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IN VITRO REGENERATION OF MOSAIC VIRUS FREE *CATHARANTHUS ROSEUS* (L.) G. DON. PLANTS THROUGH CALLUS CULTURE

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Shoot tip meristem from mosaic virus infected *Catharanthus roseus* were cultured on modified MS-medium to raise callus. Friable, regenerative and fast grwoing callus was obtained on MS- medium supplemented with 2, 4-D (2.0 mg/1) and Kn (0.5 mg/1). Regeneration of virus free plants from callus cultrures was observed on MS-medium supplemented with BAP (2.0 mg/1) and NAA (0.1 mg/1). Regenerated shoots were rooted on half strength MS-medium fortified with IBA (0.5 mg/1). Rooted plantlets so developed were transfered successfully to the pots. The *in vitro* raised plantlets gave negative results in diagnostic tests on the local lesion host *Chenopodium amaranticolor*, indicating the absence of mosaic virus in regenerated plantlets.

Keywords: Callus differentiation; *Catharanthus roseus*; Mosaic virus; Shoot tip meristem.

Introduction

Catharanthus roseus (L) G. Don (Preriwinkle) is an important medicinal as well as ornamental plant. It is cultivated world wide for its medicinal importance. About 90-95 alkaloids are extracted from it. It is known to cure diseases like diabetes, leukamia, cancer and urinary troubles and also used in the treatment of hypertension. Mosaic disease of *Catharanthus* poses serious problem to its cultivation and is wide spread in occurance. The initial symptoms consisted of slight mottling of young leaves followed by clear mosaic mottling consisting of greenish yellow and normal green patches[1].

Morel and Martin[2] first used the meristem culture for production of virus free Dahlias. It is known that shoot tip meristems are generally free of viruses. Since then, this technique is of great relevance and has been successfully used as an efficient and reliable method, either alone[3] or in combination with thermotherapy[4]/chemotherapy[5] in eliminating viral pathogens from ornamental plants [6-7], wide range of economically important plants[8] and medicinally important plants [9-10]. In the present investigation, we report the regeneration of mosaic virus free plants of *Catharanthus roseus* through callus, raised from shoot tip meristem.

Meterials and Methods

Shoot tips were excised from mosaic infected *Catharanthus* plants, maintained in the green house of Department of Botany, University of Rajasthan, Jaipur. These were trimmed and rinsed in running tap water, treated with mild detergent solution (extran 1 ml/100 ml distilled water) for 10 minutes and rinsed thoroughly with sterile distilled water. Each shoot tip was aseptically excised under stereomicroscope and shoot tip meristem measuring about (0.5 mm) with two leaf primordia were used as explants. These explants were surface sterilized with 70% ethanol for 10 seconds, and washed with sterile distilled water several times. Explants were implanted vertically on the medium. For callus induction and organogenesis, the basic medium used was modified Murashige and Skoog's[11] medium supplemented with various combinations and concentrations of auxins and cytokinins. The pH of the medium was adjusted to 5.8 before autoclave and sterilized at 15 Psi for 20 minutes. All the cultures were incubated at 28 ± 2°C and exposed to 16 hr photoperiod at a

light intensity of 3000 lux and 50-70% relative humidity. Callus was subcultured at least three times before its final subculture for organogenesis. At each subculture passage it was assayed on local lesion host *Chenopodium amaranticolor* Coste and Reyn. Sap inoculation tests were conducted by macerating 100 gms of callus, following standard inoculation technique.

The shoots differentiated from callus cultures were carefully seperated and rooted on half strength MS-medium fortified with various auxins (0.1 - 5.0 mg/1). Rooted shoots were transfered to 6" earthern pots containing mixture of soilrite and soil (3:1). These plantlets were alternately watered with half MS inorganic solution and distilled water, hardened for 2-3 weeks in the glass house.

In vitro raised plantlets of *Catharanthus roseus* were assayed on the diagnostic local lesion host, *Chenopodium amaranticolor*. Sap inoculation tests were conducted by homogenizing leaves of regenerated plantlets in chilled 0.1 M phosphate buffer (pH - 7.0) adding a pinch of cerite powder as an abressive. The inoculum was applied to the leaves of vigorously growing *C. amaranticolor* at the 6-8 leaf stage.

Results and Discussion

Callus initiation was observed from the shoot tip meristem after 8-10 days of inoculation. Various auxins viz. 2, 4-D, NAA and IAA when incorporated in MS-medium alone at different concentrations (0.5 - 5.0 mg/1) produced callus with slow or fast growth. However, 2, 4-D (2.0 mg/l) along with Kn (0.5 mg/1) was found best for producing friable, regenerative and fast growing callus (Fig. 1). The amount of callus produced on IAA and NAA augmented media was less and turned brown after 3-4 weeks. It was incapable of regeneration. On virus indexing, the callus obtained on above mentioned medium was found free of the virus infection in second and third subcultures.

Differentiation of shoot buds from the callus tissues was observed on MS-medium supplemented with different concentration of BAP/Kn (1.0 - 5.0 mg/1) and NAA (0.1 mg/1). Initially the entire piece of callus turned green and gave rise to number of shoot buds which further developed into shoots (Fig. 2). Maximum proliferation of shoots (20-25) from callus cultures was obtained on MS-medium supplemented with BAP (2.0 mg/1) and NAA (0.1 mg/1). For shoot proliferation use of cytokinin in combination with auxin gave best result and similar results were reported on Carnation[12]. Further elongation of shoots (6-7 cm) was achieved by lowering the concentration of BAP from 2.0 mg/1 to 1.0 mg/1 and eliminating NAA (Fig. 3).

The regenerated shoots on transfer to a hormone free MS-medium failed to form roots even after 40 days of culture. Incorporation of auxins IAA, IBA and NAA singly at concentrations ranging from 0.1 - 5.0 mg/1 to MS or half strength MS with reduced sucrose concentration induced root formation. Profuse root formation from proximal end of the shoot was observed on half strength MS-medium with 2% sucrose and IBA 0.5 mg/1 (Fig. 4). Higher levels (more than 2 mg/1) of all auxins (IAA, IBA and NAA) induced callusing followed by root formation.

The *in vitro* raised plantlets were subjected to infectivity assay tests on the diagnostic local lesion host, *Chenopodium amaranticolor*. Inoculations were made with homogenized leaf tissues from regenerated

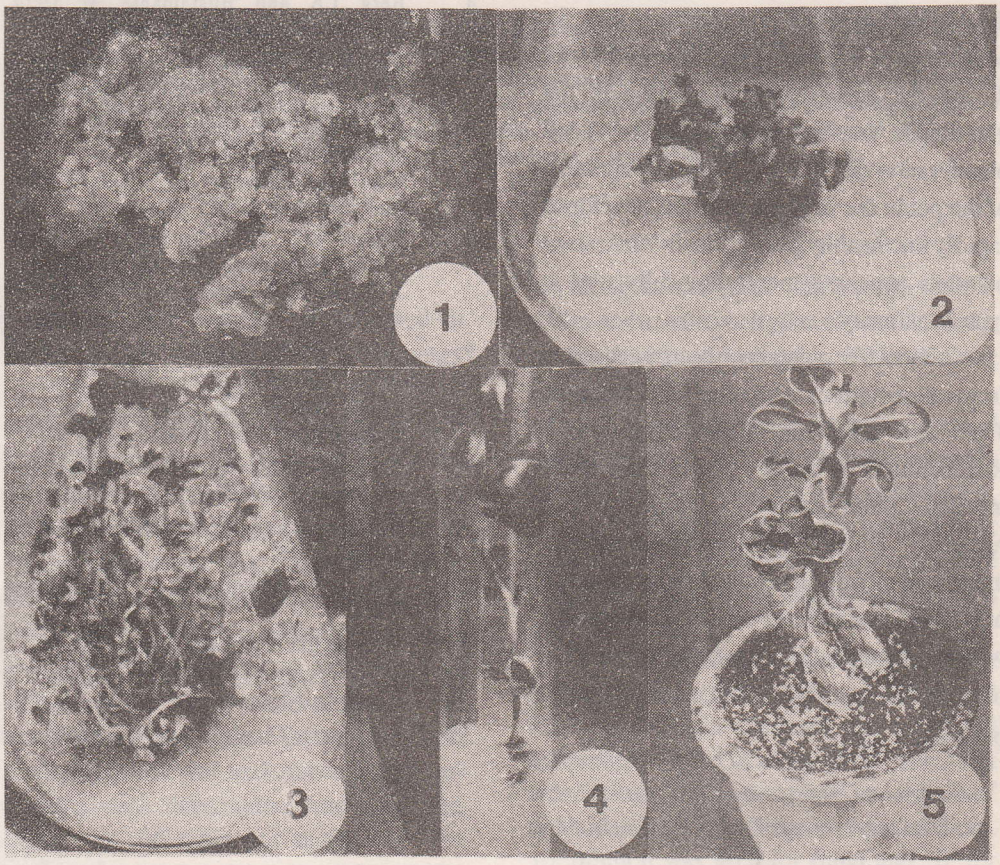

Figure 1-5 *In vitro* regeneration of mosaic virus free *Catharanthus roseus*)L) G. Don. plants through callus culture. 1. Callus induction on MS-medium supplemented with 2,4-D (2.0 mg/1) and Kn (0.5 mg/1); 2. Shoot buds regeneration on BAP (2.0 mg/1 and NAA (0.1 m/1) augmented MS-medium; 3. Proliferated virus free multiple shoots (20-25) on MS-medium fortified with BAP (1.0 mg/1); 4. A rooted shoot on half strength MS-medium containing IBA 0.5 mg/1 and 2% sucrose; 5. *In vitro* regenerated mosaic virus free plant of *C. roseus* transfered to earthern pot.

plants, the lesions do not appeared on the host thus giving negative reaction on *C. amaranticolor* leaves.

Once a vigorously growing root system was developed on half strength MS-medium fortified with 2% sucrose and 0.5 mg/1 IBA, the plantlets were transfered to pots (Fig. 5). Maintenance of high humidity (80-90%) during the early hardening phase in the glasshouse was found essential for good plantlet survival (90-95%). On transplantation of the pot in the field the plantlets developed rapidly into healthy plants. The *in vitro* raised plantlets appeared morphologically true to type at maturity.

Virus free plants have been regenerated from callus cultures in tobacco[13], in Caladium, Taro and Cocoyam[14] and in potato[15]. Regeneration of healthy plants from MLO infected *Catharanthus roseus* has also been reported[16]. However *in vitro* regeneration of mosaic virus free plants of *Catharanthus* has not been accomplished earlier. These results will also be useful in long term preservation of virus free germplasm and for quarantine purposes.

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