Padmavathy S. / Vol 4 / Issue 1/ 2014 / 41-45.

International Journal of

Innovative Drug Discovery

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

www.ijidd.com

CALLUS PRODUCTION STUDIES IN *STROBILANTHUS FOLIOSUS* AND *SMILAX WIGHTII*

S.Padmavathy

PG & Research Department of Botany, Nirmala College for Women, Coimbatore, Tamilnadu, South India.

ABSTRACT

Strobilanthus foliosus and *Smilax wightii* are two important endemic medicinal plants which are extensively used in Ayurvedic medicine and are highly demand able in pharmaceutical industries. Induction of callus in *Strobilanthus foliosus* and *Smilax wightii* was carried out using leaf segment explants. The explants were tried with various concentration and combinations of phytohormones. In *Strobilanthus foliosus*, stock callus developed from leaf segment on MS-medium supplemented with 2, 4, D (2.0mg/l) and BAP (0.5 mg/l) which was used for further experimentation. The callus so formed was green, soft, healthy and fast growing. Whereas in case of *Smilax wightii* leaf segment explants were used as explants for callus induction and its establishment with various concentrations and combinations of phytohormone. Stock callus developed from leaf segment on MS medium supplemented with 2, 4 D (1.0mg/l) and NAA (1.5 mg/l) was used for further experimentation. The callus so formed on this medium was light green, compact, hard healthy and fast growing. The stock calli (non-differentiating calli) developed were maintained on MS medium by frequent subculturings after every 4-5 weeks.

KEY WORDS: Tissue culture, Medicinal plants, Auxin, Napthalene Acetic Acid.

INTRODUCTION

Medicinal Plants are a valuable source of a vast array of chemical compounds; they synthesize and accumulate extractable organic substances in quantities sufficient to be economically useful as raw materials for various commercial applications. Plant tissue culture technology holds great promise for micropropagation, conservation, and enhancement of the natural levels of valuable secondary plant products and to meet pharmaceutical demands and reduce the in situ harvesting of natural forest resources

In vitro cell and tissue culture methodologies are envisaged as a mean for germplasm conservation to ensure the existantance of endangered plant species, rapid mass propagation for large scale regeneration, and for genetic manipulation studies.

Combinations of *in vitro* propagation techniques [1] may help in conservation of biodiversity of locally used m edicinal plants. In-vitro propagation of plants holds tremend ous potential for the production of high-quality plant-based medicines [2].

Callus culture consists of an undifferentiated, proliferating mass of cells usually arising on wounds of differentiated tissues and cells. Normally juvenile and hence physiologically the most active tissues give better callus formation. The exogenous plant growth regulator is required for callus formation [3]. The strongest callus induction factor is the growth/nutrient medium supplemented with plant growth regulators [4]. There are various reports on in vitro induction of callus and production of secondary metabolites in medicinal plants [5].

The present investigation on tissue culture of *Strobilanthus foliosus* and *Smilax wightii* was carried out in-vitro keeping in view raising callus cultures from leaves as explants under defined nutritional, hormonal and cultural conditions.

MATERIALS AND METHODS

Corresponding Author:- **S.Padmavathy** Email:- rajpadmah@gmail.com

Plant materials

The medicinal plants were collected from Shola forests, Kothagiri, Tamilnadu District and plants were authenticated by Dr. S.Padmavathy, Associate Professor, PG & Research Department of Botany, and Nirmala College for Women, Coimbatore, and Tamilnadu.

Inoculation

All the experimental inoculations were carried out under aseptic conditions in a Laminar Airflow. The bench of the chamber was thoroughly scrubbed with cotton dipped in rectified spirit (alcohol). Surface of all objects (spirit, lamp, coupling jar, forceps, clinical knives, flasks containing distilled water, sterilizing agents, culture vessels, instrument tray etc.) were thoroughly cleaned before placing them in the chamber. Laminar flow cabinet was also sterilized by ultra violet light (UV) for 30 minutes. Fresh unsterilized leaves from *Strobilanthus foliosus* and *Smilax wightii* were washed under tap water, then with detergent and finally rinsed with distilled water to remove every trace of detergent. These explants pre-treated with antioxidants were surface sterilized in a sterile flask using mercuric chloride (0.1%) for 3-5 minutes with continuous shaking. They were repeatedly washed with sterile distilled water and then were inoculated vertically or horizontally on the surface of the medium. The inoculated culture flasks/tubes were kept in culture room under controlled conditions.

Culture conditions

The cultures were maintained at a temperature of 30±2°C under 55±5% relative humidity in the culture room. A rhythmic cycle of 16 hours light followed by 8 hours darkness was given to the cultures. Light was provided by a combination of cool white fluorescent tubes and incandescent bulbs in the ratio of 3:1. The light intensity as irradiance varied according to experimentation in with range of 2500 lux to 3000 lux. The temperature light and relative humidity conditions varied according to the experiment to get optimal regenerations.

Callus induction and establishment

Leaves of both the plants were collected from mature plants and were used as explants for callus formation, establishment and development. Initially explants were washed with running tap water and then cut into small pieces of appropriate size (8-10 mm). These explants were then washed with 2% commercial detergent and rinsed thoroughly with sterile distilled water. Explants were surface sterilized with 0.1% $HgCl_2$ solution for 3-5 minutes followed by several washings in sterile distilled water. The surface sterilized explants were finally inoculated on MS-medium supplemented with different growth regulators. All experimental manipulations were carried out under aseptic conditions. The medium was pre-adjusted to pH 5.8 and autoclaved at 15 psi for 20 min. Callus of each plant raised on MS-medium *in vitro* was maintained for six month by frequent subculturings on fresh medium after every 4-5 weeks, for its biomass production.

Callus was also subcultured on fresh medium in order to evaluate whether the callus showed any morphogenic response or continued to proliferate as such. For maintenance of stock callus, non-embryogenic and rhizogenic callus was discarded in every passage and only regenerable callus pieces were used for further subcultures on fresh medium at an interval of 4-5 weeks. Fresh weight of callus was recorded by weighing the callus tissue after separating it from the plant. All the cultures were incubated at 30±2°C under 16 hr. photoperiod, illuminated by fluorescent light of about 2500-3000 lux intensity and 55±5% relative humidity. The cultures were regularly subcultured after 4-5 weeks.

Table 1. Callus proliferation from leaf explants of *Strobilanthus folisus* on MS medium supplemented with 2, 4, D (2mg/l) and BAP (0.5 mg/l)

Hormone concentrations (mg/l)		**Callus response (%)**
2,4-D	**BAP**	**leaf**
0.1		10
0.5		21
1.0		35
1.5		59
2.0		**78**
2.5		64
3.0		51
	0.1	5
	0.2	18
	0.3	22
	0.4	28
	0.5	**36**
	0.6	25
0.1	0.5	31

0.5	0.5	42
1.0	0.5	58
1.5	0.5	78
2.0	**0.5**	**89**
2.5	0.5	73
3.0	0.5	63

Table 2. Callus initiation from leaf explants of *Smilax wightii* on MS medium supplemented with NAA (1.5mg/l) and 2, 4, D (1 mg/l)

Hormone concentrations (mg/l)		Callus response (%)
NAA	**2,4,D**	**leaf**
0.1		12
0.5		19
1.0		24
1.5		**48**
2.0		32
2.5		30
3.0		26
	0.1	7
	0.5	13
	1.0	**35**
	0.4	30
	0.5	23
	0.6	20
0.1	1.0	27
0.5	1.0	40
1.0	1.0	59
1.5	**1.0**	**84**
2.0	1.0	71
2.5	1.0	63
3.0	1.0	58

Figure 1. Callus induction of *Strobilanthus folisus*

10 Days

20 Days

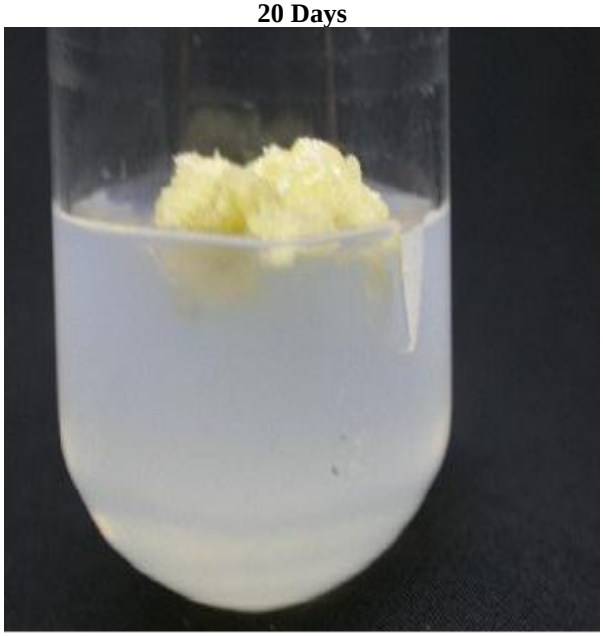
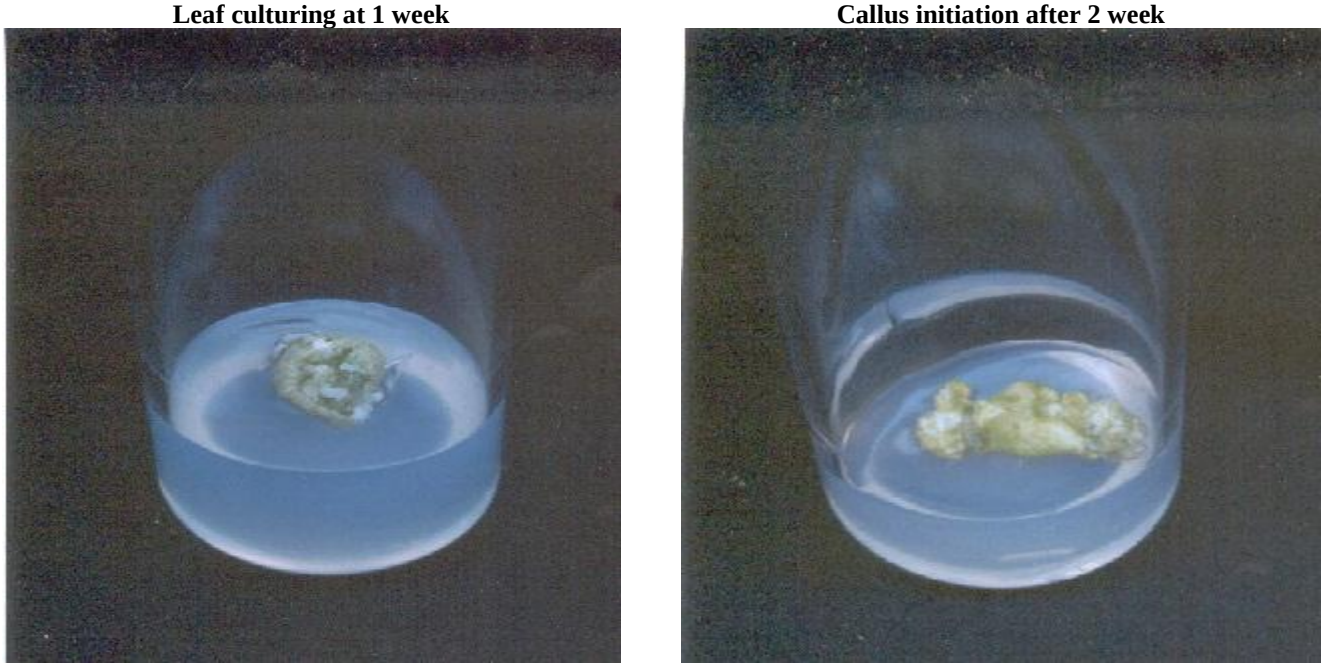


Figure 2. Callus induction of *Smilax wightii*

Leaf culturing at 1 week **Callus initiation after 2 week**



RESULTS AND DISCUSSION

In this study, two different plants for tissue culture and various combinations or concentrations of growth regulators were used to find out their effects on callus induction. As shown in Figure 1&2 callus formation started from cotyledon hypocotyls explants after one week of culturing on the callus induction media. Various auxins viz. 2, 4-D, NAA, BAP when incorporated on MS-medium separately at different concentrations (0.1-3.0 mg/l) produced callus with variable growth and yellowish white in color (Table- 1 &2). Among these auxins 2, 4-D was the best for callusing 2, 4-D at concentration 2.0 mg/l was optimum for callus formation from leaf segment explants. 2, 4-D (2.0 mg/L) which was found optimal in previous set of experiments was combined with BAP (0.5-0.5 mg/l) of NAA (0.1-3.0 mg/l). It was observed that 2, 4-D (2.0 mg/l) in combination with BAP (0.5 mg/l) was best for callus induction and establishment, the callus so produced was healthy, fast growing and light green in color.

Auxins and cytokinins are the most widely used plant growth regulators in plant tissue culture and usually used together [6]. It was revealed that auxins played an important role in the callus induction and different types of auxins had various effects [7,8], and the cytokinins facilitated the effect of auxin in callus induction [9,10]. Similarly callus induction on BAP and NAA were observed in many plants by several workers viz. *Momordica dioica* [11]; *Rauwolfia serpentine* [12] and *Asteracantha longifolia* [13]. NAA was best suited for continuous and sustained growth of callus among the various auxins tested (2, 4-D, IAA, NAA and IBA). Superiority of NAA for induction of callus cultures has been reported in different plant species by Burbulis et al [14] in flax; Lei *et al* [15] in *Erigeron breviscapus*.

In conclusion, we reported an efficient *in vitro* regeneration system of *Strobilanthus foliosus* and *Smilax wightii* and the results could be helpful not only for large scale vegetative propagation, but also for genetic improvement of both the plants through transformation studies. Optimized callus induction media for *Strobilanthus foliosus* and *Smilax wightii* consisted of the MS supplemented with 2.0 mg/l 2, 4 D and 0.5 mg/l NAA or BAP. Effective in-vitro callus mass production can be beneficial for high yield of secondary metabolites and optimization of alkaloid production. In-vitro generation of callus can encourage in-vitro mass production of bioactive compounds of health benefits from *Strobilanthus foliosus* and *Smilax wightii* plants.

ACKNOWLEDGEMENT

I acknowledge UGC (File No. 39-352/2010- dated: 31st Dec.2010) for the financial support to carry out this project.

REFERENCES

1. Fay MF. Conservation of rare and endangered plants using in vitro methods. In Vitro Cell Dev. *Biol. Plant,* 28, 1992, 1- 4.
2. Anand SP, Jayakumar E, Jeyachandran R, Nandagobalan, V. and Doss A. Direct Organogenesis of *Passiflora foetida* L. through Nodal Explants. *Plant Tissue Cult. & Biotech*, 22(1), 2012, 87-91.

3. Pierik RLM. *In-vitro* culture of higher plants. Martinus Nijhoff Publishers, Dordrecht, the Netherlands, 1987.
4. Gibson DM, Ketchum REB and Hirasuna TJ, Shuler ML. Potential of plant cell culture for Taxol production. Science and Applications, 1995.
5. Hina T, Ali S and Asi MR. Appraisal of an important Flavonoid, Quercetin, in callus cultures of *Citrullus colocynthis*. *Int. J. Agri. Biol*., 14(4), 2012, 528-532.
6. Gang YY, Du GS, Shi DJ, Wang MZ, Li XD and Hua ZL. Establishment of *in vitro* regeneration system of the *Atrichum mosses*. *Acta Bot. Sin*., 45, 2003, 1475-1480.
7. Skoog F and Armstrong DJ. Cytokinin annual review. *Plant Physiol*, 21, 1970, 359-384.
8. Baskaran P, Raja Rajeswari B and Jayabalan N. Development of an in vitro regeneration system in sorghum (*Sorghum bicolor* (L.) Moench using root transverse thin cell layers (tTCLs). *Turk. J. Bot*, 30, 2006, 1-9.
9. Rao AQ, Hussain SS, Shahzad MS, Bokhari SYA, Raza MH, Rakha A, Majeed A, Shahid AA, Saleem Z, Husnain T and Riazuddin S (2006).Somatic embryogenesis in wild relatives of cotton (*Gossypium* spp.). *J. Zhejiang Univ. Sci. B*, 7, 2006, 291-298.
10. Yang J, Gong ZC and Tan X. Induction of callus and extraction of alkaloid from Yi Mu Cao (*Leonurus heterophylus* Sw.) culture. *Afr. J.Biotechnol*., 7, 2008, 1157-1162.
11. Nabi SA, Rashid MM, Al-Amin M and Rasul, MG. Organogenesis in Teasle Gourd (*Momordica dioica* Roxb.). *Plant Tissue Cult*, 12(2), 2002, 173-180.
12. Tomar R. and Tiwari S. In vitro morphogenesis of *Rauwolfia serpentina* L. through cotyledons. *Plant Cell Biotechnol. Mol. Biol*, 7, 2006, 53-58.
13. Panigrahi J. Mishra RR. and Behera, M. In vitro multiplication of *Asteracantha longifolia* (L.) Nees – A medicinal herb. *Ind. J. of Biotechnol*, 5, 2006, 562-564.
14. Burbulis N, Blinstrubiene A, Sliesaravicius A and Kupriene R. Some factors affecting callus induction in ovary culture of flax (*Linum usitatissimum* L.). *Biologija*, 53(2), 2007, 21-23.
15. Lei Z, Chenghong L, Ling L and Wanshing C. (2007): Callus induction and adventitious shoot regeneration from petiole of *Erigeron breviscapus*. *Plant Production Science*, 10(3), 2007, 343-345.