Research Article

An Efficient Protocol Devised for Rapid Callus Induction From Leaf Explants of *Canthium parviflorum* Lamk

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Accepted on: 19-12-2013; **Finalized on:** 28-02-2014.

ABSTRACT

The callus culture of *Canthium parviflorum* Lamk. was generated from leaf explants. Different growth regulators greatly influenced the growth of callus cultures. The callus from leaf explants is induced by inoculating the young leaf bits on MS medium supplemented with various auxins (2, 4- Dichlorophenoxyacetic acid (2, 4-D), α-Naphthalene Acetic Acid (NAA) and Indole Buteric Acid (IBA), cytokinins (6-Benzyladenine (BA) and Kinetin (KN) and cytokinin-auxin combination (BA+NAA) in different concentrations were (0.5 to 5.0 mg/l) used. The supplementation of medium with 2 mg/ l 2, 4-D was suitable for callus induction, and most efficient callus produced with the combination of 2, 4-D (2.0 mg/l) and BA (0.2 mg/l).

Keywords: *Canthium parviflorum* callus, leaf explants, growth regulators.

INTRODUCTION

Canthium parviflorum Lam. (syn: *Plectoria parviflora*) of Rubiaceae is commonly called as Carray cheddie in English, Balusu in Telugu. *Canthium parviflorum is* a thorny subscandent shrub with spreading branches distributed throughout India in scrub forests and dry plains. The leaves and roots are astringent, sweet, thermogenic, diuretic, febrifuge, constipating, anthelmintic, and tonic. The ethanolic extract of *Canthium parviflorum* has wound healing properties.[1] It is traditionally used for snake bites.[2] Leaf paste is externally applied twice a day to treat scabies and the ring worm infection[3]. In the wild plants leaf extracts of *Canthium parviflorum* contain phytochemicals such as alkaloids, flavonoids, tannins, steroids, saponins, terpenoids, sanranetin-4-o-glycoside long chain acids and cardiac glycosides in *Canthium* leaf extracts[4]. Zhao *et al*., 2001[5] reported that callus culture could provide alternative supply of secondary metabolites for use in medicine and stimulating the production of novel compounds not found *in vivo*.

This is because of cells in callus cultures are undifferentiated and may not under specific control. [6] Although cell cultures offer a suitable biological system in a controlled environment wherein the morphogenic events can be maintained and regulated by growth regulators in the nutrient medium with a rapid production of plant metabolites of pharmaceutical value. So the secondary metabolite production was most important from callus cultures.[7] *Canthium parviflorum* plant is having germination problems and it's frequently attacked by Meliola fungi.[8] We are previously reported on the *Canthium parviflorum* leaf explants cultured with 2mg/l of 2, 4-D alone medium was most suitable for callus induction and released most valuable phytochemicals,[9] so further going to production of secondary metabolites using with different cytokinins and auxins combinations of using leaf explants, so there is no reports about *in vitro* productions of secondary metabolites.

MATERIALS AND METHODS

Preparation of plant material

The fresh matured plants (100 no.) of the *Canthium parviflorum* collected from A.N.U Campus, Guntur District were used as a source of explants. The leaf explants were excised into 1 cm long segments and were washed with liquid detergent (5% Teepol, Qualigens, India) followed by Bavistin (1% w/v) for 3 min and then mercuric chloride (0.1% w/v) for 1 min. Finally the explants were sterilized with 70% ethanol followed by three times with sterile distilled water and the explants were aseptically inoculated on Murashige & Skoog[10] medium supplemented with various concentrations and combinations of phytohormones for induction of callus.

Callus culture

The explants were cultured on MS (Murashige & Skoog) [10] basal medium supplemented with different concentrations of auxins alone and in combinations with cytokinins. Considering the quantity and quality of callus and percentage of response, best explants were selected. Further callus studies were confirmed with that explants only. About 30 day, old callus was collected and sub cultured on fresh medium with same growth regulator combinations and repeated twice with two week time interval. All the cultures were incubated at 24±2° C under 16 h photoperiod provided by cool white florescent lights. Leaf explants were excised aseptically and cultured on MS medium supplemented with different concentrations of auxins alone and in combinations with cytokinins. Considering the quantity and quality of callus and percentage of response, best explants were selected. Further callus studies were confirmed with that explants only.

Data analysis

All the experiments were repeated thrice with 15 replicates. The effect of different treatments was analyzed using one way analysis of variance (ANOVA), and means were compared using the Tukey test at the 0.05 level of significance.

RESULTS AND DISCUSSION

Callus Culture Studies

Callus is a dedifferentiated and unorganized mass of parenchyma cells formed by the proliferation of parent tissue. It is a good source of genetic variability and adventitious shoot formation.

Table 1: Effect of different concentrations of plant growth regulators on callus induction from leaf of *Canthium parviflorum*

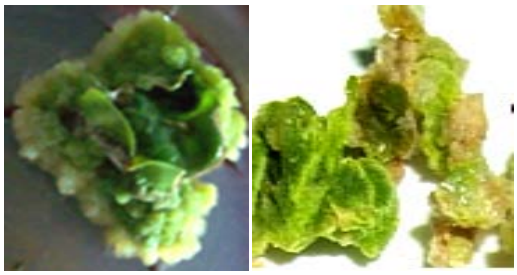
Plant growth regulators	Concentration of plant growth Regulators (mg/L)	Intensity of Callus formation	Leaf explants	
			Mean callus (weight ± SD)	Nature of callus
Control	-	-	-	-
BA	0.5	+	0.18±0.12	White green friable
	1.0	+	0.24±0.14	White green friable
	2.0	+++	0.70±0.23	White green friable
	3.0	++	0.62±0.32	White green friable
	4.0	+	0.28±0.26	White green friable
	5.0	+	0.16±0.42	Compact green friable
KN	0.5	++	0.58±0.10	White friable
	1.0	+	0.50±0.41	White friable
	2.0	+	0.32±0.21	White friable
	3.0	+	0.22±0.13	White friable
	4.0	+	0.30±0.12	White friable
	5.0	+	0.18±0.24	White friable
2.4-D	0.5	+	0.28±0.09	Cream friable
	1.0	+	0.24±0.14	Cream friable
	2.0	+++	0.76±0.26	Cream friable
	3.0	++	0.62±0.07	Light green compact
	4.0	+	0.48±0.22	Light green compact
	5.0	+	0.26±0.60	Light green compact
NAA	0.5	+	0.30±0.24	Light green compact
	1.0	+	0.28±0.40	Light green compact
	2.0	+	0.35±0.06	Light green compact
	3.0	+	0.30±0.22	Light green compact
	4.0	-	-	No callus formed
	5.0	-	-	No callus formed
IBA	0.5	+	0.18±0.20	White friable
	1.0	+	0.35±0.41	White friable
	2.0	+	0.30±0.22	White friable
	3.0	+	0.06±0.05	White friable
	4.0	+	0.08±0.09	White friable
	5.0	+	0.25±0.21	White friable
BA+NAA	0.5+0.5	+	0.12±0.20	Green compact
	1.0+1.0	+	0.32±0.31	Green compact
	2.0+2.0	++	0.66±0.22	Green compact
	3.0+3.0	++	0.62±0.60	Green compact
	4.0+4.0	+	0.14±0.05	Green compact
	5.0+5.0	-	-	No callus formed
BA+2,4-D	0.1+2.0	+	0.30±0.24	Green, compact
	0.2+2.0	+++	1.82±0.21	Green compact
	0.3+2.0	+	0.38±0.70	Green compact
	0.4+2.0	+	0.28±0.04	Green compact
	0.5+2.0	+	0.22±0.16	Green compact

Intensity of callus: (+) low; (++) moderate; (+++) high.

These results indicated that all the growth regulators alone not able to induce a callus from *C.parviflorum* leaf explants. While the combinations of growth regulators showed maximum callus production. The young moderate sized leaf explants were well responded for rapid callogenesis after incubation period of about 4 weeks.

The results indicated that 2, 4-D at 2.0 mg/l on MS medium induced high amounts of callus with high frequency of regeneration interms of their fresh weight and dry weight (Table-1), (Fig-1; A). The 2, 4-D (2.0 mg/l) in combination with BA (2.0 mg/l) also produced maximum amount of callus (Table-1), (Fig-1; A, B).

Morphological and phytochemical differences in callus are attributing to culture conditions, composition of the medium.[11] Variations in the callus forming ability of different explants types, has been reported in many plants.[12] The high auxin and low cytokinin ratio proved their importance for callusing in various explants. Further increase in the concentration of plant growth regulators did not show any significant improvement in callusing. These results agree with previous studies of *Withania somnifera*[13] and *Hypericum heterophyllum.*[14] Cell cultures that have been used extensively for *in vitro* secondary metabolite production were obtained from callus through cell suspension culture.



A B

Figure 1: Proliferation of callus from leaf explants of *Canthium parviflorum.*

A) Callus after 4 weeks (MS medium supplemented with 2, 4-D (2.0 mg/l).

B) Callus formation after 8 weeks (MS medium supplemented with BA+2, 4-D (0.2+2.0 mg/l).

CONCLUSION

Callus culture system offer many advantages as a model system for several biological investigations. Here, in the present investigation an efficient protocol has been devised for *in vitro* callus induction of an important medicinal plant, *Canthium parviflorum* from young leaf explants. Callus culture system offer many advantages as a model system for several biological investigations.

REFERENCES

1. Mohideen, S., Ilavarasan, R, Hemalatha, S. Anitha, N. and Sasikala, E. Wound healing and diuretic activities of *Canthium parviflorum* Lam., Natural Product Sci. 9(2), 2003, 102-104.
2. Parinitha Mahishi, B.H., Srinivasa and M.B. Shivanna. Medicinal plant wealth of local communities in some villages in Shimoga District of Karnataka, India. J. Ethnopharmacol., 98(3), 2005, 307-312.
3. Anitha, B., Mohan, V.R., Athiperumalsami, T. and S. Sutha. Ethnomedicinal plants used by the kanikkars of Tirunelveli District, Tamil Nadu, India to treat skin diseases. Ethnobotanical Leaflets, 12, 2008, 171-180.
4. Sathish Kumar, T. Shanmugam, S. Palvannan, Bharathi Kumar, V.M. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. Leaves, Natural Product Radiance, 7(2), 2008, 122-126.
5. Zhao, J., Hu, Q., Guo, Q., Zhu, W.H. Effects of stress factors, bio-regulators, and synthetic precursor on indole alkaloid production in compact callus clusters cultures of callus clusters cultures of *Catharanthus roseus***.** Appl. Microbiol. and Biotechnol., 55, 2001, 693-698.
6. Wink, M. Physiology of secondary product formation in plants. *In* Secondary Products from plant tissue culture. Edited by Charlwood, B.V. and Rhodes, M.J.C. Clarendon Press, Oxford, 1990, 23-41.
7. Sirigiri Chandra Kala, Kokkanti Mallikarjuna, Challa Siva Reddy. *In vitro* pharmacological investigations of *Biophytum sensitivum* callus extract: Lack of potent activities. J Pharm Negative Results, 4, 2013, 60-5.
8. Hosagoudar, V.B. and Archana, G.R. (2009). Host range of meliolaceous fungi in India. J. Threatened Taxa, 1(5), 2009, 269-282.
9. Chandra Kala S, Mallikarjuna K, and Aruna P. Qualitative Phytochemical Analysis of Seed and Leaf Callus Extracts of *Canthium Parviflorum* Lam. Guntur District, Andhra Pradesh. Int. J. Pharm. Bio. Sci. 3(4), 2012, 177-182.
10. Murashige T. and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol., 150, 1962, 473-497.
11. Murashige, T. Plant propagation through tissue cultures. Ann. Rev. Plant Physiol., 25, 1974, 135-166.
12. Khatun MM, Ali MH, Desamero NV, 2003. Effect of genotype and culture media on callus formation and plant regeneration from mature seed *Scutella* culture in rice. Plant Tiss. Cult., 13, 2003, 99-107.
13. Abhyankar, G.A. and Chinchanikar, G.S. Response of *Withania somnifera* Dunal leaf explants. *In vitro* Phytomorphol., 46, 1996, 249-252.
14. Ali Kemal Ayan and Cuneyt Cirak. *In vitro* Multiplication of *Hypericum heterophyllum*, an Endemic Turkish species. Amer. Jour. Plant Physiol., 1, 2006, 76-81.

Source of Support: Nil, **Conflict of Interest:** None.