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In vitro studies of *Mitragyna parvifolia* normal and galled leaf

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Abstract
It is a fact that the tissue culture technique has been very useful in studies with problems of pathological growth in general and plant tumours in particular and in revealing the intricacies of the host-pathogen relationship at the cellular level, particularly the transformation of normal growth into abnormal growth. During the present investigations normal and gall callus cultures of *Mitragyna parvifolia* were raised and established on a suitable medium.

Keywords: Tissue culture, host-pathogen, gall callus

Introduction
Interaction between cytokinins and other growth regulators has been considered important in the regulation of cellular differentiation, growth and morphogenesis. Kinetin is particularly active as an interactant with IAA or 2,4-D in the culture of tobacco pith. During the present experimentations, growth of normal tissue was observed on several dilutions of BAP and kinetin in association with NAA. With increasing concentration of auxin even in the absence of BAP, growth was evident. However, for good growth, in the present studies both auxin and BAP were required. It has been observed that a combination of auxin and cytokinin usually caused an interaction that resulted in altered morphogenetic response *Mitragyna parvifolia* callus was produced and developed using leaf explants on MS media supplemented with NAA (3.0 mg/l) and BAP (0.5 mg/l). Gall calli was formed from MSmedium amplified with 2,4-D (4.0 mg/l) and 2,4-D (5.0 mg/l) from leaf and fruit explants, respectively. The effects of different auxins on normal and gall callus growth were also investigated. 2,4-D (4.0-6.0 mg/l) was shown to be the best for *Mitragyna parvifola* gall tissue growth. NAA (3.0-6.0 mg/l) was discovered to be the optimum for normal tissue growth. Different researchers have conducted *in vitro* investigations involving the isolation of normal and gallbladder tissues using MS-media enhanced with several growth regulators. The present investigation showed that the metabolism of the host was altered by the insect. There was obvious derangement in the physiology of galled leaf. As a result of this alteration, differential behaviour, histochemical and biochemical changes in normal and gall tissues were evident. However, there is a need to carry out further studies on identifying and isolating specific gallinducing compounds. Materials and Methods

Normal Callus Culture
For initiation of normal callus, leaves were collected from the *Mitragyna parvifolia* plant and retained beneath running tap water for approximately 30 minutes. Eventually, these were divided into pieces of suitable size. Following several rinses in sterile distilled water, these pieces were rinsed with a 2 per cent detergent solution (Extran, E. Merck- a commercialgrade detergent). These steps were taken to remove any infection that may have been present on the surface of the explant. These explants were sterilised in 0.01 per cent mercuric chloride for 1-2 minutes, then rinsed three times in sterile distilled water to eliminate any leftover mercuric chloride. These sterile explants were placed in a 100 mL " Erlenmeyer " flask with 40 mL stiffened MS-medium supplemented with growth regulators (NAA/2,4-D/ IAA/IBA). The cultures were incubated in a culture chamber at 282°C and 60% relative humidity for 16 hours of light and 8 hours of darkness, with 16 hours of light and 8 hours of nightfall.

Gall Callus Culture
Leaf galls from *Mitragyna parvifolia* plants were collected for the initiation of gall callus. Young galls were cut in half and rinsed with water. Cecidozoa were removed from the cavities using a fine brush. Following many rinses in sterile distilled water, these were rinsed in a 2 per cent detergent solution (Extron, E. Merck- a commercial-grade detergent). These dis-infected explants were sterilised for 1-2 minutes with 0.01 per cent mercuric chloride, followed by three rinses in sterile distilled water to remove any remaining mercuric chloride. These sterile explants were cut into minor fragments and aseptically transferred to a 100 ml " Erlenmeyer " flask containing 40 ml of stiffened MSmedium supplemented with growth regulators (NAA/2,4-D/IAA/IBA). The cultures were incubated in a culture chamber at 282°C and 60 per cent relative humidity, with 16 hours of light and 8 hours of dusk. For each combination, eight imitates were used, and the tests were repeated three times to confirm the results.

Observations and Results
Callus Establishment
During the current investigation, healthy leaves and leaf galls were utilized as explants for the induction of callus and its establishment. These explants were attempted with numerous concentrations and amalgamations of phytohormones.

1. Effect of phytohormones on callus initiation and establishment of normal leaf

1.1 Effect of auxins
To obtain callus during the current deposit of experiments, all explants were inculcated on MS- medium with varying dilutions (0.5-6.0 mg/l) of all auxins, namely 2,4-D, NAA,

IAA, and IBA. After a 7–10-day incubation period, normal callus formation was observed on the cut ends of leaf segments. Table 7 shows the retaliation of leaf explants to various auxins. On MS media supplemented with 2,4-D (2.0-6.0 mg/l), a variable amount of callus was formed. On MS-media supplemented with 2, 4-D (4.0 mg/l), a soft, whitish brown callus was formed [Plate 1, Figs. A-C]. This callus eventually turned brown. Callus was formed at various NAA concentrations (1.0-6.0 mg/l). Callus grew quickly and was whitish-green on MS medium supplemented with NAA (3.0mg/l) [Plate 1, Fig.D]. After 3-4 weeks, IAA (4.0 mg/l) produced callus that was compact and brown, with slow growth [Plate 1, Fig.E]. IBA (2.0-6.0 mg/l) caused poor callusing [Plate 1, Fig.F].

2. Effect of NAA in Combination with Cytokinins

The integrated response of auxin and cytokinin was seen in the second deposit of studies. NAA at 3.0 mg/l concentration was found beneficial in former deposit of studies, was amalgamated with BAP (0.5-6.0 mg/l) or Kinetin (0.5-6.0mg/l). Table 8 contains the results. NAA (3.0 mg/l) in combination with BAP (0.5mg/l) was shown to be the most effective for callus inoculation. The created calluswas green, friable, vigorous, and growing rapidly [Plate 2, Figs. A-D]. Kinetin was found to be ineffective in the production of green, healthy callus.

Table 1: Effect of different auxins on callus induction in normal leaf of *Mitragyna parvifolia*

Medium : MS + Sucrose (3.0%) + 2,4-D/NAA/IAA/IBA (0.5-6.0 mg/l)
Explant : Leaf
Incubation: At 28±2°C under 16 hours photoperiod (2000-3000 lux) upto 4 weeks

S. No.	Auxins concentration (mg/l)	Response	Remarks
1.	Control: MS basal medium (without auxins)	Nil	Nil
2.	2,4-D 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 - - C^{+} C^{++} C^{+++} C^{++} C^{++}	Soft, whitish brown callus was produced which later on turned brown.
3.	NAA 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 - C^{+} C^{++} C^{+++} C^{+++} C^{+++} C^{+++}	Callus was whitish-green in colour and fast-growing.
4.	IAA 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 - C^{+} C^{+} C^{++} C^{+++} C^{+++} C^{+++}	Compact brown callus was produced but the growth was slow after 3-4 weeks
5.	IBA 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 C^{-} C^{-} C^{+} C^{++} C^{++} C^{+} C^{+}	Poor callusing was observed.

C : Callusing response
- : No callusing
+ : Slight callusing
++ : Moderate callusing
+++ : Profuse callusing

Table 2: Effect of different auxins on leaf gall callus induction in *Mitragyna parvifolia*

Medium : MS + Sucrose (3%) +2,4-D/NAA/IAA/IBA(0.5-6.0 mg/l)
Explant : Leaf gall
Incubation: At 28±2°C under 16 hours photoperiod (2000-3000 lux) upto 4 weeks

S. No.	Auxins concentrations (mg/l)	Response	Remarks
1.	Control: MS basal medium (without auxins)	Nil	Nil
2.	2, 4-D 0.5 1.0 2.0 3.0 **4.0** **5.0** 6.0	 - - $C^{+}R^{-}$ $C^{++}R^{-}$ $C^{+++}R^{-}$ $C^{++}R^{+}$ $C^{++}R^{+}$	Compact, fast-growing nodular callus, greenish-brown in colour Rhizogenic response.
3.	NAA 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 _ $C^{+}R^{-}$ $C^{++}R^{-}$ $C^{++}R^{-}$ $C^{+++}R^{-}$ $C^{+++}R^{-}$ $C^{+++}R^{-}$	Yellow-green callus was obtained and there is slow growth after 3-4 weeks
4.	IAA 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 - - - $C^{+}R^{-}$ $C^{++}R^{-}$ $C^{+++}R^{-}$ $C^{+++}R^{-}$	Yellowish white callus produced and showed retarded growth
5.	IBA 0.5 1.0 2.0 3.0 4.0 5.0 6.0	 - - - - $C^{+}R^{-}$ $C^{++}R^{-}$ $C^{++}R^{-}$	Poor callusing. Callus was dark brown.

C : Callusing response
- : No callus
+ : Slight callusing
++ : Moderate callusing
+++ : Profuse callusing
R: Rhizogenic response
- : No rooting
+ : Slight rooting
++ : Moderate rooting
+++ : Profuse rooting

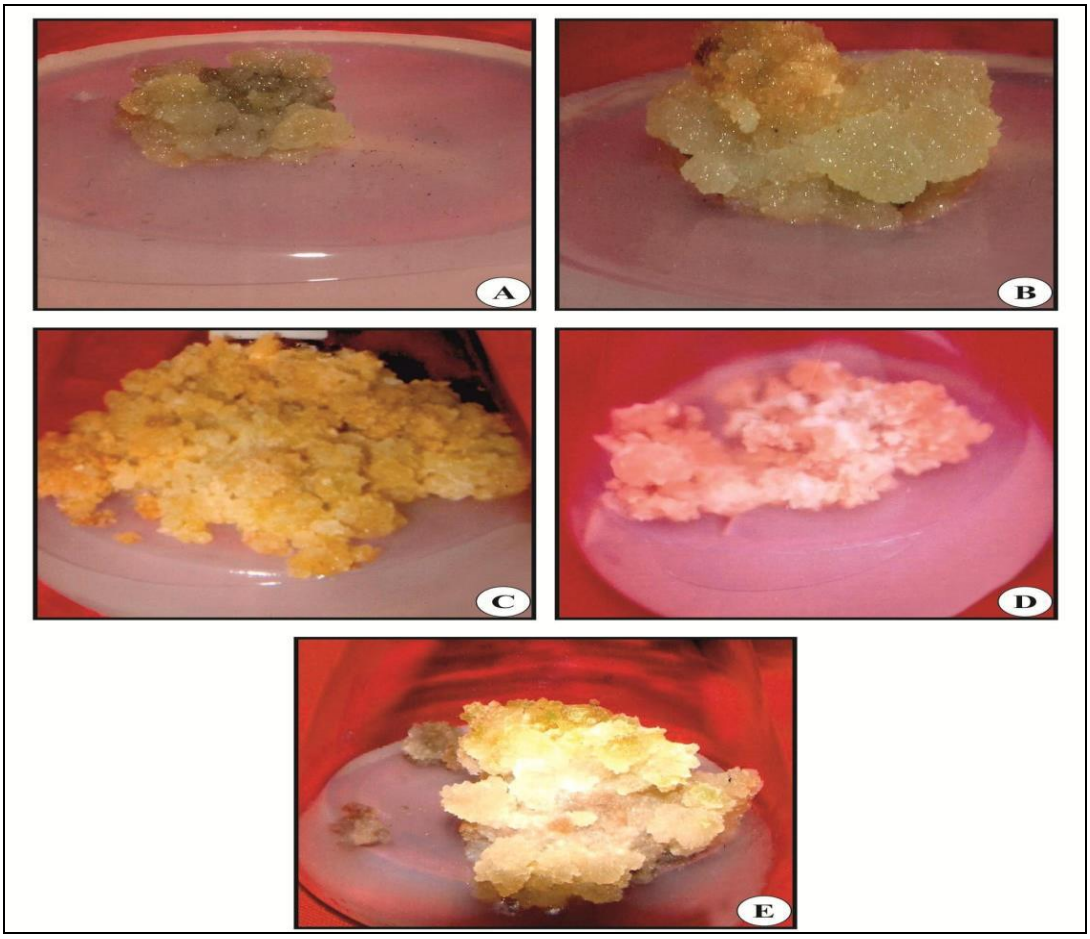

Plate 1 Fig to E

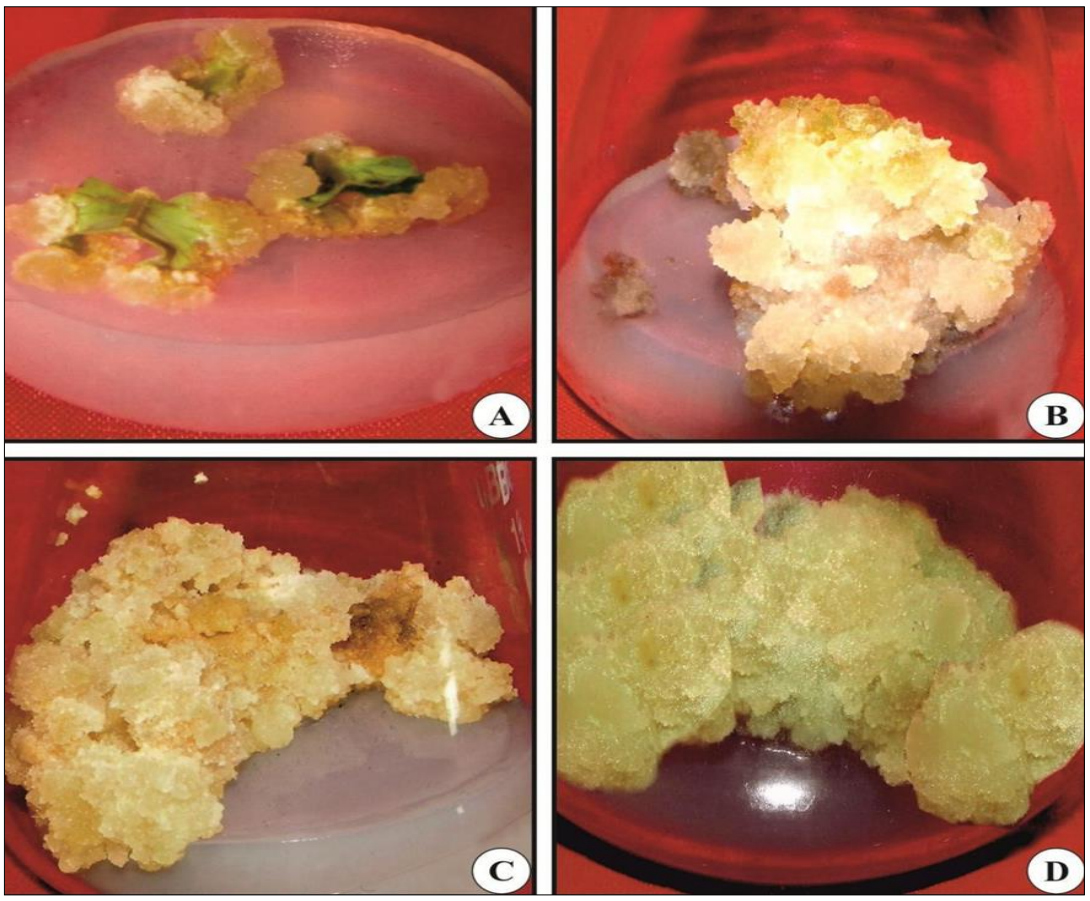

Plate 2: Fig A-D: Effect of NAA (3.0 mg/l) in union with BAP (0.5 mg/l)

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