The Bioscan 6(1) : 39-42, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

IN VITRO MICROPROPAGATION AND CALLUS INDUCTION OF *LAWSONIA INERMIS* L. FROM SHOOT TIPS

NAZRA PAIKER* AND KUNUL KANDIR
Department of Botany,
Ranchi University, Ranchi - 834 008
E-mail: nazrapaiker@gmail.com

KEY WORDS
Lawsonia inermis L.
IAA, IBA, 2, 4-D, KN
Callus

Received on :
21.08.2010
Accepted on :
27.12.2010

***Corresponding author**

ABSTRACT
Different concentration of phytohormones affected Callus formation of *Lawsonia inermis* L. Explant of *Lawsonia inermis* was cultured in MS medium supplemented with different concentration of IAA, IBA, 2,4-D, NAA, KN, BAP. Medium supplemented with various phytohormones for organogenesis. Cultures were kept on 25 ± 2^{o} temperature and 16 hr photoperiod while callus was observed on different concentration of auxin or cytokinin alone or in combination. Most suitable medium for callus formation from shoot tips was that 2, 4 – D (2.5 mgL^{-1}) + IAA (0.5 mgL^{-1}).

INTRODUCTION

Lawsonia inermis L. (Lythraceae) commonly has known as Henna, Mehandi an important medicinal plant. It is a glabrous much branched deciduous shrub (Muhammad and Mustafa, 1994). This plant is worldwide known cosmetic agent used to colour hair, skin and nails (Hanna *et al.*, 1998). However it is not only relevant to cosmetic Henna was also reported to have tuber culostatic activity. The leaves are also used as prophylactic against skin disease. They are used extremely in the form of paste or decoction against boils, burns, bruises and skin inflammation. A decoction is used as a gargle against sore throat (Rout *et al.*, 2001). The roots of this plant are useful in burning sensation, leprosy, strangury and premature graying of hair. The major phytochemical constituents of Henna, Lowsone is found to posses significant anti-inflammatory, analgestic and antiphyretic activities (Ali *et al.*, 1995)

Recently, this compound has been reported to have growth inhibitory effect against human colon carcinoma, HCT – 15 cells (Kamei *et al.*, 1998).

Although the medicinal value of *Lawsonia inermis* L. is widely cultivated as a hedge plant. The flower of Lowsonia 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 be set with many problems that restrict multiplication on a large scale. Preservation of genetic stability in germplasm. Collections and micropropagation of elite plant of the ulmost importance and propagation of plants through apical or axillary meristem culture allows recovery of genetically stable and true to type progeny. At the present communication describes a successful protocol for mass propagation of *L. inermis* L. Tissue culture and micropropogation protocols have been describe a for a number of woody species (Prez and Ochao Alezo, 1997). *In vitro* culture of woody species have low rate of bud opening and high rates of ethylene induces leaf abscission. Browning of media is also a serious concern in micropropagation of woody species. (Resai *et al.,* 1994). The impact of cytokinin and auxin in solution and also in different combination on micropropagation of the species they studied.

Long term usefulness of tissue culture for woody species has been described as limited. Thus, it is important to determine empirically the utility of tissue culture for various woody plants that have been deemed to be of medicinal value. The benefit of a successful tissue culture protocol, would allow the deemed commercially and medicinally valuable woody species such as *Lawsonia inermis* L. to be propagated much more rapidly than other more traditional methods.

In this paper, we report the effect of various phytohormones at different concentration in Murashige and Skoog medium.

MATERIALS AND METHODS

Green young leaves explants of 4 - 8 months old *Lawsonia inermis* L. were collected from gardens. The explants were kept under running top water to 15 - 20 minutes. They were washed with 1 - 2 drops of savlon for 2 minutes. They were surface sterilized in 70% ethanol for 30 second and washed 3 - 4 times autoclaved distilled water. They were immerged in 0.1% $HgCl_2$ for ½ minutes. They were rinsed with 3 - 4 times autoclaved distilled water. Leaves were inoculated in test tube containing MS basal media, callus having different morphology was also used as culture material.

Table 1: Effects of various concentrations of auxin incorporated in MS Medium on callus induction from shoot tip culture of *Lawsonia inermis* L. after 4 weeks

Conc. of Phytohormones concentration in MgL^{-1}	% culture showing callus formation 2, 4 – D			IAA			IBA			NAA		
	A	B	C	A	B	C	A	B	C	A	B	C
0.5	9	9	8	10	8	9	5	6	8	NR	NR	NR
1.0	21	17	16	18	22	16	10	7	17	10	9	10
1.5	35	33	36	24	29	35	25	21	22	16	21	20
2.0	71	77	76	40	49	56	32	31	29	21	34	34
2.5	96	98	91	60	70	69	38	34	31	38	48	46
3.0	81	83	81	83	80	84	34	40	41	45	57	55
3.5	64	63	65	80	78	76	48	40	46	63	62	61
4.0	58	55	57	79	72	71	46	44	45	75	76	78
4.5	55	56	54	71	69	64	42	40	41	82	81	83
5.0	50	46	45	60	58	52	40	36	37	87	86	86
5.5	38	34	37	45	47	45	38	31	34	86	84	82
6.0	21	25	27	32	35	37	35	34	30	77	78	77
6.5	18	19	21	25	28	23	30	29	25	68	63	61
7.0	15	17	20	20	19	16	23	21	20	56	59	54
7.5	12	11	10	19	17	21	20	20	19	44	46	46
8.0	9	10	9	10	10	7	16	18	14	31	35	32
8.5	8	6	8	8	8	9	12	12	10	25	35	32
9.0	4	3	4	7	6	7	9	10	5	25	28	26
9.5	2	1.5	1.5	5	7	6	5	6	5	16	14	11
10	NR	NR	2	2	1	1	3	2	3	10	9	6

Culture media

Semisolid MS media (Murashige and Skoog, 1962) containing 3% sucrose with varying concentration of phytohormones was used for callus formation and roots and shoot regeneration. Combination of auxin (IAA, IBA, NAA, 2, 4-D) was also used for root and shoot regeneration. Coconut milk (CW – 20% v/v) was also used in MS basal medium for regeneration system. The pH of media was adjusted to 5.8 before gelling with Agar – Agar (0.8 w/v) by adding 0.1% NaOH or 0.1% HCl and autoclaved for 15 to 20 minutes at 15^{PSI} and 121^{0} surface sterilized leaves were inoculated into the culture medium in culture room.

Culture condition

Culture was inoculated at $25 \pm 2^{o}C$ under cool fluorescent light (1500 – 2000 Lux) with a 16 hr/8 hr light dark cycle. Each treatment consisted of minimum 15 explants and all experiments were carried out under sterile condition. All inoculation tools like forceps, needle, scissors, blade, scalpel etc. were thoroughly autoclaved. Culture area, glass wares and all the tools used were properly sterilized with UV light and floor surface were swabble with 95% ethyl alcohol. Inoculation tools were flamed time to time.

RESULTS AND DISCUSSION

Shoot tips and young leaf plants were cultures on MS medium. Supplemented with various phytohormones with different concentration of 2, 4-D, IAA, IBA, KN and BAP. These growth regulators were used singly or in combination.

Effect of auxins

The effect of auxins on the induction of callusing from shoot tip and leaf was different. The callus developed was white in colour in nature. Only callusing and no organogenesis were seen in on medium containing 2, 4–D (Fig. 1). The best result was observed at $2.5mgL^{-1}$ concentration of auxins hundred percent cultures exhibited callusing at this concentration. IAA proved to be more effective than IBA in inducing callus from shoot tip culture. The shoot tip culture on MS Medium containing IAA ($0.5mgL^{-1}$ – $10mgL^{-1}$) showed callusing from its cut end. Incorporation of NAA on MS medium at concentration ranging from 0.5 to $10mgL^{-1}$ yielded callusing from cut ends of shoot tips. $5mgL^{-1}$ was recorded as the best concentration for callus induction and 100% culture exhibited callusing (Fig. 2; Table 1).

Effect of Cytokinins

When MS medium was supplemented with $5mgL^{-1}$ concentration of cytokinins (KN, BAP) callus formation was induced. The callus was not fast growing one and did not grow further sub-culture in medium even similar composition. Root differentiation was never observed in any such cultures.

Differentiation of shoot bud from shoot tips was observed in 45% cultures on MS medium supplemented with $1.5mgL^{-1}$ concentration of BAP (Table 2). Kinetin was found moist effective in differentiation of shoot buds from shoot tip in comparison to BAP (Table 2).

Effect of combinations of auxin and cytokinin

MS basal medium supplemented with combination of cytokinin and auxin shooting and rooting was observed. Kinetin was found to be the most effective cytokinin for shoot differentiation in combination with auxin. Most suitable medium found for shoot formation from shoot tips was a combination kinetin ($3mgL^{-1}$) + IAA ($1.5mgL^{-1}$). 90% of cultures exhibited shoot differentiation in above combination (Table 2; Fig. 3 and 4). Kinetin in combination with auxin was also found very effective in stimulating shoot formation from shoot tip (Fig. 2 and 4). The synergistic effect of higher concentration of cytokinin with lower concentration of auxin induced better shoot organogenic response has been reported by various

workers in different plant species (Mishra *et al.*, 2004 and Vidya *et al.*, 2005). It was found that in higher cytokinin supplementation shoots were recorded with stunted growth, short internodes and crowded leaves which are in confirmatory with the findings reported for few other medicinal plants (Rudra and Jewarkar, 2002). Similar result was obtained by Nair *et al.*,

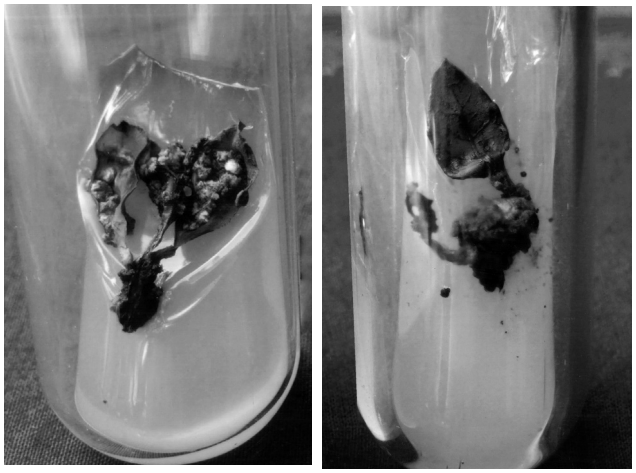

Figure 1 and 2: (1) 14 days old callus from shoot tip culture on MS medium containing 2, 4-D; (2) 3 weeks old callus from shoot tip on MS medium containing NAA

Figure 3 and 4: (3) 3 weeks old callus on MS + Kinetin + IAA; (4) 4 weeks old culture of callus on MS + Kinetin + NAA

Table 2: Effects of various concentrations of cytokinin amd combination of cytokinin and auxin on cellus induction and shoot differentiation from shoot tip culture of *Lawsonia inermis* L.

MS Medium supplemented with	Group	% culture showing shoot formation			
		1st week	2nd week	3rd week	4th week
KN (1mgL^{-1})	A	10	12	19	22
	B	9	13	18	23
	C	8	15	20	24
KN (5mgL^{-1})	A	7	15	20	28
	B	8	16	21	29
	C	9	17	22	27
BAP (1mgL^{-1})	A	13	22	34	37
	B	15	23	33	36
	C	14	21	35	38
BAP (1.5mgL^{-1})	A	16	30	42	59
	B	15	31	45	60
	C	19	29	44	59
BAP (2mgL^{-1})	A	14	30	35	58
	B	15	29	34	60
	C	17	31	33	59
BAP (5mgL^{-1})	A	7	14	18	21
	B	8	15	19	22
	C	6	13	17	20
KN (0.5mgL^{-1}) + NAA (0.5mgL^{-1})	A	41	59	72	84
	B	42	61	75	86
	C	43	59	74	87
KN (1mgL^{-1}) + IAA (2mgL^{-1})	A	21	45	53	63
	B	24	46	55	64
	C	25	44	54	62
KN (2mgL^{-1}) + IAA (1mgL^{-1})	A	19	27	40	55
	B	20	28	41	53
	C	18	26	42	54
KN (3mgL^{-1}) + IAA (1.5mgL^{-1})	A	42	60	73	94
	B	40	62	74	93
	C	41	61	75	95
KN (5mgL^{-1}) + IAA (2.5mgL^{-1})	A	13	28	36	51
	B	15	29	35	52
	C	14	27	37	50
KN (10mgL^{-1}) + IAA (5mgL^{-1})	A	6	13	19	24
	B	7	14	20	25
	C	4	16	18	23

(1983). Many authors reported that several plant species highly responded on MS + auxin and cytokinin combination (Radhamani and Chandel, 1992).

In the present study, media containing coconut water (20% v/v) showed similar result. Shoot growth and multiple shoot production induced by medium containing coconut water have been reported in a number of tree species of Fobaccae (Nadgair *et al.*, 1984). Effects of cytokinins and auxin on micropropagation *Clitoria ternatea* L. Which also belongs to the family Fabaceae was observed by Rout (2004). Callus formation was greatly effected by type and age of explants and growth regulators used. The exogeneons growth regulators requirement (types, concentrations, auxin to cytokinin ratio) for callus formation depends, upon the genotype and endogenous hormone contents of the tissue (Pierik, 1987).

Disease free plants of *Lawsonia inermis* L. could be produced in large scale in lesser time on the basis of the present study.

REFERENCES

Ali, B. H., Bashir and Tanira, M. O. 1995. Anti inflammatory, antipyretic and analgesic effect of *Lawsonia inermis* L. (henna) in rats. *Pharmacology.* **51:** 356 - 363.

Hanna, R., Maciej, J. N., Lapinsky, L. and Adamowicz, L. 1998. Molecular structure and infra red specta of 2-hydroxyl, 4-naphthaquinone: Experimental matrix isolation and theoretical Hartree Fock and Post Hartree Fock study. *Spec. Act.* **54:** 1091 - 1103.

Kamei, H., Koide, T., Kojima, T., Hashimoto, Y. and Hasegawa, M. 1998. Inhibition of cell growth in culture by quinones. Cancer Biother. *Radiopharm.* **13:** 185 - 188.

Mishra, P., Datta, S. K., Johri, J. K., Singh, H. B. and Srivastava, A. 2004. An improved method *in vitro* large scale propagation of *Piper betle* L. *J. Plant Biochemistry and Biotechnology.* **13:** 161 - 164.

Muhammad, Z. and Mustafa, A. M. 1994. Traditional Malay Medicinal plants. Kuala Lumpur : Fajar Bakti Sdn. Bhd. '" 2

Nadgair, A. L., Phadke, C. H., Gupta, P. K., Parsharam, V. A., Nair, S. and Mascarehas, A. 1984. Rapid multiplication bamboo by tissue culture. *Silvae Genet.* **33:** 219. - 223.

Nair, S., Gupta, P. K. and Mascarnhas, A. F. 1983. Haploid plants from *in vitro* anther culture of *Annona squamosa* Linn. *Plant cell Rep.* **3(4):** 198 - 200.

Pierik, R. L. M. 1987. *In vitro* culture of Higher Plants. Martinus Nijhoff Publishers. Dordrecht, NL.

Prez - Molphe – Balch, F. and Ochao - Alezo, N. 1997. *In vitro* plant regeneration of Maxican Lime and mandarin by direct organogenesis. *Hort. Sci.* **32(5):** 931 - 934.

Radhamani, J. and Chandel, K. P. S. 1992. Cryoperservation of embryonic axes of trifoliate orange (*Cponcirus trifoliata* L. RA. F.) *Plant Cell Rep.* **11:** 372 - 374.

Resai, S., Kantharajah, A. S. and Dodds, W. A. 1994. The effect of growth regulators, sucrose of explants and irradiance of *In vitro* regeneration of atemoya. *Aust. Bot.* **42:** 330 - 340.

Rout, G. R. 2004. Effect of cytokinins and auxin on micropropagation of *Clitoria ternatea* L. *Biol. Lett.* **41(1):** 21 - 26.

Rout, G. R., Das, G., Samantoray, S. and Das, P. 2001. *In vitro* micropropagation of *Lawsonia inermis* (Lythraceae Int. *J. Trop. Biol. Conserv.* **49:** 1 - 7.

Rudra, J. and Jewarkar, A. S. 2002. *In vitro* multiplication of *Punica granatum* Linn. CV GBG - 1 (Ganesh), through axilary bud culture. *Adv. Plant. Sci.* **15(1):** 5 - 10.

Vidya, S. M., Krishna V., Manjunatha, B. K. and Shankarmurthy, K. 2005. Micropropagation of *Entada pursaetha* DC - An endongered medicinal plant of Western Ghats. *Indian J. Biotechnology.* **4:** 561 - 564.