BULL. BOT. SURV. INDIA
Vol. 24, Nos. 1-4 : pp. 100-105, 1982

TISSUE CULTURE STUDIES ON SHANKHPUSHPI

(*Convolvulus pluricaulis* Chois.)

V. MUDGAL

Botanical Survey of India, Howrah

ABSTRACT

Callus from root portion of seedlings of *Convolvulus pluricaulis* has been cultured on a medium (BM) based on Murashige and Skoog's (1962) medium. Sucrose, 1.5% ; casein hydrolysate, 1250 mg/l ; IAA, 15 mg/l ; kinetin 1.5 mg/l and GA, 0.5 mg/l, individually were found optimum for the maximum growth of callus.

Maximum hypotensive activity was noticed in the extract of callus tissue cultured on BM supplemented with a higher concentration of casein hydrolysate.

Plant tissue culture procedures have wide application for the study of biosynthesis of primary and secondary constituents of medicinal plants (Carew and Staba, 1965 ; Butcher, 1976 ; Staba, 1976 ; Tabata, 1977 ; Misawa and Samejima, 1978). Our objectives of cultivating the tissues from medicinal plants is to understand the optimum nutrient requirements for their maximum growth and to find out the possibility whether the cultivated tissues could biosynthesize the medicinally active compounds. A survey of literature revealed, that a detailed work has been done on root culture and controlled organogenesis of *Convolvulus arvensis* (Bonnett and Torrey, 1965, Earle and Torrey, 1965a, b), but not on *C. pluricaulis*. The present study deals with the isolation of callus from seedlings of *C. pluricaulis*. In view of the previous reports (Chaturvedi *et al.*, 1966 ; Mudgal, 1975), the hypotensive activity was taken as a parameter for assessing the medicinal value of cultured callus tissue of *C. pluricaulis*.

Date of receipt : 11.2.80. Date of acceptance : 22.2.82.

MATERIAL AND METHOD

The constituents of MS medium (Murashige and Skoog, 1962) were used in all the experiments reported here. The medium was supplemented with IAA (10 mg/l), kinetin (1 mg/l) and Casein hydrolysate (500 mg/l). The medium has been referred as basal medium (BM) in the present paper.

For the initiation of primary culture of *Convolvulus pluricaulis* three slices of about 1 mm size from root portions of 10 days old seedlings, germinated in sterilized conditions, were transferred to culture tubes (100 × 20 mm) containing 20 ml of the basal medium (BM) solidified with 1% 'Difco' agar.

For determination of optimal pH, cultures were maintained in continuous fluorescent light (850 Lux) at temperature 26 ± 2°C. To study the effect of light on callus growth, 6 tubes were kept in continuous dark, 6 in continuous light (850 Lux) and 6 in 10 hrs. light/14 hrs. dark period.

For the study of growth of primary and secondary cultures, the dry weight was determined after drying the tissue in an oven at 60°C for 24 hrs.

Effect of addition of 5, 10, 15, 20 and 25% v/v of coconut milk into the basal medium on the growth of callus was studied. For the study of the effect of different concentrations of sucrose on the callus growth, BM without sucrose served as control and 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% concentrations of sucrose were added to control medium. Similarly for the study of the effect of casein hydrolysate on callus growth, BM without casein hydrolysate served as control and 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500 and 2750 mg/l casein hydrolysate were added to the control medium. Callus tissue weighing 30 mg from primary cultures was used as inoculum in each case.

Effect of common growth regulators on the growth of callus: Inoculum weighing 30 mg was transferred to the replicated subculture tubes containing the basal medium supplemented with different amount of IAA, IBA, 2, 4-D, NAA, GA and kinetin as expressed in the following chart:

S. N.	*IAA*	*IBA*	2, 4-*D*	*NAA*	*GA*	*Kinetin*
1.	BM-IAA (Control)	(BM-IAA) +10 mg/l	(BM-IAA) +10 mg/l	(BM-IAA) +10 mg/l	BM+0.5 mg/l	BM-K (Control)
2.	Control+5 mg/l	BM+1 mg/l	BM+1 mg/l	BM+1 mg/l	BM+1 mg/l	Control+ 0.5 mg/l
3.	Control+10 mg/l	BM+5 mg/l	BM+5 mg/l	BM+5 mg/l	BM+1.5 mg/l	Control+1 mg/l
4.	Control+15 mg/l	BM+10 mg/l	BM+10 mg/l	BM+10 mg/l	BM+2 mg/l	Control+ 1.5 mg/l
5.	Control+20 mg/l	BM+15 mg/l	BM+15 mg/l	BM+15 mg/l	—	Control+ 2 mg/l
6.	—	BM+20 mg/l	BM+20 mg/l	BM+20 mg/l	—	—

BM = Basal medium

Fresh and dry weights of callus were recorded after 35 days of growth. Each observation is the mean of 6 sample in all the experiments reported here.

Hypotensive activity of callus tissue: Alcoholic extracts of the harvested and dried tissues of different sets shown in Table-1 were prepared. They were made completely free from alcohol. Required amount of extracts was weighed and dissolved in distilled water. Test drugs were prepared and hypotensive activity was assessed as per procedure described earlier (Mudgal, 1975).

EXPERIMENTAL RESULTS

Experiments involving the variations in the pH of BM indicated that optimum growth of callus could be noticed at the pH 6.5. Experiments involving three light conditions revealed that maximum growth of callus could be obtained in continuous light (850 Lux). (Fig. 1)

Under these conditions of pH and light, initiation of callusing was observed after the 8th day. However, the growth rate remained extremely low till the 30th day. Induction of callusing in root slices was discerned in the second month. Fresh and dry weights of the callus increased simultaneously. However, an initial reduction in the dry weight percentage was evident, followed by a sharp rise in this parameter (Fig. 2). In few instances, single shoot differentiation was observed during the

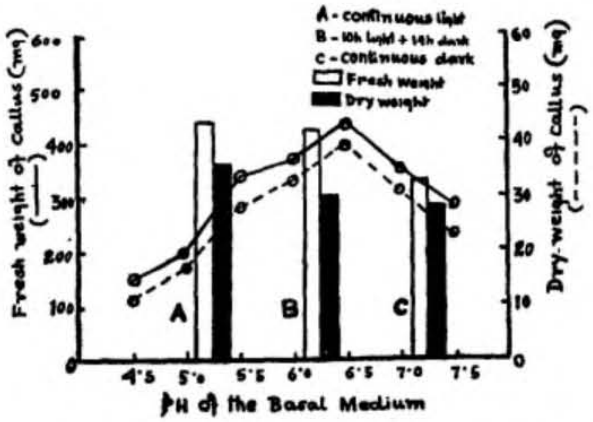


Fig. 1 : Effect of pH and different light conditions on callus growth of *C. pluricaulis*.

second half of the second month (Plate I). The yield of callus in subsequent passages was higher (Fig. 2).

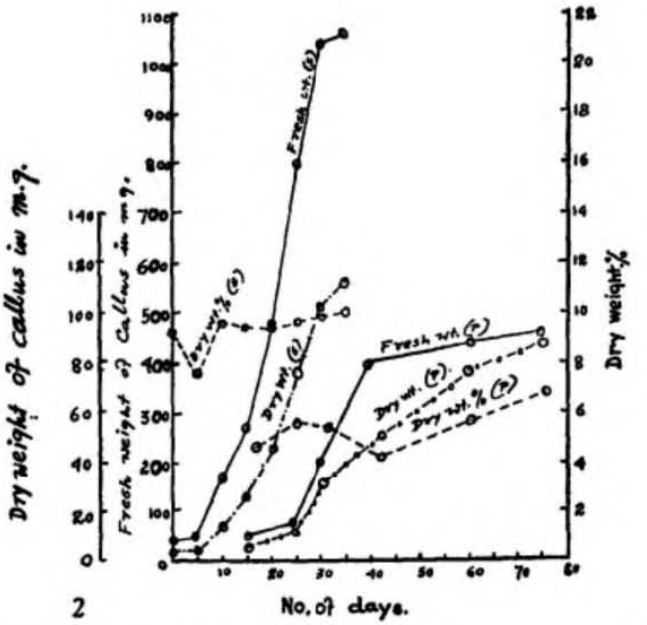


Fig.2 : Growth of callus in primary and sub-cultures P = Primary culture, S = Sub-culture.

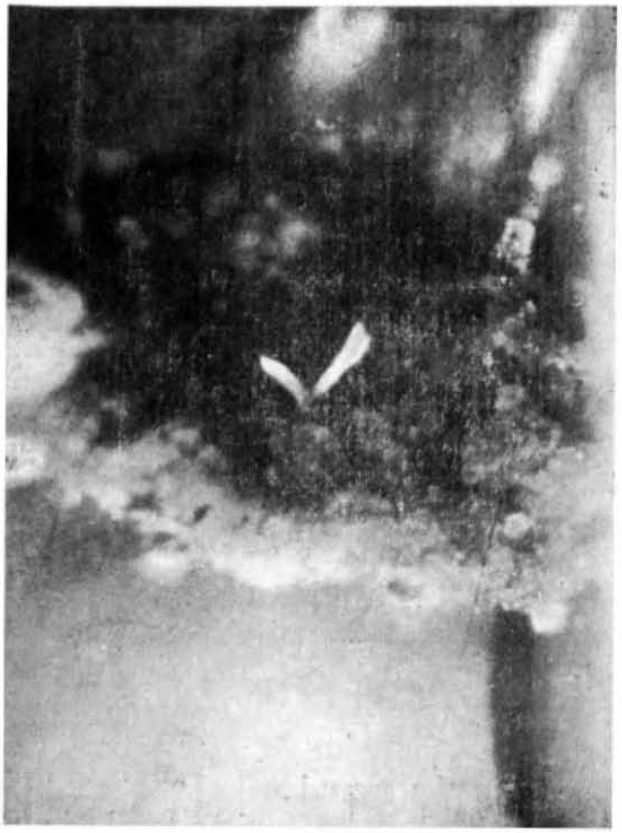


Plate I: Differentiation in callus of *Convolvulus pluricaulis*.

Addition of 5 and 10% coconut milk to the BM resulted in a little increase in the growth rate of callus, whereas a gradual decrease in the fresh and dry weights at a higher concentration of CM was evident (Fig. 3). The inoculum could not grow in BM where sucrose was not added. With increasing concentrations of sucrose in BM a progressive increase in fresh and dry weights of callus was observed upto a concentration of 1.5%. An increase in the concentration of casein hydrolysate beyond 500 mg/l in the BM brought about a further increase in the fresh and dry weights of callus. 1250 mg/l of casein hydrolysate in the BM appeared to be the optimal amount (Fig. 3).

Studies involving the role of various growth regulators in the BM indicated that addition of 15 mg/l of IAA induced a greater growth of the callus in comparison with that of 10 mg/l (Fig. 3). It is evident that IBA, NAA or 2, 4-D cannot replace IAA in BM for callus culture of *C. pluricaulis*. However, an increase in fresh and dry weights of callus could be obtained by supplementing 1-5 mg IBA to BM. At

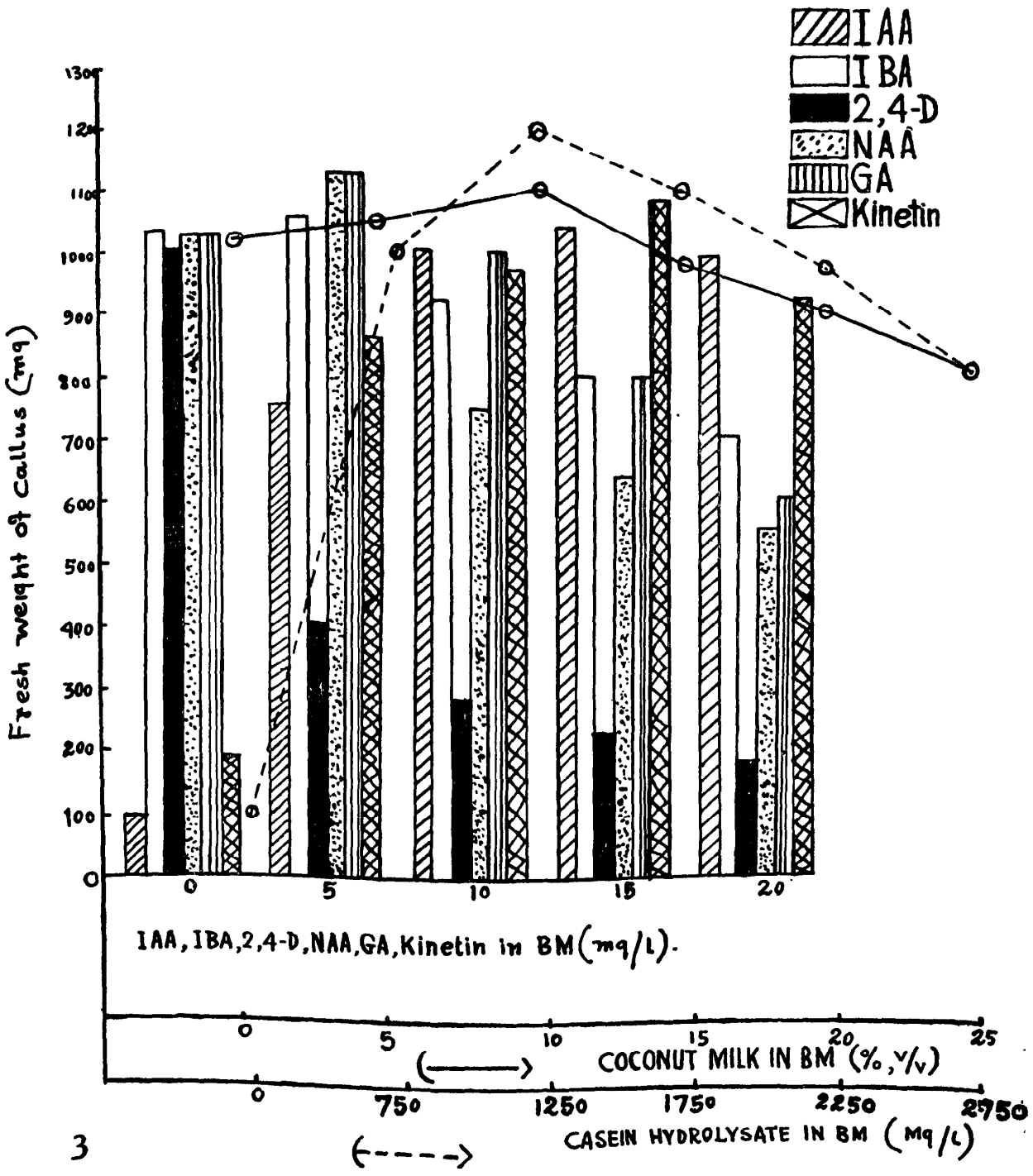


Fig. 3 : Effect of coconut milk, casein hydrolysate, IAA, IBA, 2, 4-D, NAA, GA and Kinetin on the growth of callus in sub-culture. For GA and Kinetin concentration of supplements are figured 10×.

higher concentrations of 2, 4-D, a gradual decrease in the fresh and dry weights of callus was observed. A concentration of 5 mg/l NAA in the BM proved to be beneficial with regard to the final yield of callus. GA, 0.5 mg/l, increased the growth of callus while a higher concentration of GA brought about a decline in the growth. Kinetin, 1.5 mg/l appeared to be stimulatory in comparison to 1 mg/l used in the

BM, with regard to callus growth (Fig. 3).

Hypotensive activity of callus : The results are shown in Table I. Cultures grown on basal medium supplemented with high amount of casein hydrolysate were found to possess maximum hypotensive activity. Cultures grown in continuous dark were devoid of any hypotensive activity while other were possessing an insignificant fall of blood pressure of 3 to 5 mm of Hg. Therefore the hypotensive activity of seedling was also tested to observe whether the source of inoculum possesses the activity or not. The extracts of seedling did not show any significant hypotensive activity as reported earlier (Mudgal *et al.*, 1972) in different parts of this plant (Table 1).

TABLE I

Hypotensive activity of *in vitro* cultured Calli of *C. pluricaulis*

Callus cultured on	*Blood pressure in mm of Hg (Mean)*		
	Before drug	*After drug*	*Decrease in B.P.*
BM in continuous light	102	96	6
BM in 10 hrs. light/14 hrs. dark	102	98	4
BM in continuous dark	102	102	0
BM+10% v/v coconut milk	101	98	3
BM+1.5% sucrose	101	98	3
BM+1250 mg/l casein hydrolysate	100	90	10
BM+5 mg/l IBA	102	99	3
BM+1 mg/l 2,4-D	102	100	2
BM+5 mg/l NAA	101	98	3
BM+0.5 mg/l GA	102	98	4
BM containing 1.5 mg/l kinetin	101	97	4
BM containing 15 mg/l IAA	102	97	5
Seedling of *C. pluricaulis*	100	95	5
(Source of the following data : Mudgal *et al.* 1972)			
Whole plant	104	77	27
Leaves & Flowers	104	60	44
Roots	109	81	28
Stems	106	84	22

Dose = 48 mg /kg
Number of samples for each observation = 2.

Srivastava (1970) concluded on the basis of his unsuccessful attempts of isolating the active principle of this plant that presumably a nitrogen containing derivative of scopoletin may be responsible for hypotensive activity of *C. pluricaulis*. This may be cited as a possible reason for the enhanced activity in callus grown on BM supplemented with high concentration of casein hydrolysate.

ACKNOWLEDGEMENTS

The present work was carried out at Department of Botany and Institute of Medical Sciences, Banaras Hindu University. The author is grateful to Prof. E.R.S. Talpasayi, Department of Botany, BHU for guidance and encouragements. Thanks are also due to Lt. Prof. R. N. Singh, Head of the Department of Botany, BHU and Prof. K. N. Udupa, Director, Institute of Medical Sciences, BHU for facilities. Thanks to the Council of Scientific and Industrial Research, New Delhi for providing financial help.

REFERENCES

BONNETT, H. J. JR. AND J. G. TORREY. Chemical control of organ formation in root segments of *Convolvulus* cultured *in vitro*. *Plant Physiol.* 40 : 1228-1236. 1965.

BUTCHER, D. N. Secondary products in tissue cultures. Verlag, Berlin. pp. 668-693. 1976.

CAREW, D. P. AND E. J. STABA. Plant tissue culture. *Lloydia* 28(1) : 1-26. 1965.

CHATURVEDI, G. N., R. K. SHARMA AND S. P. SEN. Hypotensive effect of certain Indigenous drugs with special reference to Shankhpushpi (*Convolvulus pluricaulis*) in anaesthetised dogs. *Journ. Res. Ind. Med.* 1(1) : 57-66. 1966.

EARLE, E. D. AND J. G. TORREY. Colony formation by isolated *Convolvulus* cells plated on defined media. *Plant Physiol.* 40 : 520-528. 1965a.

—— AND —— Morphogenesis in cell colonies grown from *Convolvulus* cell suspensions plated on synthetic media. *Am. Journ. Bot.* 52 : 891-899. 1965b.

MISAWA, M. AND H. SAMEJIMA. Production of biologically active substances by plant tissue culture. In : Frontiers of Plant Tissue Culture. Ed. T. A. Thorpe, The Bookstore, University of Calgary, Alberta, Canada, pp. 353-362. 1978.

MUDGAL, V. Studies on medicinal properties of *Convolvulus pluricaulis* and *Boerhaavia diffusa*. *Planta Medica* 26(1) : 62-68. 1975.

——, D. N. SRIVASTAVA, R. H. SINGH AND K. N. UDUPA. Comparative studies on the hypotensive action and potentiation of barbiturate hypnosis with different parts of the plant *Convolvulus pluricaulis*. *Journ. Res. Ind. Med.* VII (4) : 74-77. 1972.

MURASHINGE, T. AND F. A. SKOOG. Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum* 15 : 473-497. 1962.

STABA, E. J. Tissue culture and pharmacy. Tissue and Organ culture. Verlag, Berlin. pp. 694-702. 1976.

TABATA, M. Recent advances in the production of medicinal substances by plant cell cultures. Plant Tissue Culture and Its Bio-technological Application. Verlag, Berlin. pp. 3-16. 1977.