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In Vitro Regeneration of Inflorescence and Callus Enhancement of *Celastrus paniculatus* Willd.

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ABSTRACT

A protocol for plant regeneration with an intervening callus phase was developed for *Celastrus paniculatus* Willd. using immature inflorescence segments and flower buds. The floral explants are proliferate into callus mass on Linsmaier and Skoog (LS) medium augmented with 3% fructose as the carbon source and the plant growth regulators kinetin (Kn) and indole-3-butyric acid (IBA) in a range of 6-8 mg L^{-1} and 0.25 to 0.75 mg L^{-1}, respectively. The interaction of these cytokinins and auxins at a lower level (1-4 mg L^{-1} Kn and 0.1-0.3 mg L^{-1} IBA) induced the calli to differentiate into shoot buds. The maximum number of shoots from immature inflorescences (9.3 ± 2.21 per callus) was noticed at 3.0 mg L^{-1} Kn and 0.2 mg L^{-1} IBA. Microshoots developed a good root system on half-strength LS medium fortified with 0.3 mg L^{-1} IBA. The regenerants derived from inflorescence culture exhibit vegetative morphology different to that of the donor plant. Callus from leaf and stem was further enhanced by augmenting the combination of Kn and IBA. There was a significant increase in the callus mass of both leaf and stem. The fresh and dry weight of stem calli (57.36 ± 5.58 g and 1.15 ± 0.12 g) was higher than that of leaf (51.76 ± 1.02 g and 1.04 ± 0.11 g).

Keywords: *Celastrus paniculatus*, inflorescence culture, leaf calli, plant regeneration, stem calli
Abbreviations: ANOVA, analysis of variance; **BA**, 6-benzyl adenine; **DW**, dry weight; **FW**, fresh weight; **$HgCl_2$**, mercuric chloride; **HSD**, honestly significant difference; **IBA**, indole-3-butyric acid; **Kn**, kinetin; **LS**, Linsmaier and Skoog; **NAA**, α-naphthaleneacetic acid

INTRODUCTION

Celastrus paniculatus Willd. (Celastraceae) is a woody climbing shrub, sparsely distributed in the hilly regions of India and South East Asia, up to an altitude of 1200 m. The fruits are yellow coloured capsules and seeds are brown, covered by scarlet arils. In Indian system of medicine this plant is popularly known as 'Jyothishmathi'. The seeds are therapeutically used to treat tranquilization, sedation, hypothermia, anxiety, beriberi and anticonvulsant activity (Kirthikar and Basu 1995; Kumar *et al*. 2002). The seed yields dark brown coloured empyreumatic oil, which is popularly known as malkanguni oil. The oil is used as a powerful stimulant, to cure beriberi, scabies, eczema, primary tuberculosis, leucoderma and rheumatic pains. It is beneficial as a chief ingredient of drugs in treating mental depression and hysteria (Chopra *et al.* 1994; Rekha *et al.* 2005). Leaves are emmenagogue, appetizer, emetic, mild laxative, sex invigoration, headache, various fevers and as an antidote for opium poisoning. Bark is used to treat swollen veins, gastric complaints, bronchitis and as an abortifacient (Singh and Abrar 1989).

The biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated (Krings and Berger 1998; Ravishankar and Ramachandra Rao 2000). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations.

This species is conventionally propagated through the seeds. Unscientific collection of fruits, leaves and roots in large quantities for medicinal purposes and destruction of habitat have made it to become an endangered species (Begum 1997). Hence, efforts are needed to propagate this species on commercial scale to meet the demands of pharmaceutical industries and to protect the existing natural population. Lakshmi and Seeni (2001) made attempt in obtaining adventitious shoot buds from the nodal explants of *C. paniculatus* on Murashige and Skoog medium supplemented with 6-benzyladenine (BA) alone. Martin *et al.* (2006) confirmed the qualitative chemical similarity of tissue culture regenerants with the mother plant using high performance thin-layer chromatographic profiling. Rao and Purohit (2006) reported *in vitro* shoot bud differentiation and plantlet regeneration in *C. paniculatus*. Lal and Singh (2010) reported mass multiplication of *C. paniculatus* using nodal segments. We reported the high frequency of regeneration of plantlets following the culture of hypocotyls (Maruthi *et al.* 2004) and leaf explants (Maruthi *et al.* 2004). In the present paper we report the rapid regeneration of plantlets by the culture of immature inflorescence explant and enhancement of callus for the production of secondary metabolites from both leaf and stem explants.

MATERIALS AND METHODS

The inflorescence segments with immature flower buds, leaf and stem of *C. paniculatus* were collected from the Kuvempu University campus, Shankaraghatta, Karnataka, India. The surface particles adhere to the inflorescence segments were washed with running tap water for 10 min and then washed with 'Tween-20' solution (2 drops per 100 ml, Himedia, Mumbai, India) as detergent

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for exactly 5 min. Then they were washed with double distilled water 4-5 times. Immature inflorescence explants were reduced in length to 6 cm and surface sterilized in a laminar flow hood by immersion in 0.1% $HgCl_2$ (Himedia, Mumbai, India) for 3 min. After rinsing 4 to 6 times with sterilized double distilled water, inflorescence segments and the immature flower bud explants were aseptically inoculated onto the media.

Culture media consisted of Linsmaier and Skoog (LS) (1965) basal nutrients augmented with 3% fructose as the carbon source. The different concentrations of auxins and cytokinins tested induces callogenesis and caulogenesis at 1.0-10 mg L^{-1} each of 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), 6-benzyl adenine (BA) (Hi-Media, Mumbai, India). The combinations of kinetin (Kn) and IBA were also tested at the range of 6-8 mg L^{-1} Kn and 0.1-1.5 mg L^{-1} IBA for callogenesis from the explants. The caulogenic media consists of lower levels of 1 to 4 mg L^{-1} Kn and 0.1-1 mg L^{-1} IBA. The pH of the media was adjusted to 5.8 before autoclaving and gelled with 0.8% (w/v) bacteriological grade agar (Hi-Media). The molten media was poured into culture tubes (25 × 150 mm) and conical flasks (250 ml) of about 20 ml and 75 ml respectively and autoclaved at 121°C and at 15 psi (1.06 Kg cm^{-2}) pressure for 15 min. For each treatment consists of 10 replicates. At the end of four weeks of incubation the number of shoot buds per callus were counted and the data were evaluated by one-way ANOVA followed by Tukey's HSD Test (Tukey's Honestly Significant Difference test is a pair-wise comparison that attempts to control for multiple comparisons) (ezANOVA software package) and the results were considered significant when $P < 0.05$. Subculturing of the cultures was routinely carried out at the interval of four weeks. All the cultures were incubated at 25 ± 2°C by providing white fluorescent light (1000 Lux, Phillips, India) for 12 h per day, with 70–80% relative humidity.

Microshoots taller than 4 cm in height were aseptically excised and inoculated into the conical flasks containing LS half-strength medium augmented with 3% fructose as carbon source and growth hormone with 3.0 mg L^{-1} IBA. The base of the culture flasks was covered with the black paper to facilitate rhizogenesis from the shoots. The aerial part of shoot was exposed to 12 hrs photoperiod. Agar was washed-off carefully under running tap water and regenerated plantlets with good root and shoot system were allowed to grow in small plastic pots containing the mixture of sterilized sand, soil and litter compost (1: 2: 2, v/v). Regenerants were covered with a perforated polythene bag and maintained at 22 ± 2°C, 12-h photoperiod with a light intensity of 2000 lux (Philips cool-white fluorescent tubes, India) and 65 to 70% relative humidity for a period of 10 days, providing $1/10^{th}$ basal nutrient water and then transferred to the natural environment.

RESULTS

The combination of higher level of Kn with lower level of IBA was most effective for callogenesis. Optimal callus induction was observed at the concentration of 5 mg L^{-1} Kn and 0.5 mg L^{-1} IBA. Initiation of the callus was first noticed from the pedicel of the flower buds (**Fig. 1A**), gradually, it proliferated all over the surface of the inflorescence explants. In case of isolated immature flower bud culture callogenesis was first noticed from the sepals (**Fig. 1B**) then it proceeded towards the petal and other floral parts. At the end of three weeks the entire inflorescence modified into fleshy nodular pale yellowish mass with photosynthetic nodules (**Fig. 1C**).

The interaction of Kn and IBA at lower levels (1-4 mg L^{-1} Kn and 0.1-0.4 mg L^{-1} IBA) induce shoot bud differentiation from the inflorescence calli (**Fig. 1D**). On caulogeneic medium the fleshy callus become very hard and exhibits the tendency towards regeneration of shoots. The data on caulogeneic response at different concentration of Kn and IBA is depicted in **Table 1**. Among the treatments, the highest number of shoots (9.3 ± 2.21 per callus) was noticed at the concentration of 3 mg L^{-1} Kn and 0.2 mg L^{-1} IBA. In the above combinations when IBA was deleted the caulogenic potency of the calli was suppressed. The differentiating callus becomes converted into nodular photosynthetic mass and these photosynthetic nodules were unable to sprout into shoot buds.

In *C. paniculatus*, IBA is the prime rooting hormone induces root initials from the base of the excised microshoots at the concentration of 0.1-0.4 mg L^{-1} IBA. The best root response was observed at concentration 0.3 mg L^{-1} IBA (**Fig. 1E**). The *in vitro* regenerants were successfully hardened and transferred to the pots containing sterilized soil. A month old transformed regenerant exhibited all the vegetative morphological similarity with the *in vivo* plants (**Fig. 1F**).

The leaf and stem explants of *C. paniculatus* were proliferated into callus mass on LS medium supplemented with a range of 1-5 mg L^{-1} Kn and 0.1-0.5 mg L^{-1} IBA. In leaf

Table 1 Effect of different concentrations of Kn and IBA on shoot bud organogenesis from immature inflorescence callus of *Celastrus paniculatus* Willd.

Growth regulators		Mean number of shoots
Kn	**IBA**	**Mean ± SD**
1.5	0.1	0.2 ± 0.42
1.5	0.2	0.6 ± 0.52
1.5	0.3	1.6 ± 1.07
1.5	0.4	1.1 ± 0.88
2.0	0.1	0.3 ± 0.48
2.0	0.2	1.2 ± 1.14
2.0	0.3	3.4 ± 1.34
2.0	0.4	1.9 ± 0.74
2.5	0.1	0.6 ± 0.7
2.5	0.2	1.5 ± 0.85
2.5	0.3	4.6 ± 0.97
2.5	0.4	3.7 ± 1.42
3.0	0.1	6.0±1.41
3.0	0.2	9.3 ± 2.21
3.0	0.3	5.5 ± 1.08
3.0	0.4	2.1 ± 1.6
3.5	0.1	3.1 ± 0.74
3.5	0.2	4.2 ± 1.23
3.5	0.3	2.2 ± 1.62
3.5	0.4	1.2 ± 0.92
4.0	0.1	1.4 ± 0.7
4.0	0.2	2.3 ± 1.34
4.0	0.3	0.9 ± 0.74
4.0	0.4	0.2 ± 0.42
	F-Value	1.94

The value of each concentration consisted of ± S.D. of 10 replicates.
The F value is significantly different at 0.05%.

Table 2 Effect of Kinetin and IBA on the mass production of callus from leaf of *Celastrus paniculatus*.

Growth regulators (mg/L)		Leaf callus weight (g) Mean ± SD	
Kn	**IBA**	**Fresh**	**Dry**
2	0.1	38.46 ± 1.27	0.77 ± 0.04
2	0.2	51.76 ± 1.02	1.04 ± 0.11
2	0.3	25.36 ± 2.53	0.62 ± 0.08
2	0.4	21.10 ± 2.78	0.51 ± 0.06
2	0.5	10.30 ± 2.80	0.21 ± 0.06
F-Value		82. 8	68. 1

The value of each concentration consists of Mean ± SD of 5 replicates.
The F value is significantly different at 0.05%.

Table 3 Effect of Kinetin and IBA on the mass production of callus from stem of *Celastrus paniculatus*.

Growth regulators (mg/L)		Stem callus weight (g) Mean ± SD	
Kn	**IBA**	**Fresh**	**Dry**
5	0.1	57.36 ± 5.58	1.15 ± 0.12
5	0.2	45.70 ± 4.69	0.91 ± 0.09
5	0.3	29.04 ± 4.03	0.58 ± 0.08
5	0.4	22.70 ± 4.35	0.45 ± 0.09
5	0.5	18.40 ± 2.26	0.37 ± 0.05
F-Value		54. 9	54. 8

The value of each concentration consists of Mean ± SD of 5 replicates.
The F value is significantly different at 0.05%.

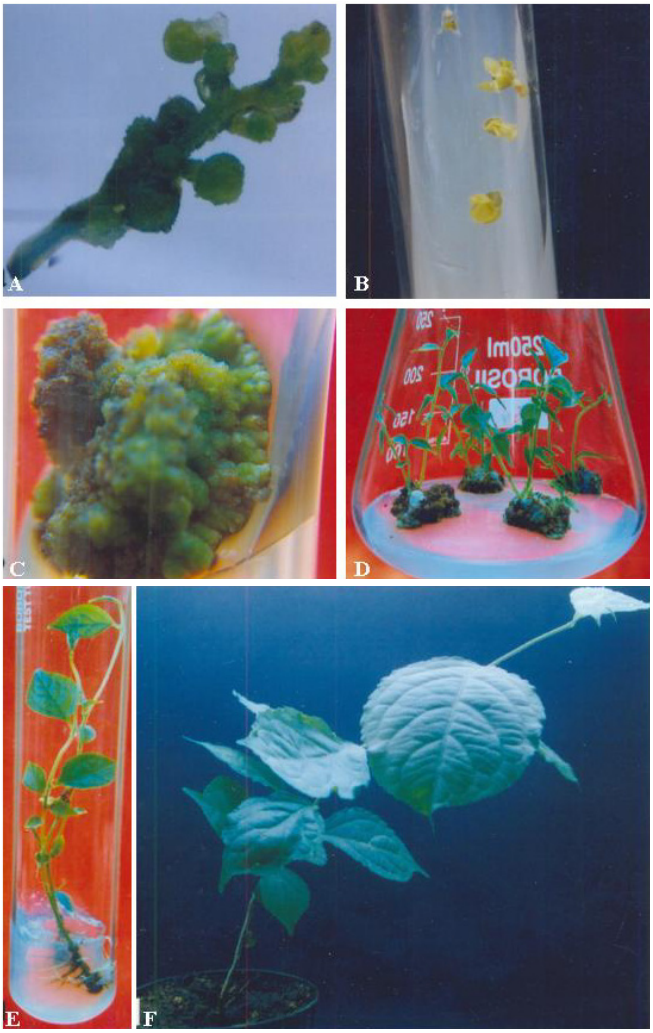

Fig. 1 (**A**) Immature inflorescence explant of *Celastrus paniculatus* showing swollen pedicel of the flower buds and initiation of callus on LS + 5 mg L^{-1} Kn + 0.5 mg L^{-1} IBA. (**B**) Callus initiation from the pedicel, sepals and petals of flower on LS + 5 mg L^{-1} Kn + 0. 5 mg L^{-1} IBA. (**C**) Fleshy greenish nodular callus of immature inflorescence, after four weeks of incubation on same callogenic media. (**D**) Differentiation of shoot buds from the inflorescence callus on caulogeneic media LS + 3 mg L^{-1} Kn + 0.2 mg L^{-1} IBA. (**E**) Initiation of root initials from the microshoots on LS half-strength medium augmented with 0.3 mg L^{-1} IBA. (**F**) A month old transformed regenerant.

explants, callusing was optimized at 2 mg L^{-1} Kn and 0.2 mg L^{-1} IBA. Prior to callusing the excised leaf segments showed curling and became enlarged into more than 10 times its normal size. Sprouting of the callus was initiated from the margin and excised cut end of the explants in the form of nodular mass (**Fig. 2A**). After four weeks of incubation callogenesis was completed all over the surface of the explant and it appear in the form of fleshy whitish nodular mass (**Fig. 2B**). The interaction of Kn and IBA on the intensity of callogenesis was further evaluated by subculturing the leaf calli on the same media containing 2 mg L^{-1} Kn and 0.1-0.5 mg L^{-1} IBA in 20-cm diameter Petri dishes for mass multiplication. The intensity of callogenesis was maintained however, the fleshy whitish callus turned to yellow nodular mass with photosynthetic nodules (**Fig. 2C**). The fresh weight of the total mass of the calli was evaluated between the day one of inoculation and up to 45 days of incubation. The total dry weight of the calli mass was evaluated after complete drying of the callus at 50°C in a hot air oven. Among the different concentrations of IBA tested with Kn for callus mass production, the interaction of 0.2 mg L^{-1} IBA with 2 mg L^{-1} Kn yielded more callus (51.76 ± 1.02 g FW and 1.04 ± 0.11 g DW). The data on the fresh and dry weight of the leaf calli is depicted in the **Table 2**.

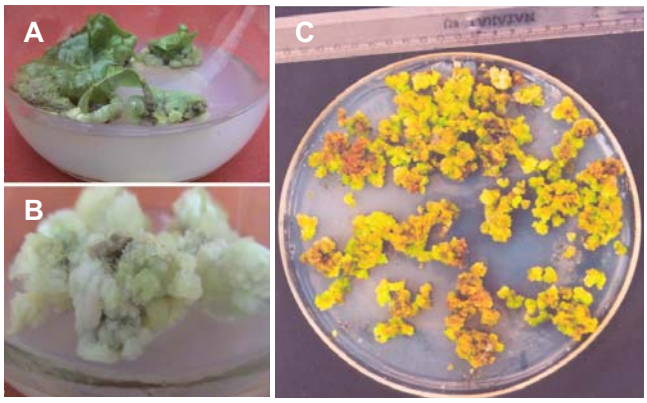

Fig. 2 (**A**) Callus initiation from the margin and excised cut end of the leaf explants of *Celastrus paniculatus*. (**B**) A four weeks of incubated culture showing proliferation of fleshy whitish nodular mass of callus on LS + 2 mg L^{-1} Kn + 0.2 mg L^{-1} IBA. (**C**) Mass multiplication of callus grown in 20-cm diameter Petri plate at the concentration of LS + 2 mg L^{-1} Kn + 0.2 mg L^{-1} IBA.

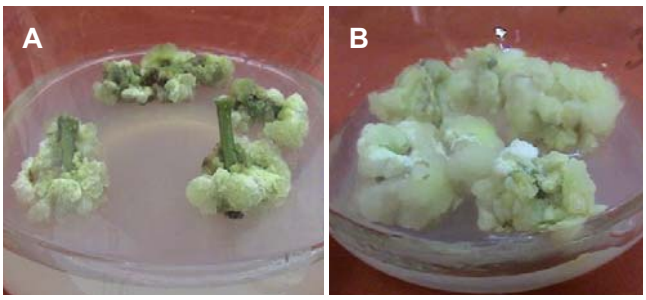

Fig. 3 (**A**) Callus was initiated from the excised end of the stem explant of *Celastrus paniculatus* on LS + 5 mg L^{-1} Kn + 0.1 mg L^{-1} IBA. (**B**) 30 days of incubated culture showing whitish hard nodular mass of callus on LS + 5 mg L^{-1} Kn + 0.1 mg L^{-1} IBA.

When compared to leaf culture callus induction efficiency was more in stem explants. Callus was initiated from the excised end of the stem explant which were in contact with the medium (**Fig. 3A**) and the process was initiated after 10 days of incubation and completed within 30 days of incubation whereas in leaf culture callusing was delayed by a week. The texture of the callus was similar with that of leaf callus in the form of whitish hard nodular mass (**Fig. 3B**). Among the different concentrations of Kn and IBA tested, a combination of 5 mg L^{-1} Kn and 0.1 mg L^{-1} IBA proved best for callus production. Mass production and multiplication of stem calli was achieved by subculturing it onto media supplemented with 5 mg L^{-1} Kn and 0.1-0.5 mg L^{-1} IBA. The quantity of fresh and dry weight of the calli was more (57.36 ± 5.58 g FW and 1.15 ± 0.12 g DW) at 5 mg L^{-1} Kn and 0.1 mg L^{-1} IBA (**Table 3**).

DISCUSSION

Immature inflorescence explants were cultured on LS medium supplemented with 2,4-D, Kn, IBA, NAA at the range of 1-10 mg L^{-1} to explore the callogenic and caulogeneic potentialities. No response was observed on the medium fortified with 2,4-D. In most of the dicotyledonous species 2,4-D is the principle hormone used for the initiation and maintenance of callus (Rajasubramaniam and Pardha Saradhi 1994). Whereas, in *C.s paniculatus* 2,4-D does not show any effect on callogenesis from the inflorescence explants. On NAA supplemented medium the explants were unable to show the morphogenic response. Within a week of culture they become brown and dried on the medium. Kn at higher concentrations (6-8 mg L^{-1}) was effective in inducing callogenic response from the inflorescence explants. The reason for effectiveness of the Kn may lie in the ability to stimulate the plant tissue to metabolize

the natural endogenous hormones or could induce the production of natural hormone system for callus initiation. The mode of interaction between auxins and cytokinins can therefore be synergistic, antagonistic, or additive and is dependent on the type of tissue and on the plant species in which the interaction occurs. Although the molecular mechanisms underlying most of these auxin-cytokinin interactions are unknown, they are thought to include mutual control of auxin and cytokinin metabolism, interactions in the control of gene expression, and posttranscriptional interactions (Coenen and Lomax 1997). The effect of interaction of cytokinin with auxin on improved shoot regeneration was allowed in many medicinally important climbing species such as *Naravelia zeylanica* (Vaghna) (Rajanaika *et al.* 2007), *Jatropha curcas* (Purging Nut) (Timirbaran *et al*. 2007), *Decalepis hamiltonii* (swollen root) (Bais *et al.* 2000) and *Holostemma ada-kodien* (*Holostemma* Creeper) (Martin 2002). In six-weeks-old cultures, 4 to 6 shoots grew above 8 cm with 6-7 leaves and the leaves exhibited serrate venation, which is one of the characteristics of *C. paniculatus*. In most woody species, retaining the microshoots on medium supplemented with IBA for a longer duration hindered the growth and caused etiolation of leaves whereas in *C. paniculatus*, IBA induces profuse root system from the microshoots.

Most of the investigators subjected the vegetative explants *in vitro* for the regeneration of plantlets. Although, early in 1942, La Rue cultured the floral parts *in vitro* for the regeneration of plantlets so far, only a few reports available on the regeneration of plantlets from the inflorescence explant culture (Sudharshana and Shanthamma 1988; Ornstrup *et al.* 1993). It could be possible for occurrence of genetic variability among plantlets derived from the inflorescence explants. Further study on the evaluation of genetic variability will be carried out in future using various genetic markers.

Successful establishment of cell lines for the production of secondary metabolites has been reported in various medicinal plant species. The production of solasodine from calli of *Solanum eleagnifolium* (Nigra *et al.* 1987) and pyrrolizidine alkaloids from root calli of *Senecio* sp. (Toppel *et al.* 1987). Cephaelin and emetine were isolated from callus cultures of *Cephaelis ipecacuanha* (Jha *et al.* 1988). Callus culture also provides excellent tool for biochemical investigation. Callus growth and development are influenced by a complex relationship between the explants used, the constituents of the medium and the proper environmental conditions (Brar and Khush 1994).

The leaf and stem explants of *C. paniculatus* were cultured on LS medium supplemented with different concentrations and combinations of auxins and cytokinins to explore its callogenic and morphogenic potentialities. The callogenic response was nil on the medium supplemented with the auxins 2,4-D, IAA and NAA, tested alone in different concentrations. Within a week of incubation exudation of polyphenol takes place from the excised end of the explants and they turned brown. IBA at the range of 0.1 -0.5 mg L^{-1} induced a meager callus from the leaf and stem explants. Among cytokinins the callogenic response was nil on the media fortified with BAP alone. However, combination of BAP (1-6 mg L^{-1}) and IBA (0.1-0.5 mg L^{-1}) induced meager hard nodular callus mass. The significant increase in the callus mass is due to the action of growth regulators *in vitro* condition is highly specific, it may also be due to the synergetic effect of BAP with the endogenous growth regulators of the explants.

Micropropagated plants derived either from direct or indirect organogenesis can be used to supplement the natural stock of plants in wild populations as well as to provide a ready supply to the herbal medicinal trade, thereby alleviating pressure on existing resources already over-exploited by uncontrolled harvesting. The *in vitro* protocols developed for the enhanced production of these secondary metabolites, so that the active constituent is obtained in large scale without destroying the natural plant population. The above protocol may be useful for the conservation of other medicinally important threatened and endangered species.

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REFERENCES

Bais HP, George J, Ravishankar GA (2000) *In vitro* propagation of *Decalepis hamiltonii* Wight & Arn.- An endangered shrub, through axillary bud culture. *Current Science* **79**, 408-410

Bapat VA, Mahtre M, Rao PS (1986) Regeneration of plantlets from protoplast cultures of *Pergularia pallida. Journal of Plant Physiology* **124**, 413-417

Begum SN (1997) Distribution of Red listed plants of Southern India. *Amruthalaya* **3**, 2-13

Brar DS, Khush GS (1994) *Cell and tissue culture for plant improvement.* In: Basra AS (ed) *Mechanism of Plant Growth and Improved Productivity - Modern Approaches*, Marcel Dekker Inc., New York, Basel, Hong Kong, 229 pp

Coenen C, Lomax TL (1997) Auxin-cytokinin interactions in higher plants: Old problems and new tools. *Trends in Plant Science* **2**, 351-356

Jha S, Sahu NP, Mahato SB (1988) Production of the alkaloids - emetine and cephaeline in callus culture of *Cephaelis ipecacuanha. Planta Medica* **54 (6)**, 504-506

Kirthikar KR, Basu BD (1995) *Indian Medicinal Plants* (Vol 1), International Book Distributors, Deharadun, India, 97 pp

Komalavalli N, Rao MV (1997) *In vitro* micropropagation of *Gymnema elegans* Wight & Arn – A rare medicinal plant. *Indian Journal of Experimental Biology* **35**, 1088-1092

Krings U, Berger RG (1998) Biotechnological production of flavours and fragrances. *Applied Microbiology and Biotechnology* **49**, 1-8

Kumar MHV, Gupta YK (2002) Antioxidant property of *Celastrus paniculatus* Willd. – A possible mechanism in enhancing cognition. *Phytomedicine* **9**, 302-311

La Rue CD (1942) Rooting of flowers in sterile cultures. *Bulletin of Torrey Botanical Club* **69**, 332-341

Lakshimi GN, Seeni S (2001) Rapid *in vitro* multiplication and restoration of *Celastrus paniculatus* sp. (Celastrace) – A medicinal woody climber. *Indian Journal of Experimental Biology* **39**, 697-704

Lal D, Singh N (2010) Mass multiplication of *Celastrus paniculatus* Willd – An important medicinal plant under *in vitro* conditions using nodal segments. *Journal of American Science* **6 (7)**, 55-61

Linsmaier EM, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. *Physiology of Plant* **18**, 100-128

Martin G, Geetha SP, Sudhakar S, Raja, Raghu AV, Indira B, Ravindran PN (2006) An efficient micropropagation system for *Celastrus paniculatus* Willd. – a vulnerable medicinal plant. *Journal of Forest Research* **11 (6)**, 461-465

Martin KP (2002) Rapid propagation of *Holostemma ada-kodien* Schult - A rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Reports* **21**, 112-117

Maruthi KR, Krishna V, Nagaraja YP, Rahiman BA, Pullaiah T (2004) *In vitro* regeneration of *Celastrus paniculatus* Willd. - A rare medicinal plant. *Plant Cell Biotechnology and Molecular Biology* **5 (1&2)**, 33-38

Maruthi KR, Krishna V, Shankarmurthy K, Rahiman BA (2004) Micropropagation of *Celastrus paniculatus* Willd. through hypocotyl explant culture. *Journal of Tropical Medicinal Plants* **5 (2)**, 249-254

Nigra HM, Caso OH, Guilietti AM (1987) Production of solasodine by calli form different parts of *Solanum eleaginifolium* Cav. plants. *Plant Cell Reports* **6**, 135-137

Ornstrup H, Molgaard JP, Farestveit B (1993) Somatic embryogenesis and regeneration from cell suspension of *Exacum affine*. *Plant Cell, Tissue and Organ Culture* **35**, 37-41

Raja Naika H, Krishna V, Harish BG, Khadeer Ahamed BM, Mahadevan KM (2007) Antimicrobial activity of bioactive constituents isolated from the leaves of *Naravelia zeylanica* (L.) DC. *International Journal of Biomedical and Pharmaceutical Sciences* **1 (2)**, 153-159

Rajasubramaniam S, Pardha Saradhi (1994) Organic nitrogen stimulates caulogenesis from hypocotyl callus of *Phylanthus fraternus. Plant Cell Reports* **13**, 619-622

Rao MS, Purohit SD (2006) *In vitro* shoot bud differentiation and plantlet regeneration in *Celastrus paniculatus* Willd. *Biologia Plantarum* **50 (4)**, 501-506

Ravishankar GA, Ramachandra Rao S (2000) Biotechnological production

of phyto-pharmaceuticals. *Journal of Biochemistry, Molecular Biology and Biophysics* **4**, 73-102

Rekha K, Bhan MK, Balyan SS, Dhar AK (2005) Cultivation prospects of endangered species *Celastrus paniculatus* Willd. *Natural Product Radiance* **4**, 483-486

Singh VK, Abrar MK (1989) *Medicinal Plants and Folklores* (1st Edn), Today and Tomorrows Printers & Publishers, New Delhi, 220 pp

Sudarshana MS, Shanthamma C (1988) Plant regeneration from inflorescence culture of *Boerhaavia diffusa* L. *Current Science* **57 (5)**, 268-270

Timirbaran J, Mukherjee P, Mukul MD (2007) Somatic embryogenesis in *Jatropha curcas* Linn. – an important biofuel plant. *Plant Biotechnology Reports* **1 (3)**, 135-140

Toppel G, Witte L, Riebesehl B, Von Borstel K, Hartman T (1987) Alkaloid patterns and biosynthetic capacity of root cultures from some pyrrolizidine alkaloid producing *Senecio* spp. *Plant Cell Reports* **6**, 466-469