DIFFERENTIATION AND ANATOMICAL CHANGES IN CALLUS CULTURES OF *RAUWOLFIA SERPENTINA* BENTH

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Abstract: *Callus induced in shoot pieces of* Rauwolfia Serpentina *on Knop's medium supplemented with 1 mg/1 of NAA exhibited vigorous growth when transferred to MS medium supplemented with 1 mg/1 both of Naphthalene acetic acid (NAA) and Benzyl amino purine (BAP). The effect of varying concentrations of NAA & BAP alone and in combination with each other was studied on further growth and differentiation of the callus. At NAA 1 mg/1, BAP 0.5 mg/1, NAA 0.5 + BAP 0.5 mg/1 roots developed. At 0.5 mg/1 of BAP & NAA 0.5 + 1.0 mg/1 of BAP shoot buds also developed.*

Callus pieces cultured on MS medium supplemented with different combinations of NAA & BAP showed progressive anatomical changes accompanying differentiation of roots and shoot buds.

Introduction: In a previous communication (Perveen & Ilahi, 1978) the effect of different growth hormones, coconut milk (CM) and casein hydrolysate (CH) was studied on callus induction and its further growth in stem and leaf segments of *Rauwolfia serpentina* using Knop's solution as the basal medium (BM). In present studies callus was transferred to Murashige & Skoog's (MS) medium (Murashige & Skoog 1962), and the effect of different concentration of NAA & BAP was studied on further callus growth and differentiation.

Differentiation of shoot buds and roots from callus cultures have been reported (Skoog & Miller 1957, Murashige *et al*, 1970; Mehra & Mehra 1974). It has also been observed that morphological changes noticed during such differentiation were accompanied by changes in the callus tissue (Wetmore & Sorokin 1955, Wetmore & Rier 1963; Earle 1968; Pillai & Hildebrandt, 1969). In this article growth and differentiation of *Rauwolfia serpentina* callus and a parallel study of the anatomy of callus is reported.

Materials and Methods: Callus induced on shoot segments of *Rauwolfia serpentina* was used in these studies. MS medium was prepared according to Gamborg & Wetter (1975). Two per cent sucrose was added as a carbon source. Difco-Bacto agar was used at 0.8% concentration. The experiments were conducted in light (16 hrs. light period). The cultures were kept in a room with temperature regulated at 26 ± 1°C.

The effects of NAA (0.5, 1.0 mg/1), BAP (0.5, 1.0 mg/1) alone or in combination with each other were studied on callus growth and differentiation.

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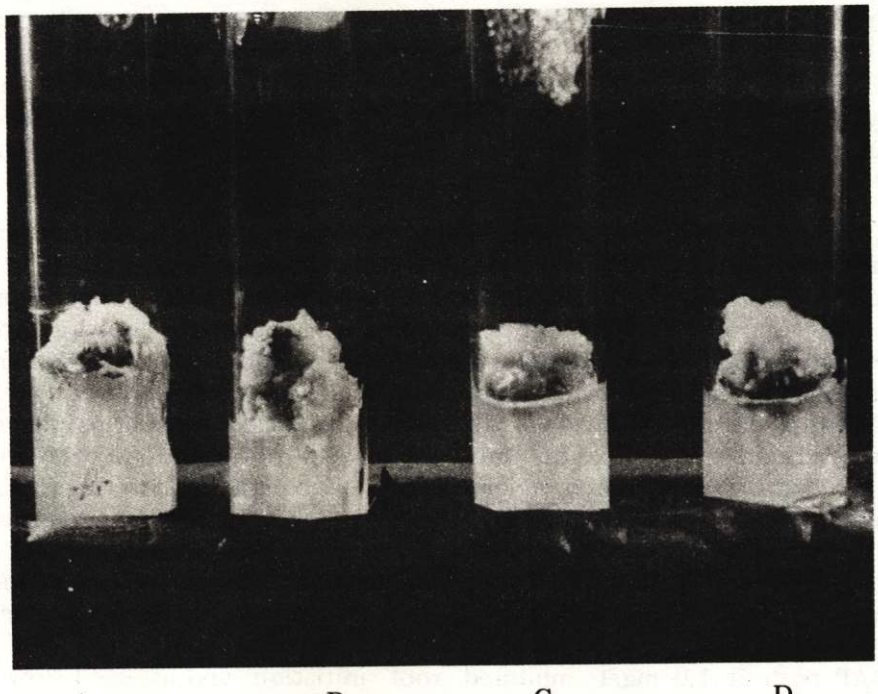
For Anatomical studies callus was fixed in 2% formeldehyde solution. Free hand sections of callus were cut by a razor. Sections were stained with saffranin and light green (Johanson, 1940).

Results: Callus induced on shoot segments on Knop's medium supplemented with 1 mg/1 of NAA exhibited slow growth, therefore callus from this medium was transferred to the MS medium supplemented with 1 mg/1 of NAA and 1 mg/1 of BAP. Callus growth was enhanced and copious callus resulted after 6 weeks of culture. The callus formed was yellowish white in colour hard and nodular in appearance. This calus was transferred to BAP 0.5, 1.0 mg/1 and 1.0 mg/1 of NAA either with 0.5 or 1.0 mg/1 of BAP.

Effect of BAP: At 0.5 mg/1 of BAP numerous roots and some buds developed from callus after 3 weeks of culture (Fig 1A). BAP 1.0 mg/1 inhibited root development and the growth of callus was also slow than that on 0.5 mg/1 of BAP (Fig. 1C). The callus from 0.5 mg/1 of BAP on transfer to the same fresh medium exhibited very slow growth and no differentiation was observed.



A B C D

Fig. 1 Callus growth and its differentiation on MS supplemented with (A) 0.5 mg/1 of BAP (B), 0.5 mg/1 of BAP + 1.0 mg/1 of NAA (C) 1.0 mg/1 of BAP (D) 1.0 mg/1 of BAP + 1.0 mg/1 of NAA (½x).
R = Roots, B = Bud.

Effect of NAA and BAP: At NAA 1.0 mg/1 and BAP 0.5 mg/1 callus growth was better than NAA alone. Some roots developed on this medium, but soon after initiation their growth ceased (Fig. 1B). NAA 1.0 mg/1 with 1 mg/1 of BAP exhibited slower callus growth than NAA at 1.0 mg/1 with 0.5 mg/1 of BAP. No differentiation was observed, however in some cultures a few roots developed but their growth ceased soon after their initiation (Fig.1D). At 0.5 mg/1 of NAA with 0.5 mg/1 of BAP, callus developed further and from it roots with numerous lateral branches originated, after 4-5 weeks of culture. NAA at 0.5 mg/1 with 1.0 mg/1 of BAP exhibited better callus growth than 0.5 mg/1 of BAP. Roots also developed but their growth was slow, shoot buds developed as well at this concentration of NAA and BAP.

Anatomy of the callus: Callus pieces cultured on MS medium supplemented with different concentrations of NAA and BAP showed progressive anatomical changes accompanying differentiation of roots and shoot buds. Anatomy of the undifferentiated callus showed, that it had thin walled parenchymatous cells of varying size and shape. At the periphery callus had small meristematic cells densely filled with cytoplasm, having large nuclei. In the centre it had comparatively large vacoulated mature cells. During differentiation some of these mature parenchymatous cells more or less deep seated in the callus, first elongated slightly, followed by thickening of cell walls and then differentiated into conducting tissue (trachieds) which were arranged regularly or irregularly in the callus.

As differentiation progressed many such vascular groups of tissue developed scattered within the callus tissue. This vascular tissue differentiated later on into xylem tissue, which formed complete ring structures (as vascular bundles). From some of the vascular bundles, conducting elements developed acropetally towards nodules of the callus. Transverse section of nodules also showed organized tissue in the centre. During the organization of roots, meristematic areas got differentiated deep in the callus. This tissue developed growing points, which gradually pushed itself out of the callus and formed roots. T.S. of a rooted region of callus exhibited a triarch xylem. It was also noticed that the roots and differentiated tissue of nodules were in connection with these vascular tissue.

Discussion: In these studies it was noticed that NAA 1.0 mg/1 induced rooting in callus. However with the addition of 0.5 mg/1 of BAP to the medium, though callus growth was enhanced, but that of roots slightly inhibited. NAA and BAP both at 1.0 mg/1 inhibited root initiation and affected slow callus growth as compared to 0.5 mg/1 of BAP + NAA 1.0 mg/1. However 1.0 mg/1 of BAP with 0.5 mg/1 of NAA resulted in good callus growth and induced shoot buds, but this combination inhibited root growth. At 1.0 mg/1 of BAP alone, growth of callus was slow. At 0.5 mg/1 of BAP alone shoot buds and roots developed from callus. BAP at 0.5 mg/1 with 0.5 mg/1 of NAA promoted callus growth and roots also developed from callus. However, low concentrations of

BAP e.g., 0.5 mg/1 with 0.5 mg/1 of NAA promoted root growth and lateral root formation. Root formation at BAP 0.5 mg/1 alone might be due to some endogenous NAA or early differentiation of root primordia in the callus when it was cultured on NAA + BAP. However, at high concentration of BAP root initiation was inhibited. These results are in agreement with those of Skoog & Miller (1957) and Doershuz & Miller (1967), where addition of Kinetin to the medium inhibited root growth.

From anatomical studies, it was observed that at first the growth of callus was only due to the division of peripheral meristematic cells, but later on with the development of vascular tissue and other maristematic area, growth was also due to the cambial cells and newly developed meristems in addition to peripheral cells. First step towards differentiation was the slight elongation and development of thick walls in deeper cells of the callus. These cells later on differentiated into tracheids. In agreement with Vasil & Hildebrandt (1966) the initiation of roots was not from the superficial cells, but seated deep in the callus.

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