). Agricultural experimentation. John Wiley and sons, New York

Mathur J and Mukunthakumar S (1992). Micropropagation of *Bauhinia variegata* and *Parkinsonia aculeate* from nodal explants of mature trees. *Plant cell Tissue and Organ Culture* **28** 119-121.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15** 473-499.

Muruganantham M, Amutha S and Ganapathi A (2010). Somatic embryo productions by liquid shake culture of embryogenic calluses in *Vigna mungo* (L.) Hepper. *In Vitro Cell Developmental Biology- Plant* **46** 34-40.

Parekh J and Chanda S (2007). Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. *African Journal of Biomedical Research* **10** 175-181.

Picciarelli P, Ceccarelli N, Paolicchi F and Calistri G (2005). Endogenous auxins and embryogenesis in *Phaseolus coccineus*. *Australian Journal of Plant Physiology* **28** (1) 73-78.

Rajkapoor B, Jayakar B, Murugesh N and Saktisekharan D (2006). Chemopreservation and cytotoxic effect of *Bauhiunia variegata* against N –nitrosodiethylamine induced liver tumors and human cancer cell lines. *Journal of Ethnopharmacology* **104** 407-409.

Sinha S, Mazumdar K and Sinha RK (2000). Somatic embryogenesis and plantlet regeneration from leaf explants of Sapindus mukorossi Gaerten: A soapnut tree. *Current Science* **78** 620-623.

Srikhande M, Thengane SR, Mascarenhas AF (1993). Somatic embryogenesis and plant regeneration in *Azadirachta indica* A. Juss. *In vitro Cell Developmental Biology- Plant* **29** 38-42.

Upreti J and Dhar U (1996). Micropropagation of *Bauhinia vahlii* Wight & Arnott. - a leguminous liana. *Plant Cell Reports* **16** 250-254.

Venkatachalam P, Geetha N, Khandelwal A, Shaila MS and Lakshmi Sita G (1999). Induction of direct somatic embryogenesis and plant regeneration from mature cotyledon explants of *Arachis hypogaea* L. *Current Science* **77** 269-273.

Venkatesh K, Rani R A, Baburao N and Padmaja G (2009). Effect of auxins and auxin polar transport inhibitor (TIBA) on somatic embryogenesis in groundnut (*Arachis hypogaea* L.). *African Journal of Plant Science* **3** 288-293.

Zambre MA, Deelarq J, Vranova E and Dilen W (1998). Plant regeneration from embryo derived callus in *Phaseolus vulgaris* L. and *P. acutifolius* A. *Plant cell Reports* **17** 626-630.