Rev. Biol. Trop. 49(3-4): 957-963, 2001
www.ucr.ac.cr www.ots.ac.cr www.ots.duke.edu

In vitro micropropagation of *Lawsonia inermis* (Lythraceae)

G.R. Rout[1], G. Das[1], S. Samantaray[2] and P. Das[1]

1 Plant Biotechnology Division, Plant Tissue Culture Laboratory, Regional Plant Resource Centre, Bhubaneswar-751 015, India. Fax: 0091-674-550274. E-mail: grrout@hotmail.com

2 Plant Physiology and Biochemistry Laboratory, Bhubaneswar- 751 015, Orissa, India, Fax: 0091-674-550274.

Received 08-III-2000. Corrected 12-III-2001. Accepted 08-V-2001.

Abstract: A successful protocol was developed for mass propagation of *Lawsonia inermis* Linn., an important medicinal plant. Multiple shoots were induced in apical and axillary meristems derived from mature explants of *L. inermis* on Murashige and Skoog (1962) medium supplemented with 0.25 mg/l 6-benzylaminopurine (BA), 0.25 mg/l Kinetin (Kn), 0.5 mg/l ascorbic acid and 3 % (w/v) sucrose. The rate of multiplication was higher when the cultures were incubated under continuous light rather than the 14 hr photoperiod. Rooting was readily achieved upon transferring the microshoots onto MS basal semi-solid medium supplemented with 0.25 mg/l indole-3-butyric acid (IBA) after ten days of culture. Micropropagated plantlets were acclimatized and successfully grown in soil.

Key words: *In vitro*, micropropagation, medicinal plants, shoot multiplication.

Lawsonia inermis Linn. (Lythraceae) is an important medicinal plant, distributed in northern Africa and south-west Asia (Anonymous 1962). The leaves are used as a prophylactic against skin diseases. They are used externally in the form of a paste or decoction against boils, burns, bruises and skin inflammations. A decoction is used as gurgle against sore throat. Alcoholic extracts of *Lawsonia* leaves show mild antibacterial activity against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli* (Kritikar and Basu 1981). The flower of *Lawsonia* has a strong aroma with high commercial value. It is extensively used as a dye in silk and wool industry. Conventional methods of propagation of *L. inermis*, sexual as well as vegetative, are beset with many problems that restrict their multiplication on a large scale. Propagation through seed is unreliable because of disease and pest problems, short viability and heavy rains during the seeding season in the natural habitat. An unplanned exploitation by the ever growing human population has resulted in the rapid depletion of plant resources, particularly the economically important plants. Cosmetic and pharmaceutical companies largely depend upon materials procured from naturally occurring stands, raising concern about possible extinction and providing justification for development of *in vitro* techniques for mass propagation of *L. inermis*. Preservation of genetic stability in germplasm collections and micropropagation of elite plants is of utmost importance and propagation of plants through apical or axillary meristem culture allows recovery of genetically stable and true to type progeny (Hu and Wang 1983, George and Sherrington 1984). There is no report on micropropagation of *L. inermis*. The present communication describes a successful protocol for mass propagation of *L. inermis* Linn.

MATERIALS AND METHODS

Plant material and explant source: Actively growing young stems (4-5 cm) of *L. inermis* were collected from greenhouse grown plants at the Regional Plant Resource Centre, Bhubaneswar, washed with 2 % (v/v) detergent 'Teepol' (Qualigen, India) and rinsed several times with running tap water. The explants were surface sterilized in 0.1 % (w/v) aqueous mercuric chloride solution for 15 min followed by four washings with sterile distilled water. The apical and axillary meristems (~ 0.5 mm) were isolated and used as explants.

Culture medium and condition: The meristem (apical and axillary) was placed on semi-solid basal MS (Murashige and Skoog 1962) medium supplemented with different concentrations and combinations of 6-benzylaminopurine (BA: 0.0, 0.25, 0.5 and 1.0 mg/l), kinetin (Kn: 0.0, 0.25, 0.5 and 1.0 mg/l), and indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) (0.0, 0.1, 0.25 and 0.5 mg/l) for shoot proliferation and multiplication. The pH of the media was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving. Routinely, 25 ml of the molten medium was dispensed into culture tubes (25 x 150 mm), plugged with non-absorbent cotton wrapped in one layer of cheese cloth and sterilized at 121 ºC and 1.06 kg/cm^2 of pressure for 15 min. The cultures were maintained at 25±2 ºC either under a 14 hr photoperiod or continuous light (55 mmol $m^{-2}s^{-1}$) from cool, white, fluorescent lamps. The cultures were maintained by regular subcultures at 4 week intervals on fresh medium with the same compositions. To avoid blackening, the medium was supplemented with 0.5 mg/l ascorbic acid.

Induction of rooting and acclimatization: For root induction, excised microshoots (1-2 cm length) were transferred to MS basal medium supplemented with different concentrations of IAA or IBA (0.0, 0.1, 0.25 and 0.5 mg/l) and 2 % (w/v) sucrose. One excised shoot was placed in each tube (25 x 150 mm) having 15 ml of the culture media. All the cultures were incubated at 25±2 ºC under a 16 hr photoperiod with cool, white, fluorescent lamps. Rooted micro-propagules were thoroughly washed to remove the adhering gel and planted in 2.5 cm earthen pots containing a sterile mixture of sand, soil and cow-dung manure in the ratio of 1:1:1 (v/v) and kept in the greenhouse for acclimatization.

Observation of cultures and presentation of results: Twenty cultures were used per treatment and each experiment was repeated at least three times. The data pertaining to mean percentage of cultures showing response, number of shoots/culture and mean percentage of rooting were statistically analysed by the Post-Hoc Multiple Comparison test (Marascuilo and McSweency 1977). Between the treatments, the average figures followed by the same letters were not significantly different at the $P < 0.05$ level.

RESULTS

Meristem proliferation and multiplication: Meristem proliferation and multiplication was initiated from apical and axillary explants of *L. inermis* within 8-10 days of inoculation onto MS basal medium supplemented with BA, Kn and 0.5 mg/l ascorbic acid. Of the different cytokinins tested, BA + Kn was the most effective for shoot proliferation and multiplication. The maximum shoot proliferation and multiplication was observed both in apical and axillary meristems cultured on MS medium supplemented with 0.25 mg/l BA, 0.25 mg/l Kn and 0.5 mg/l ascorbic acid within 4 weeks of culture under a 14 hr photoperiod (Table 1). The apical and axillary shoots proliferated and elongated to 1.0-1.5 cm within 4 weeks of culture (Fig. 1A). There was no sign of shoot proliferation when explants were cultured in media devoid of cytokinin. At higher concentrations of BA or Kn, the rate of shoot proliferation declined. Inclusion of either IAA or IBA in the culture medium did not help in proliferation and multiplication of shoot. In most of the cases, growth was inhibited and only 1-2 shoots would elongate; some produced

TABLE 1
Effect of BA, Kn and 0.5 mg/l ascorbic acid on shoot growth from apical (A) and axillary (B) meristems of Lawsonia inermis *after 4 weeks of culture under a 14 hr photoperiod*

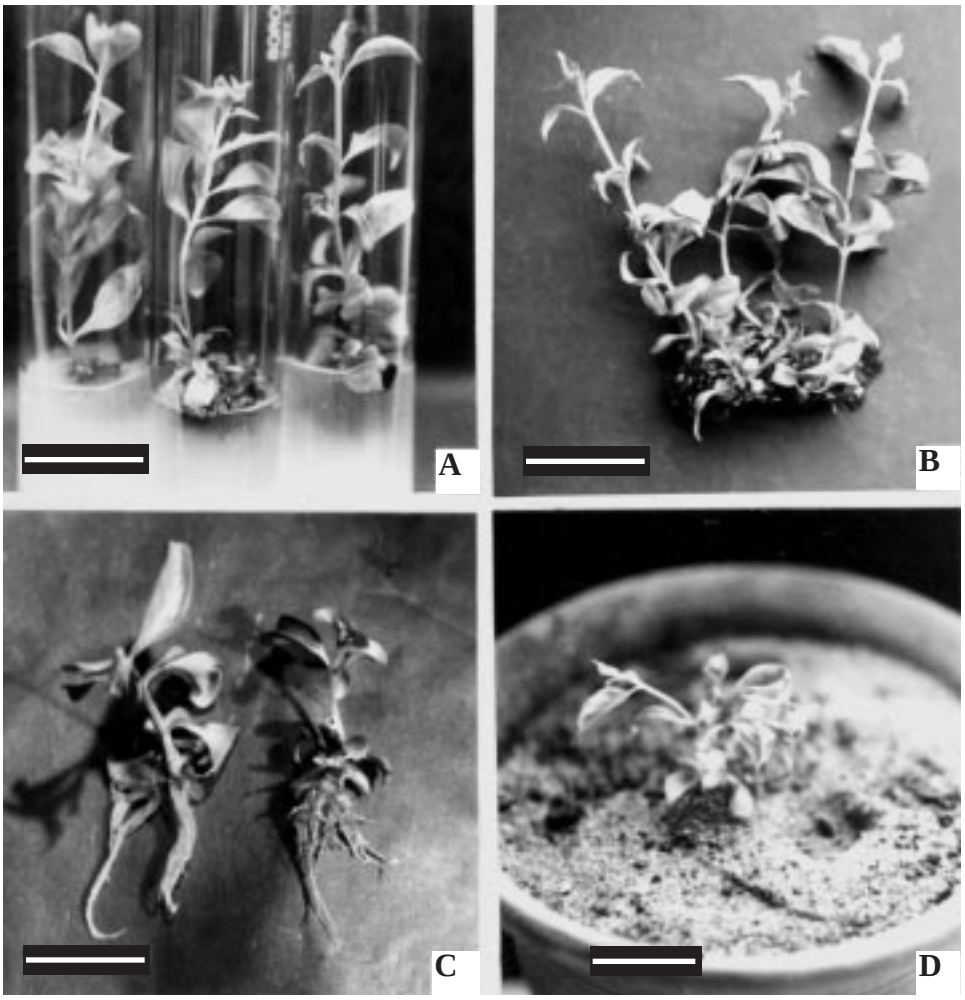
MS + Growth regulators (mg/l)		Percentage of cultures with multiple shoots (Mean ± S.E.)*		Number of shoots/explant (Mean ± S.E.)*	
BA	Kn	A	B	A	B
0	0	0	0	0	0
0.25	0	32.6 ± 0.4 d	40.4 ± 0.3 d	2.41 ± 0.2 e	2.97 ± 0.5 e
0.50	0	40.2 ± 0.5 e	44.8 ± 0.6 d, e	2.11 ± 0.4 d	2.35 ± 0.4 d
1.0	0	28.6 ± 0.7 c+	24.5 ± 0.4 b+	1.01 ± 0.5 a+	1.34 ± 0.6 a+
0	0.25	38.7 ± 0.3 e	46.7 ± 0.3 e	2.72 ± 0.5 f	2.31 ± 0.2 d
0	0.50	42.2 ± 0.5 f	47.3 ± 0.7 f	1.27 ± 0.4 b	1.76 ± 0.5 b
0	1.0	18.7 ± 0.4 a+	22.3 ± 0.6 a+	1.80 ± 0.3 c+	1.32 ± 0.6 a+
0.25	0.25	62.8 ± 0.5 h	75.7 ± 0.3 h	3.25 ± 0.2 h	4.57 ± 0.4 g
0.25	0.50	51.7 ± 0.4 g	58.6 ± 0.5 g	3.16 ± 0.4 g	3.23 ± 0.7 f
0.50	0.25	50.3 ± 0.6 g	57.4 ± 0.3 g	2.26 ± 0.7 e	2.18 ± 0.6 c
0.50	0.50	39.4 ± 0.4 e	42.8 ± 0.5 d	1.86 ± 0.6 c	1.83 ± 0.5 b
0.50	1.0	21.6 ± 0.4 b+	20.4 ± 0.5 a+	1.14 ± 0.5 a+	1.18 ± 0.3 a+
1.0	0.50	23.7 ± 0.5 b+	27.5 ± 0.4 c+	1.07 ± 0.6 a+	1.23 ± 0.6 a+

* Mean of 20 cultures per treatment; repeated thrice.
\+ Callusing at the basal end.
a-h Means having the same letter in a column were not significantly different by Post-Hoc Multiple Comparison test $P < 0.05$ level.

a compact callus at the base of the explants. Prolonged culture on the proliferation and multiplication media resulted in the blackening of the basal ends of the developing shoots. There were differences among the treatments in both the percentage of cultures with multiple shoots and the mean number of shoots/culture. The axillary meristems produced more number of shoots (4.57) than the apical meristems (3.25) (Fig. 1B) (Table 1). The study also revealed that the continuous light (24 hr) was more conducive to higher rate of shoot multiplication than the 14 hr photoperiod (Table 2). The highest percentage of cultures with multiple shoots (86.4) was observed on media containing 0.25 mg/l BA, 0.25 mg/l Kn and 0.5 mg/l ascorbic acid when the cultures were incubated in the continuous light for 4 weeks. The frequency of multiple shoots per culture varied from 1.24 to 4.42 in the case of the 14 hr photoperiod and from 2.01 to 4.78 in the case of the continuous light incubation (Table 2). The rate of multiplication was high and stable up to the 5^{th} subculture and declined in subsequent subcultures (Fig. 2).

Induction of rooting from microshoots: Elongated shoots (1-2 cm long) were rooted on MS basal medium supplemented with various concentrations of either IAA or IBA (Table 3). The rooting in the microshoots was inhibited in the medium devoid of growth regulator. Root initiation took place within 10-12 days of transfer to MS basal medium supplemented with 0.1-0.25 mg/l IAA or IBA. However, optimal rooting (75.6 %) and growth of microshoots were observed on medium containing 0.25 mg/l IBA with 2 % (w/v) sucrose (Fig. 1C). The rooting ability was reduced with the increase in the concentration of IAA or IBA in the culture medium. The percentage of shoots forming roots and days to rooting significantly varied with different concentrations of IAA or IBA.

Acclimatization and field establishment: Rooted plantlets grown *in vitro* were washed thoroughly to remove the adhering gel, transplanted to 2.5 cm earthen, sterile pots containing garden soil, sand and cowdung at the ratio of 2: 1: 1 (v/v). About 96 % of the rooted plantlets established in the greenhouse within 2-3 weeks of transfer. The



Figs 1A-D. Micropropagation of *Lawsonia inermis*. A. Proliferation of shoot from apical meristems on MS medium supplemented with 0.25 mg/l BA, 0.25 mg/l Kn, 0.5 mg/l ascorbic acid and 3 % sucrose. (Bar = 5 mm). B. Multiple shoots from apical meristems on MS medium supplemented with 0.25 mg/l BA, 0.25 mg/l Kn, 0.5 mg/l ascorbic acid and 3 % sucrose after 4 weeks of culture. (Bar = 10 mm). C. Rooting in the *in vitro* derived shoots after 10 days of culture on MS medium supplemented with 0.25 mg/l IBA and 2 % (w/v) sucrose. (Bar = 10 mm). D. Plant grown in the pot. (Bar = 50 mm).

plants grew well and attained a 6-8 cm height within 4 weeks of transfer (Fig. 1D). The acclimatized plants were established in the field condition and grew normally without morphological variation.

DISCUSSION

The present study showed that it was possible to explore the morphogenetic potential of *L. inermis* by application of growth regulators and light condition. With the use of different combinations of cytokinins, the induction and multiplication of shoots derived from apical and axillary meristems. The regulatory action of cytokinin and the apical dominance that helped the *in vitro* shoot induction and multiplication was well documented (Wickson and Thimann 1958). The maximum shoot induction and multiplication was observed both in apical and axillary meristems cultured on

TABLE 2

Effect of photoperiod on shoot multiplication of Lawsonia inermis *cultured on MS medium supplemented with various concentrations of BA, kinetin and 0.5 mg/l ascorbic acid after 4 weeks of culture*

Growth regulators (mg/l) BA	Kn	Percent of cultures with multiple shoots (Mean ± S.E.)* A	B	Number of shoots/explant (Mean ± S.E.)* A	B
14 hr Photoperiod					
0	0	0	0	0	0
0.25	0	30.4 ± 0.3 a	38.2 ±0.3 a	2.31 ± 0.3 f	2.94 ± 0.4 f
0.50	0	38.2 ± 0.6 d	43.6 ±0.4 b	2.14 ± 0.6 d	2.23 ± 0.5 c
0	0.25	36.5 ± 0.4 c	45.8 ±0.3 c	2.62 ± 0.4 h	2.36 ± 0.3 e
0	0.50	41.5 ± 0.7 f	46.3 ± 0.5 c	1.24 ± 0.7 a	1.66 ± 0.5 a
0.25	0.25	61.2 ± 0.5 l	73.4 ± 0.6 g	3.20 ± 0.4 m	4.42 ± 0.3 l
0.25	0.50	52.4 ± 0.3 k	59.4 ± 0.3 f	3.03 ± 0.5 l	3.18 ± 0.7 i
0.50	0.25	48.2 ± 0.4 h	56.5 ± 0.4 e	2.20 ± 0.6 e	2.23 ± 0.6 c
0.50	0.50	37.3 ± 0.6 c	44.5 ± 0.3 b	1.76 ± 0.5 b	3.82 ± 0.4 k
Continuous light					
0	0	0	0	0	0
0.25	0.25	33.1 ± 0.5 b	39.2 ± 0.4 a	2.71 ± 0.6 i	3.06 ± 0.4 g
0.50	0.50	42.2 ± 0.4 g	45.6 ± 0.2 c	2.41 ± 0.5 g	2.91 ± 0.2 f
0	0.25	39.6 ± 0.3 e	51.4 ± 0.4 d	2.92 ± 0.5 k	3.12 ± 0.4 h
0	0.50	43.4 ± 0.7 g	46.2 ± 0.2 c	2.17 ± 0.2 d, e	2.27 ± 0.5 d
0.25	0.25	70.4 ± 0.5 m	86.4 ± 0.4 h	3.41 ± 0.3 o	4.78 ± 0.6 m
0.25	0.50	50.3 ± 0.4 d, i	57.5 ± 0.4 e	3.21 ± 0.8 n	3.53 ± 0.3 j
0.50	0.25	51.6 ± 0.6 d, j	58.3 ± 0.3 e, f	2.82 ± 0.4 j	2.91 ± 0.4 f
0.50	0.50	42.5 ± 0.4 g	45.6 ± 0.6 c	2.01 ± 0.5 c	2.13 ± 0.6 b

A-Apical meristem; B-Axillary meristem.

* Mean of 20 cultures per treatment; repeated thrice.

a-o Means having the same letter in a column were not significantly different by Post-Hoc Multiple Comparison test $P < 0.05$ level.

TABLE 3

Effect of IAA and IBA on rooting from excised shoots of Lawsonia inermis *cultured on MS basal medium supplemented with 2 % (w/v) sucrose*

MS + Growth regulators (mg/l) IAA	IBA	Percentage of shoot rooted (Mean ± S.E)*	Days to rooting
0	0	0	0
0.1	0	48.4 ± 0.6	11-12
0.25	0	52.8 ± 0.3	12-13
0.50	0	36.4 ± 0.4	13-14 +
0	0.1	60.3 ± 0.6	11
0	0.25	75.6 ± 0.7	10
0	0.50	52.8 ± 0.4	12 +

\+ Basal callusing at the cut end.

* Data represent mean of 20 cultures/treatment; repeated thrice.

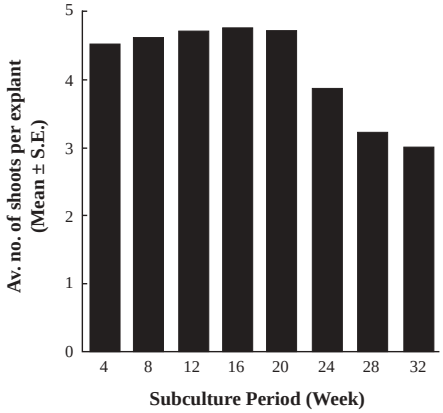


Fig. 2. Effect of subcultures on shoot multiplication of *Lawsonia inermis* cultured on MS basal medium supplemented with 0.25 mg/l BA, 0.25 mg/l Kn, 0.5 mg/l ascorbic acid and 3 % sucrose. Subcultures took place every 4 weeks. Means of 20 cultures/subculture; repeated thrice.

MS medium supplemented with 0.25 mg/l BA, 0.25 mg/l Kn and 0.5 mg/l ascorbic acid within 4 weeks of culture under a 14 hr photoperiod. At higher concentrations of BA or Kn, the rate of shoot proliferation declined. The axillary meristems produced more number of shoots than the apical meristems. Similar results were reported in *Psoralea corylifolia* (Saxena *et al.* 1998), *Picrorhiza kurroa* (Lal *et al*. 1988, Upadhyay *et al*. 1989) and *Plumbago zeylanica* (Rout *et al.* 1999). Mao *et al.* (1995) reported that BA proved superior to other cytokinins for multiple shoot induction of *Clerodendrum colebrookianum*. Our results demonstrated that the inclusion of either IAA or IBA in the culture medium did not help in shoot multiplication. Prolonged culture on the proliferation and multiplication media containing IAA or IBA resulted in the blackening of the basal ends of the developing shoots. The results are consistent with earlier reports indicating that cytokinins and auxins affect shoot multiplication in other plants using shoot tip or axillary bud explants (Mathew and Hariharan 1990, Rout *et al.* 1992, Rout and Das 1993, Rout *et al.* 1999). The results showed that the number of shoots per culture was increased in continuous light both in apical and axillary meristems than in the 14 hr photoperiod. The interaction of photoperiod and plant growth regulators had a significant effect on shoot morphogenesis as reported earlier (Baraldi *et al*. 1988, Samantaray *et al.* 1995). With the increase in the concentration of either BA or Kn, the percentage of shoot multiplication declined. The results also implied that there were differences among the treatments for both the percentage of culture developing multiple shoots and the mean numbers of shoots per culture. The variation of response was due to the varying concentrations of growth regulators used in the medium and light condition as reported earlier in *Lavandula latifolia* (Calvo and Segura 1989) and *Zingiber officinale* (Rout and Das 1997). The rate of multiplication was high and stable up to the 5th subculture and declined in subsequent subcultures. This might be due to the balancing of the endogenous and exogenous growth regulators and the ionic concentration of nutrient salts as reported earlier in other plants (Zimmerman 1985, Rout and Das 1997). The elongated shoots were rooted the maximum in MS basal salts supplemented with 0.25 mg/l IBA with 2 % sucrose. The rooting ability was reduced with the increase in the concentration of IAA or IBA in the medium. The percentage of shoots forming roots and days to rooting significantly varied with different concentrations of IAA or IBA. Similar observations were made in *Plumbago rosea* (Satheesh Kumar and Bhavanandan 1988) and *Psoralea corylifolia* (Saxena *et al.* 1998). The rooted plantlets were established in the field and grew normally.

In conclusion, an attempt was made to develop an *in vitro* protocol for mass multiplication of *L. inermis* by manipulating the nutrient salts, growth regulators and culture conditions. The pattern of morphogenesis on various phytohormonal regimes largely confirm those reported in other plant species (Koblitz *et al.* 1983, Ideada *et al.* 1988, Lal *et al.* 1988). This investigation may be useful for conservation of economic plant species.

ACKNOWLEDGMENTS

The authors acknowledge the Department of Forest and Environment, Government of Orissa for necessary facilities.

RESUMEN

Se desarrolló un protocolo exitoso para la propagación en masa de *Lawsonia inermis* Linn., una planta medicinal importante. Se indujeron múltiples tallos en meristemos apicales y axilares derivados de explantes maduros de *L. inermis* en medio de Murashige y Skoog (1962) suplementado con 0.25 mg/l 6-benzylaminopurina (BA), 0.25 mg/l quinetina (Kn), 0.5 mg/l ácido ascórbico y 3 % (w/v) sucrosa. La tasa de multiplicación fue más alta cuando los cultivos fueron incubados bajo luz continua que bajo el fotoperíodo de 14 hr. El enraizamiento se logró al transferir los microtallos al medio basal, semi-sólido MS suplementado con 0.25 mg/l de ácido indol-3-butírico

(IBA) luego de 10 días de cultivo. Las plántulas micropropagadas fueron aclimatizadas y cultivadas con éxito en el suelo.

REFERENCES

Anonymous. 1962. The wealth of India : A dictionary of Indian raw materials and industrial products. Vol. 6. Council of Scientific Industrial Research, New Delhi, India. 47-50 p.

Baraldi, R., F. Rossi & B. Lercari. 1988. *In vitro* shoot development of *Prunus* GF 6652: Interaction between light and benzyladenine. Plant Physiol. 74: 440-443.

Calvo, M.C & J. Segura. 1989. Plant regeneration from cultured leaves of *Lavandula latifolia* Medicus: Influences of growth regulators and illumination condition. Plant Cell Tiss. Org. Cult. 19: 33-42.

George, E.F. & P.D. Sherrington. 1984. Plant propagation by tissue culture. Exegetics, Eversley, England.

Hu, C.Y & P.J. Wang. 1983. Techniques for propagation and breeding, p. 177-277. *In* D.A. Evans, W.R. Sharp, P.V. Ammirato & V. Yamada (eds.). Handbook of plant cell culture. MacMillan, New York.

Ideada, K., D. Teshima., T. Aoyama, M. Satake & K. Shimomura. 1988. Clonal propagation of *Cephaelis ipecacuanha*. Plant Cell Rep. 7: 288-291.

Kirtikar, K.R. & B.D. Basu. 1981. Lythraceae, p 1076-1080. *In* K.R. Kirtikar & B.D. Basu (eds.). Indian medicinal plants. Vol. 2. Int. Book Dist., Dehradun, India.

Koblitz H., D. Koblitz, H.P. Schmauder & D. Groger. 1983. Studies on tissue cultures of the genus *Cinchona* L. Plant Cell Rep. 2: 95-97.

Lal, N., P.S. Ahuja, A.K. Kukreja & B. Pandey. 1988. Clonal propagation of *Picrorhiza kurroa* Royk ex Benth by shoot tip culture. Plant Cell Rep. 7: 202-205.

Mathew, M.K & M. Hariharan. 1990. *In vitro* multiple shoot regeneration in *Syzygium aromaticum*. Ann. Bot. 65: 277-279.

Marascuilo, L.A. & M. McSweeney. 1977. Post-Hoc Multiple Comparisons in sample preparations for test of homogenesity, p. 141-147. *In* M. McSweeney & L.A. Marascuilo (eds.). Non-parametric and distribution free methods the social sciences. Books/Cole, U.S.A.

Mao, A.H., A. Wetten, M. Fay & P.D.S. Caligari. 1995. *In vitro* propagation of *Clerodendrum colebrookianum* Walp.: A potential natural anti-hypertension medicinal plant. Plant Cell Rep. 14: 493-496.

Murashige, T & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Rout, G.R & P. Das.1993. Micropropagation of *Madhuca longifolia* (Koenig) MacBride var. latifolia Roxb. Plant Cell Rep. 12: 513-516.

Rout, G.R., U.C. Mallick & P. Das. 1992. *In vitro* plant regeneration from leaf callus of *Cephaelis ipecacuanha* A. Richard. Adv. Plant Sci. 5: 608-613.

Rout, G.R & P. Das. 1997. *In vitro* organogenesis in ginger (*Zingiber officinale* Rosc.). J. Herb. Spice. Med. Plant. 4: 41-51.

Rout, G.R, C. Saxena, S. Samantaray & P. Das. 1999. Rapid clonal propagation of *Plumbago zeylanica* Linn. Plant Growth Regul. 28: 1-4.

Samantaray, S., G.R. Rout & P. Das. 1995. An *in vitro* study on organogenesis in *Trema orientalis* (Blume) Linn. Plant Sci. 105: 87-94.

Satheesh Kumar, K. & K.V. Bhavanandan. 1988. Micropropagation of *Plumbago rosea* Linn. Plant Cell Tiss. Org. Cult. 15: 275-278.

Saxena, C., G.R. Rout & P. Das. 1998. Micropropagation of *Psoralea corylifolia*. J. Med. Arom. Plant. Sci. 20: 15-18.

Upadhyay, R, N. Arumugam & S.S. Bhojwani. 1989. *In vitro* propagation of *Picrorhiza kurroa* Royle Ex Benth- An endangered species of medicinal importance. Phytomorphology 39: 335-342.

Wickson, M. & K.V. Thimann. 1958. The antagonism of auxin and kinetin in apical dominance. Physiol. Plant. 11: 63-74.

Zimmerman, R.H. 1985. Application of tissue culture propagation to woody plants, p. 165-177. *In* R.H. Henke, K.W. Hughes, H.J. Constantin & A. Hollaender (eds.). Tissue culture in forestry and agriculture. Plenum, New York.