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PLANTLET FORMATION IN ROOT CALLUS OF *RAUWOLFIA SERPENTINA*

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Abstract

Using Benzylaminopurine (2 mg/1) and NAA (0.8 mg/1) root callus of *Rauwolfia serpentina* was induced to bud formation which further developed into shoots. Isolated shoots rooted with 24 h treatment of 3 mg/1 IAA and 3 mg/1 IBA. The plantlets transferred to soil were initially watered with half strength Knop's solution until they became autotrophic and were found to grow well in open field conditions.

Introduction

Development of plantlets through callus cultures has been reported in many plant species by using tissue from stem (Tabata *et al.*, 1972; Pareek & Chandra, 1977; Meredith, 1979) and leaf (Padmanabhan *et al.*, 1973; Prasad & Chaturvedi, 1978). Rarely root callus of *Oryza sativa* (Henke *et al.*, 1978) and *Solanum xanthocarpum* (Prasad & Chaturvedi, 1978) has been used for bud differentiation. In members of the family Apocynaceae, plantlets formation was reported from stem in *Catharanthus roseus* and *Rauwolfia serpentina* (Abu-Mandour *et al.*, 1979); Mitra & Chaturvedi, 1970); Techniques for vegetative propagation of *R. serpentina* through bud differentiation in stem callus have been developed (Akram, 1983). In the present report root callus of *R. serpentina* was induced to differentiate soil transferable plantlets.

Meterial and Methods

The plantlets differentiated in stem calli of *R. serpentina* (Akram, 1983) were used to initiate callus cultures. Plantlets of approximately 50 mm size were cultured on White's root culture (RC) medium (Thomas & Davey, 1975) containing 100 ml/1 coconut milk (CM), 10 mg/1 biotin, 1 mg/1 indole acetic acid (IAA), 10 mg/1 benzylaminopurine (BAP) 0.8 mg/1 NAA and 10 ml/1 ENS solution. Stock solution of ENS (Extra Nitrogen Source) was prepared by dissolving 0.32 gm of calcium nitrate and 10gm of potassium nitrate in 100 ml of distilled water. Sucrose was used @ 1.5% in the medium. The roots of these plantlets which produced callus were excised after 2 weeks and used.

Coconut milk was prepared by collecting liquid endosperm from unripe coconuts, heated to 80°C, filtered and stored in the freezer for use. Medium in 50 ml quantities,

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in 100 ml culture flasks were sterilized at 15 psi for 10 minutes (Meynell & Meynell, 1970). After inoculation the cultures were kept under 16 h cycle fluorescent light cooled incubators at 28 ± 1°C. Plantlets were watered in pots with half strength Knop's solution (Gautheret, 1952).

Results and Discussion

Callus before inoculation was predominantly white in colour with some pink portions. The callus exhibited growth and green coloured portions started appearing after 2 weeks. Bud differentiation was observed after 8 weeks in green coloured portion of the cultured root callus. The differentiated buds, at this stage were not large enough to be separated from the callus. Therefore, they were cultured alongwith the accompanying callus on fresh medium of the same composition. It was noticed that originally inoculated and also the newly regenerated buds, both were mostly masked and some even got lost by the overgrowth of root callus on RC medium (Fig. 1).

Presumably 2 mg/l BAP and 0.8 mg/l NAA added to the culture medium for bud differentiation at the same time favoured the growth of root callus of *R. serpentina.* Therefore, in further subcultures growth regulators (BAP and NAA) were omitted from the culture medium however, it contained CM, biotin and ENS at 100 ml, 10 mg and 10 ml per litre respectively. This technique helped to discourage copious callus growth and at the same time enhanced shoot development in differentiated buds.

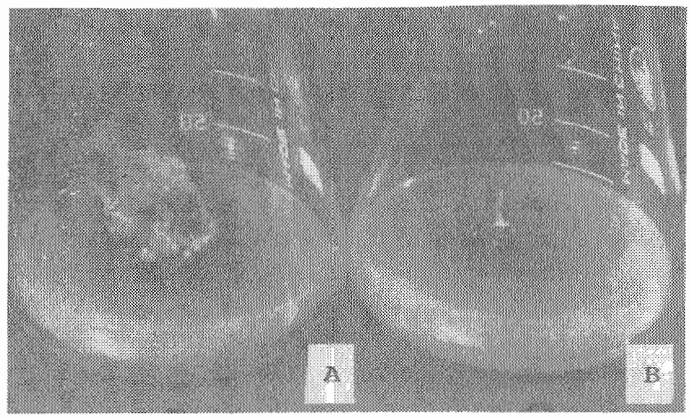

Fig. 1. Differentiated buds growing on White's Root Culture medium supplemented with 100 ml/l coconut milk, 10 mg/l biotin, 10 ml/l extra nitrogen source solution and (A) 2 mg/l BAP and 0.8 mg/l NAA (B) excluding BAP and NAA. Note disappearance of bud in (A) where it is completely masked by the overgrowth of root callus. The bud in (B) is clearly visible.

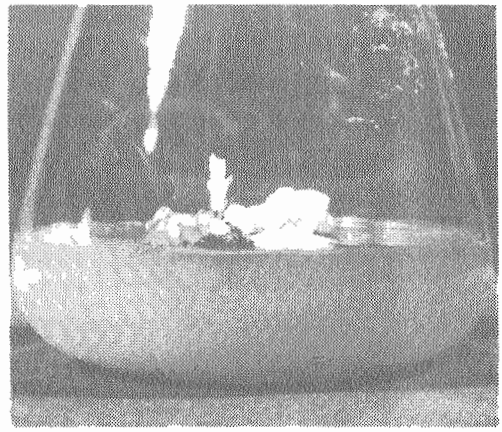

Fig. 2. Bud growth of White's root culture medium supplemented with 100 ml/l coconut milk, 10 mg/l biotin, 10 ml/l extra nitrogen source solution, 2 mg/l BAP, 0.8 mg/l NAA and 0.01 mg/l GA_3.

In other cultures conditioning of culture medium was done with gibberellic acid at 0.0001, 0.001, 0.01 and 0.1 mg/l to discourage new callus formation as reported by Morel (1964) who used GA_3 at 0.0001 mg/l to stop callus formation towards the cut end of apical shoots of *Helianthus annuus* and *Solanum tuberosum*. Gibberellic acid at 0.01 mg/l successfully balanced the development of differentiated buds in root callus of *R. serpentina* while suppressing the growth of callus (Fig. 2). The buds attained an

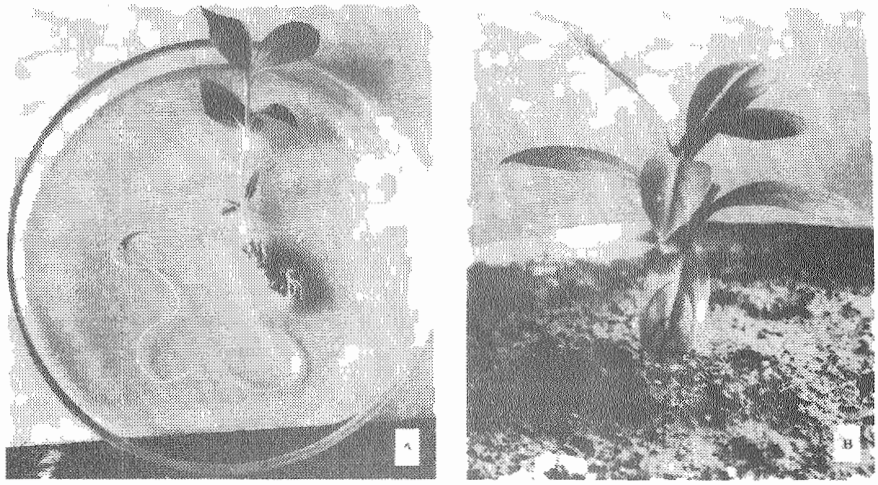

Fig. 3A. Bud differentiated in root callus, initiated rooting and shoot formation on auxinless RC medium, in 3 weeks. It was pretreated for 24 h with 3 mg/l, each of IAA and IBA. B. Four months old plantlet growing in soil.

appreciable size of 15 to 20 mm in 3 weeks of differentiation. At this stage they were isolated from the callus and treated with IAA and IBA at 3 mg/l for 24 h (Gupta *et al.*, 1980). These treated buds were then suspended in auxinless liquid medium in which initiation of roots in the buds with shoot development occurring at the same time (Fig. 3A) was observed in the 3rd week after inoculation.

The plantlets after 3 weeks of root differentiation were transferred from cultures to pots containing sandy loam soil with an equal quantity of decomposed leaf manure. The potted plantlets were kept at 28 ± 1°C and a photoperiod of 16 h with light intensity of 3,000 lux. Watering of plantlets was done twice a week with half strength Knop's solution. After 8 weeks the plantlets were given tap water and transferred to laboratory bench under diffuse sunlight where good growth was observed (Fig. 3B). The root callus is therefore, suggested as a potential tissue for the formation of plantlets in *R. serpentina.*

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