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INDUCTION OF CALLUS IN *CATHARANTHUS ROSEUS* L.

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Callus induction is one of the significant stages of plant tissue culture. Auxins and cytokinins were added to tissue culture media to raise callus culture. Leaves were found to be result oriented explants. Individual hormones did not show marked effect.

Keywords : Auxins; *Catharanthus roseus* L.; Cytokinins; Leaf explant.

Introduction

Cathranthus roseus L. is commonly called as "Vinca rosea." It grows 7-24 inches in height and wide forming a mound of colourful flowers in white, pink, and purple. The plants tolerate heat. The flowers survive even in hottest climate. It is also widely cultivated and is naturalized in subtropical and tropical area of world[1]. Fruit is a pair of follicle 2-4 cm long and 3 mm broad[2]. Medicinal importance of the plant has been exploited up to a large extent. Its effect of purified extracts on Haemotopoiesis system of rats was studied[3,4]. It was possible to isolate alkaloid from one fraction namely Vincaleublastine.[5] Cells and tissues culture of *C. roseus* L. have been studied for many years as potential source of therapeutically interesting terpenoids alkaloids. Ethylene and ancymidol are known to influence secondry metabolites in plants[6-8]. Flower extract has wound healing activity in sprouge dowley extracts[9]. In Ayurveda leaves, seeds flowers and roots are used for treatment of leukemia, diabetes menarrhagia. It was also reported that it has antioxidative potential in saline stress[10]. Its leaf juice reduces blood glucose in normal and allexan diabetic rabbits[11].

Material and Methods

Leaves were obtained from juvenile plants & confirmed from Botany Dep. of Mahatma Gandhi Institute of Applied Sciences. They were washed under running water for 20 minutes. Soap treatment was given and then washed with distilled water. Now leaves were treated with 70% acetone to remove any microbes and again treated with distilled water. Finally 0.1% $HgCl_2$ was used and at last leaves were washed with several times using autoclaved water. Leaves were inoculated aseptically over Murashige and Skoog[6] media containing various growth regulators like BAP, 2, 4-D, IAA, Kinetin and NAA. Among various concentrations and combinations of BAP, 2, 4-D, IAA, kinetin and NAA the medium containing BAP (1.0) +NAA (1.0 mg/l); BAP (0.1) + IAA (1.0 mg/l); 2, 4-D (1.0) + Kn (0.1 mg/l); 2, 4-D (0.1) + BAP (1.0 mg/l); Kn (2.0) + IAA (0.5 mg/l); Kn (1.0) + NAA (0.1 mg/l) and BAP (1.0) + IBA (0.1 mg/l) supported maximum response upto 40 days of explants inoculation. The cultures were maintained at 25 ± 2°C under continuous fluorescent light conditions (3000 lux; 24 h photoperiod).

Results and Discussion

Response in plants differs from cell to cell of different tissues. Continuous efforts have been put in on the optimization of culture media for growth and production of alkaloid by plant growth regulator[12]. Various combinations of auxins and cytokinins were found to be good for callusing.

The fastest callus growth was observed in combination of Kn (0.1) + 2, 4-D (1.0 mg/l) followed by BAP (2.0) + 2, 4-D (1.0 mg/l) up to 25 days as compared to other combinations. The culture of explants on 2,4-D alone did not show good response. The callus was fragile and white in both the combinations i.e. 2, 4-D (1.0) + Kn (0.1 mg/l) (Fig. 1) and 2, 4-D (1.0) + BAP (2.0 mg/l) (Fig. 2) where as green callus was observed in other treatments. Root formation was observed in combination containing BAP (0.1) + IAA (1.0 mg/l) up to 25 days (Fig. 3). The concentration of Kinetin (2.0) + IAA (0.5 mg/l) showed least callus response up to 40 days (Fig. 4).

Healthy and fast growing friable callus is the prerequisite of different biotechnological investigations. Callus consists of undifferentiated masses of cells developed on a semi-solid medium. The maintenance of such cultures depends on an adequate supply of nutrients, growth hormones and controlled sterile environment. By suitable manipulation of hormone and contents of the medium, it is possible to initiate the development of roots, shoots and complete plants from callus cultures[12]. The nutritional requirements of plant cells and tissues vary from species to species and therefore a number of media have been devised for specific tissues by different

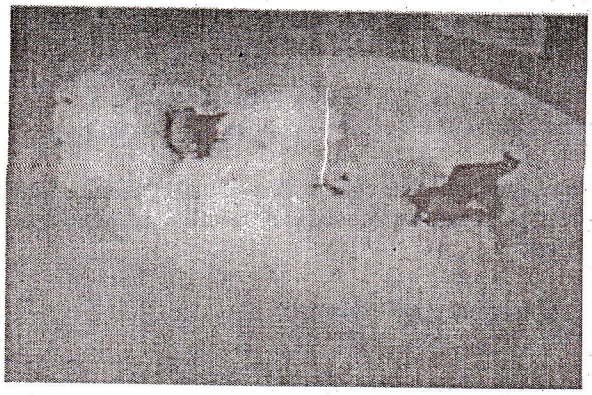

Fig.1. Kn=0.1, 2,4-D=1.0.

Fig.2. BAP=2.0, 2,4-D=1.0.

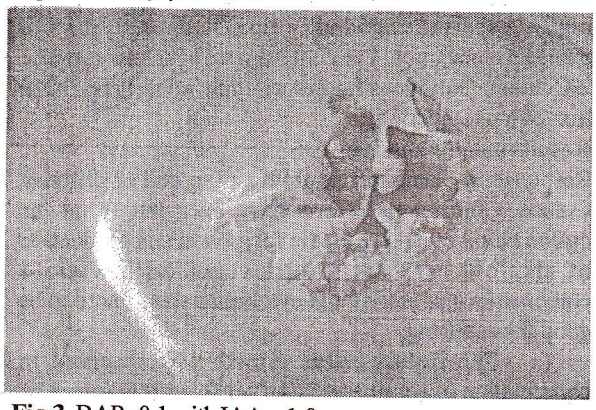

Fig.3. BAP=0.1 with IAA = 1.0.

Fig.4. Kn=2.0 with IAA=0.5

workers[13,14]. Namdeo *et al.*[15] recorded good yield of friable callus in medium containing 2,4 D (4.52 µM)+ Kn (4.65µM) from leaves of *C. roseus* incubated in MS medium. He further reported that frequency of callus initiation is slightly improved in medium containing 10% coconut water after five weeks of incubation. Higher concentration of coconut water however produced compact and hard callus.

In the present study use of kinetin at low concentration (0.1 mg/l) along with 2,4-D (1.0 mg/l) exhibited promising callus growth whereas BAP (0.1) + IAA (1.0 mg/l) was ideal for root formation in *C. roseus*. Present work will not only motivate but will also provide an opportunity to work on callus initiation and further biotechnological aspects of *C. roseus* through micropropagation.

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Table 1. Response of leaf explant of *Catharanthus roseus* L. in MS media containing growth regulators up to 40 days of inoculation.

Treatments (mg/l)	Response
2,4-D alone	Poor callusing
BAP (1.0) + NAA (1.0)	Green good callusing
BAP (0.1) + IAA (1.0)	Fast callus growth and rooting
Kn (0.1) + 2,4-D (1.0)	Fast callus growth
2,4-D (1.0) + BAP (2.0)	White callusing
Kn (1.0) + NAA (0.1)	Callusing but not good
BAP (1.0) + IBA(0.1)	Late callusing
Kn (2.0) + IAA (0.5)	Least callusing

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