

STUDIES IN *CATHARANTHUS ROSEUS* CALLUS CULTURES, CALLUS INITIATION AND DIFFERENTIATION

(MISS) BHARATI DHRUVA, T. RAMAKRISHNAN AND C. S. VAIDYANATHAN*

Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore 560012, India

THE plants of *Catharanthus* species have been reported to have about eighty alkaloids of which vincristine and vinblastine are being utilized as antitumor drugs. The callus cultures of *C. roseus* (L) G. Don produce ajamalacine, vindoline, vindoniline and other alkaloids classified into seven structural types¹. Recently Scott and Lee² have demonstrated the presence of enzymes in *C. roseus* callus that catalyse the synthesis of corynanthe alkaloids. The presence of vincristine and vinblastine in callus cultures has not, however, been reported. The observations of West and Mika with *Atropa belladone* callus³, Newmann with *Macleaya cordata* callus⁴, Newmann and Muller⁵ and Tabata and Hiraoka⁶ with *Nicotiana* callus have indicated that differentiation of callus, specially into root, enhances alkaloid synthesis. It has not, however, been shown whether synthesis of vinblastine and vincristine could be stimulated in callus under conditions favourable for differentiation. This report deals with standardization of the conditions for *C. roseus* viable callus cultures and the differentiation *in vitro*.

The leaf and shoot callus cultures of two varieties of *C. roseus*, one with pink flower (VR) and another with white flower (VA) were initiated *in vitro* following the method of White⁷. The leaf callus in both the varieties (VAI and VRI) were cultivated on half strength Murashige and Skoog's⁸ mineral solution, vitamins, 2, 4-dichlorophenoxy acetic acid (2, 4-D) and coconut milk (CM). The shoot callus in both the varieties (VAs and VRs) were initiated on Murashige and Skoog's basal medium (MSB) containing naphthalene-acetic acid (NAA) and kinetin with or without casein hydrolysate.

The VAI callus was best maintained in MSB with kinetin (1mg/l) and 2,4-D (0.5 mg/l). The callus could grow without auxin or cytokinin, but, addition of these two separately or together increased the growth by 2 to 10 fold. NAA gave equally good growth when percentage increase in dry weight of callus was compared. 6-Benzylaminopurine, kinetin and zeatin, independently, enhanced the growth (Table I) when dry weights were compared. The correlation between light conditions and auxin concentration was quite striking in this callus. When callus was grown in dark and sub-cultured into media having low concentrations (0.2 mg/l) of auxine it did not survive, but at high concentrations of auxins (0.5 and 1 mg/l)

grew profusely. The light grown callus, on the other hand, had no such remarkable change in growth pattern at low or high levels of auxins (Figs. 1 and 2, Tubes 2 and 4). Root-like projections or callus grown on NAA containing media were observed.

TABLE I

Effect of different auxins and cytokinins on the growth of *C. roseus* callus cultures

Medium: Basal medium of Murashige and Skoog's auxins and cytokinins added as mentioned in the table.
Inoculum: 80 mg fresh wt/Tube.

Sl. No.	Medium	% increase over the inoculum in mg	% dry wt in mg
1.	Basal medium (BM)	125	11.1
2.	BM + 2, 4-D (0.5 mg/l)	226	8.2
3.	BM + Kinetin (1 mg/l)	422	11.5
4.	BM + 2, 4-D (0.5 mg/l) + Kinetin (1 mg/l)	1386	5.4
5.	BM + 2, 4-D (0.5 mg/l) + BAP (1 mg/l)	1816	4.7
6.	BM + 2, 4-D (0.5 mg/l) + Zeatin (10 mg/l)	731	7.0
7.	BM + NAA (0.5 mg/l) + Kinetin (1 mg/l)	766	9.0
8.	BM + IAA (0.5 mg/l) + Kinetin (1 mg/l)	410	10.25

* Total wt. — Inoculum wt.
Inoculum wt. × 100.

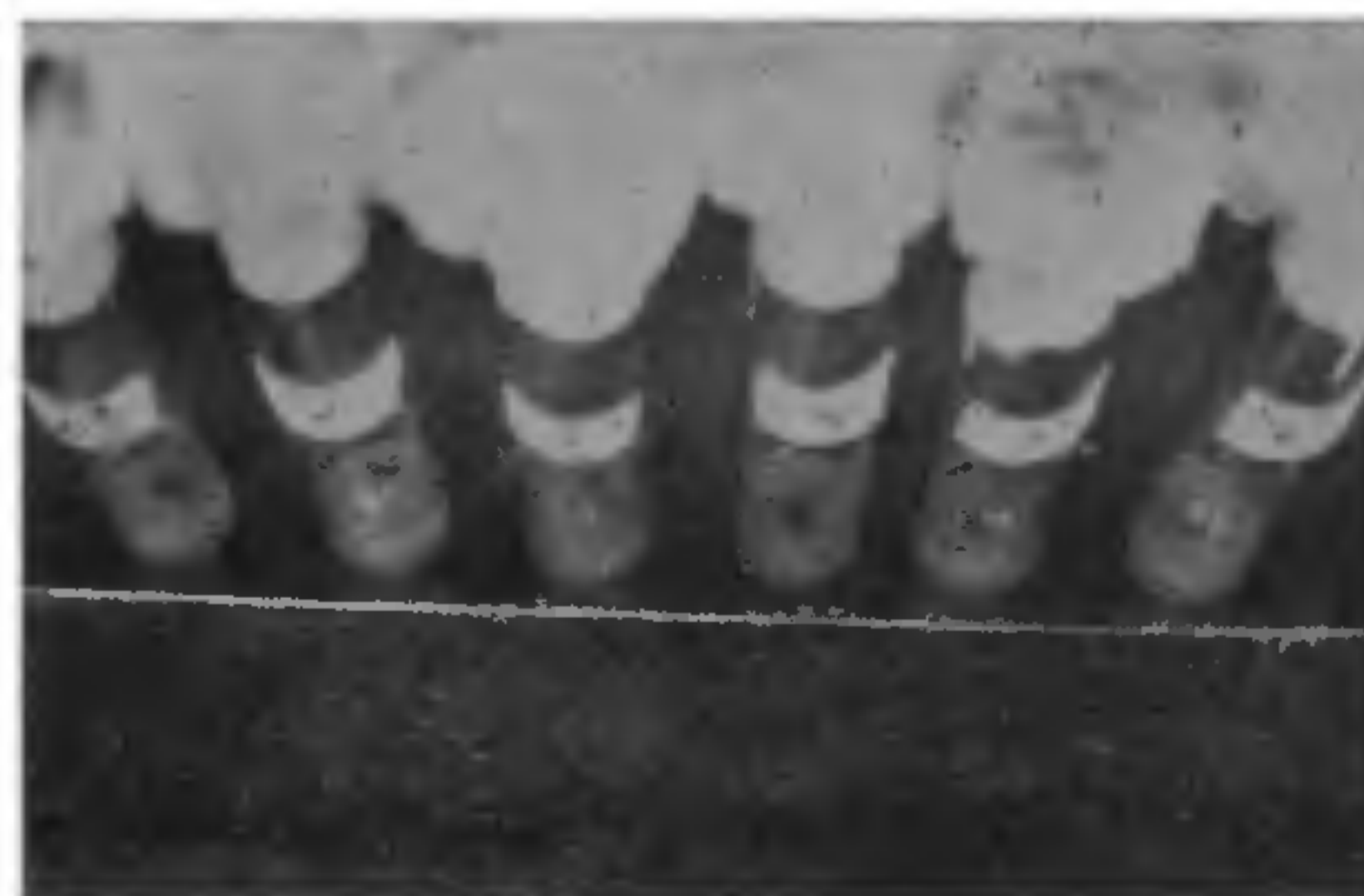


FIG. 1. VAI grown in dark and subcultured into different concentrations of auxins. Growth after 25 days. Tubes 1, 3 and 5 contain NAA and Tubes 2, 4 and 6 contain 2, 4-D at 0.2, 0.5 and 1.0 mg/l concentrations respectively.

* Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India.

The VRI callus was observed to grow well on Schenk and Hildebrandt's basal medium (SHB)⁹, containing indole acetic acid (IAA) and kinetin (1 mg/l each). This callus was brown and light, had no specific effect on its growth. The callus could differentiate into roots at high concentration of NAA (2.5 mg/l).

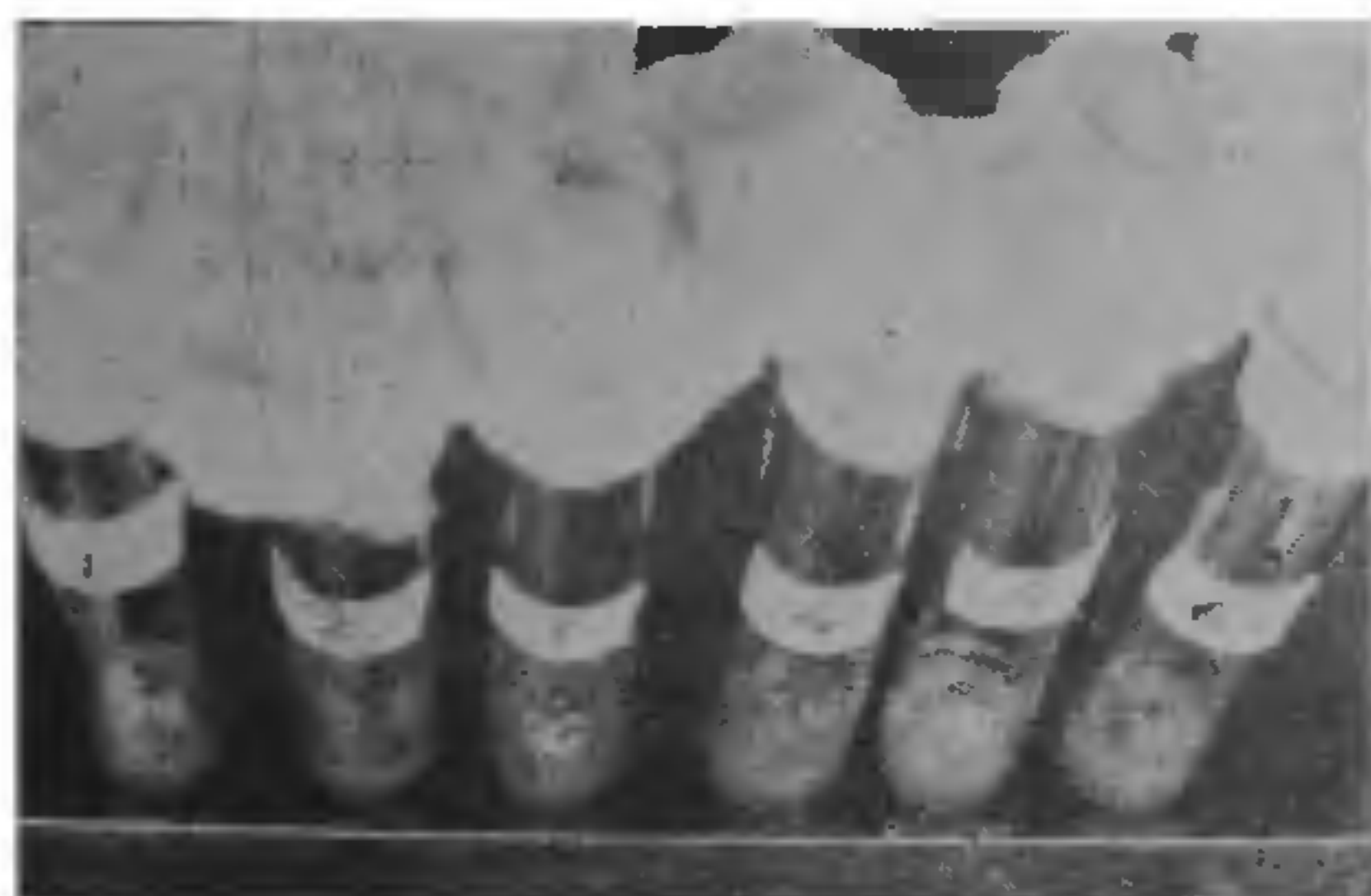


FIG. 2. VAI, grown in light and subcultured into different concentrations of auxins (details as in Fig. 1).

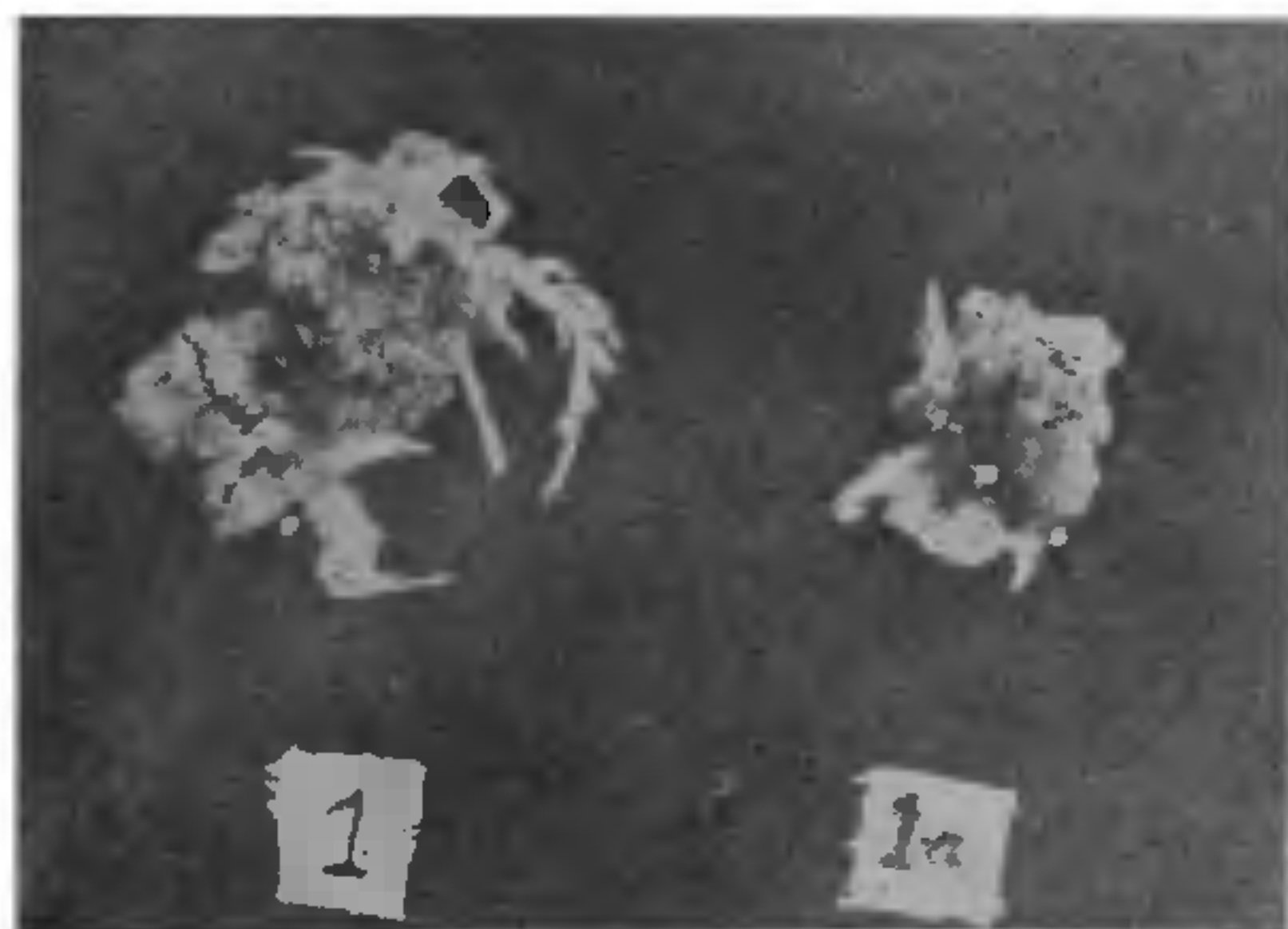


FIG. 3. Growth and rooting in VRs. 1. NAA-1 mg/l; 1a. NAA-0.1 mg/l.

The SHB media containing kinetin and 2, 4-D or NAA supported viable callus cultures of both the

varieties. The VAs cultures were white, soft and were grown on 2, 4-D containing media. When subcultured on NAA (2.5 mg/l) containing media 25% of the inoculum on this media differentiated into root-like structures.

The VRs callus differentiated very well into roots on NAA containing media. This differentiating capacity was not lost on repeated subcultures. In spite of the poor growth at low levels of NAA (0.1 mg/l), it still retained the capacity for rooting (Fig. 3). Of the auxins studied, rooting was observed in IAA containing media but was not as much as in the presence of NAA. 2, 4-D inhibited differentiation at the concentrations studied (1 mg/l).

It is hoped that the standardization of optimum conditions for the growth and differentiation of callus cultures of *C. roseus*, reported here, would pave the way for further investigations on the biosynthesis of antitumor alkaloids.

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INDIAN CHEMICAL SOCIETY—SEMINAR ON ORGANOMETALLIC CHEMISTRY

A three-day Seminar on 'Organometallic Chemistry' sponsored by the University Grants Commission will be held in the Chemistry Department of Lucknow University from Monday, October 10 to Wednesday, October 12, 1977. The programme will include six invited Session Lectures and approximately twenty-five contributed papers covering synthesis, structure, bonding, reactions and

biological or chemical uses of metal-carbon bonded compounds.

Further enquiries may be had from the Convener, Seminar on Organometallic Chemistry, Department of Chemistry, University of Lucknow, Lucknow-226007, latest by Wednesday, 15th June, 1977.