

Callus proliferation was observed initially on the pedicel (figure 1) portion and subsequently the flower buds on both MS and NM supplemented with 3 mg/l BAP and 1 mg/l NAA after a week. Hard callus developed on the pedicel and soft callus on the flower buds. Soft callus did not proliferate during subsequent cultures while the hard callus developed shoot bud on both the media with various concentrations of BAP, NAA and zeatin. Many shoots (7-8) (figure 2) were recorded on NM supplemented with 0.3 mg/l zeatin and on MS medium supplemented with 3 mg/l BAP and 1 mg/l NAA; only 4-5 shoots developed after 15 days subculture. Shoots were subcultured on these media and supplemented with GA₃ (0.5, 1, 2, 3 and 5 mg/l) for elongation prior to rooting; best response was observed at 2 mg/l (figure 4).

The shoots were transferred to liquid MS and NM supplemented with IAA (0.1, 0.2, 0.5, 1, 2, 3 and 5 mg/l) and IPA (0.2, 0.5, 1, 2 and 5 mg/l) individually. They remained *in situ* for 4-6 weeks in the liquid media without the initiation of roots. Efforts are in progress to raise the plantlets.

Histology

Nodular callus subcultured on Nitsch medium supplemented with 0.3 ppm zeatin developed differentiating callus within 2 weeks. Microtome sections of 4-week-old callus show the differentiation of shoot bud directly from the meristematic zone. The shoot bud developed as an apical dome with a pair of juvenile leaves. These structures were found to possess vasculature.

On MS medium supplemented with BAP (3 mg/l) + NAA (1 mg/l) the callus of 6-week-old was found to possess a differentiated shoot bud and several embryoids of different shapes such as globular, spindle, cordate and columnar. These embryoids as well as the shoot-buds along with the meristematic zone was found to consist of compactly packed, small and dense cytoplasmic cells (figure 3).

The organogenesis of *B. diffusa* could be easily achieved from young inflorescence explant and the technique could be exploited for biosynthesis of alkaloid-punarnavine.

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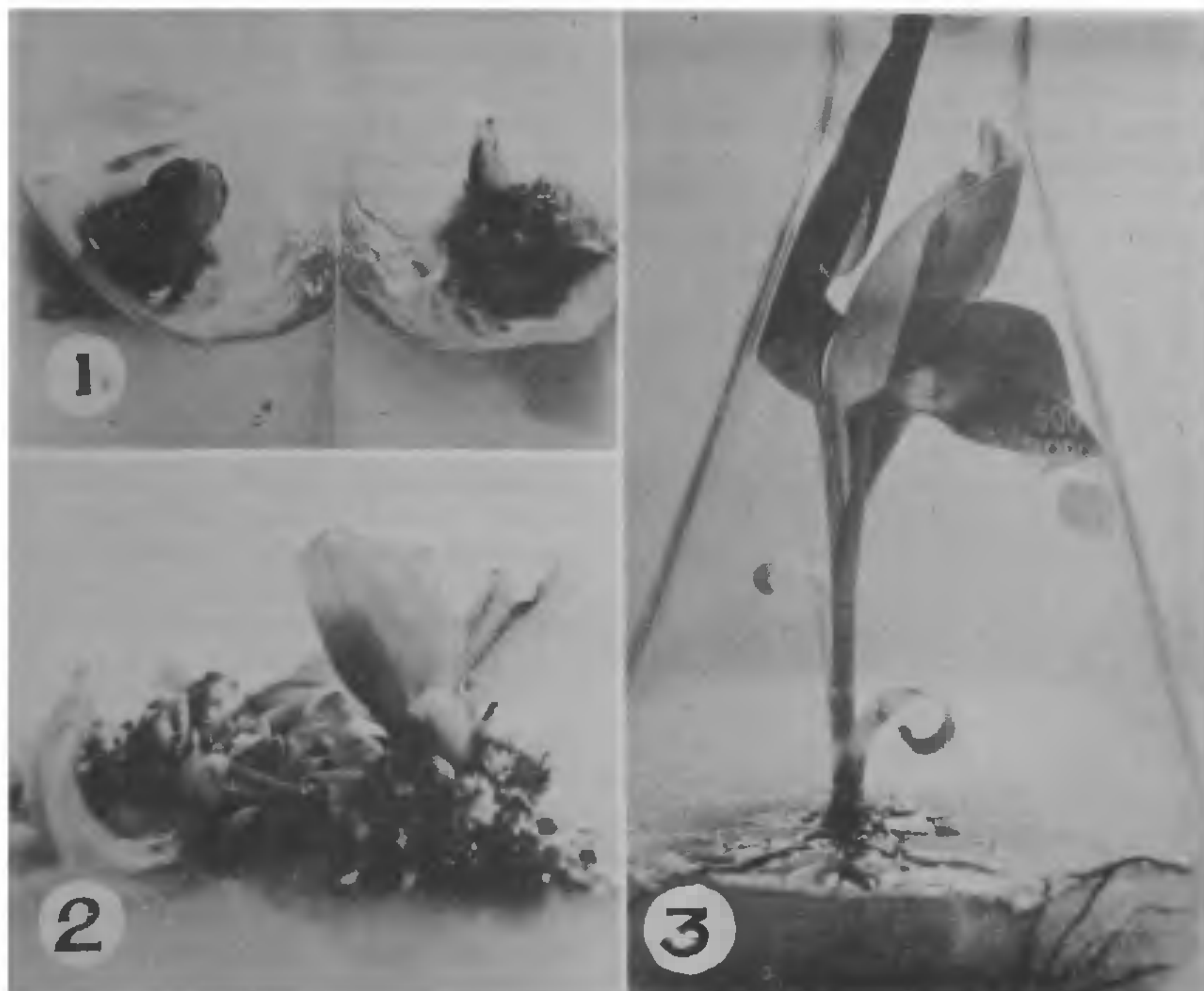
REGENERATION OF BANANA PLANTLET FROM *IN VITRO* CULTURE OF FLORAL APICES

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Of the various manipulations available for the plant scientists and plant growers, tissue culture application is most advanced in the area of clonal propagation. Therefore, both reproductive and vegetative portions of many fruit crops have been used as explants in tissue culture^{1, 2}. To study the possibilities of using tissue culture techniques for clonal propagation in banana, several plant parts have been suggested as explants. In this paper the feasibility of using floral apices of banana as explant for clonal multiplication is reported.

The terminal male flower buds of the banana cultivars Robusta (AAA) and Monthan (ABB) were collected from field-grown plants after the completion of the female phase in the bunch. The basal parts of male flowers including bracts were removed such that the flower buds measured 10-15 mm. The floral apices were then surface-sterilized with 70% alcohol for 30 s and then with 0.1% mercuric chloride solutions for 5 min, followed by 4 washings with sterile-distilled water. Finally floral apices were transferred to a solid medium containing inorganic salts of MS medium³, vitamins according to Gamborg's medium⁴, 30 g/l sucrose and 0.8% Difco bactoagar. The pH of the medium was adjusted to 5.8 before autoclaving at 1.06 kg/cm² pressure for 20 min at 121°C. Fifteen ml of the medium was used in a 25 × 150 mm test tube. Cultures were incubated at 26° ± 2°C with 14 h light (Ca 2000 lux) at 70% relative humidity. The medium was supplemented with 0, 2.5 or 5 mg/l of BAP (6-Benzylamino purine).



Figures 1–3. 1. Floral apices of banana in the culture medium; 2. Production of multiple shoots in the medium with BAP at 5 mg/l, and 3. A well-developed plantlet in the medium with NAA at 1 mg/l.

The results showed that irrespective of the concentration of BAP all floral apices survived in culture. The outermost leaf sheath of the inoculated floral apices turned green within 15–20 days of culturing. Proliferation of buds was seen only in the cultures on the medium having BAP at 2.5 and 5 mg/l, for both the cultivars. After 35 days of culturing, the floral apices (figure 1) were subdivided with scalpel by making incisions from tip downwards and grown in the same medium containing only BAP at 5 mg/l. The multiple shoot production in subculture occurred in the secondary cultures so derived and 4–12 shoots were observed. When individual shoots were transferred to the same medium with NAA at 1 mg/l rooting occurred (figure 3). The first root appeared within 7–10 days and after a month the plantlets were fully developed. The plantlets were then potted in pots containing soil, sand and farmyard manure in 2:1:1 ratio and kept in the mist chamber for a fortnight and then exposed to outside environment for another fortnight before planted in the field.

In India, banana plantations are severely affected by diseases including bunchy top virus⁵ and banana wilt diseases⁶ and pests like nematodes^{7,8}. Production of plantlets from floral apices through *in vitro* culture can provide a method for the production of disease-free plantlets for use in breeding programmes, conservation of germplasm and for rapid multiplication of a genotype soon after the fruit bunch had been evaluated, without disturbing the parent plant and its suckers. The method may prove to be particularly useful for those cultivars like Monthan which have slow rate of multiplication under *in vitro* shoot tip culture⁹.

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ROLE OF NIGROSPORA ORYZAE (BERKS & BR) PETCH IN BLAST LESIONS OF RICE CAUSED BY PYRICULARIA ORYZAE CAV.

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THE blast lesions on rice leaves caused by *Pyricularia oryzae* showed predominant colonization of the fungus *Nigrospora oryzae* in Tamil Nadu. Both young and old blast lesions on the leaves of rice were affected. The blast-infected rice leaves collected from Tirur (Chingleput Dist.), Aduthurai (Thanjavoor Dist.) and Coimbatore revealed the presence of *Nigrospora* in all types of lesions. This was seen extensively on rice varieties IR 50, white ponni, etc.

The fungus *Nigrospora* is not known as a serious plant pathogen. It is mainly a saprophyte on old and dead parts of rice plant¹. It is not commonly seen associated with the lesions of other pathogens on rice leaves. *Helminthosporium oryzae* was found to induce two types of lesions—one a typical lesion and the other a small indistinct type. *H. oryzae* was found to exist with the fungus *Nigrospora* in the indistinct type of lesions on rice leaves². The blast lesions on leaves showed the association of *Nigrospora* clearly, with dark, minute dots which give a light dark appearance and one can easily recognize

the colonization of *Nigrospora* on the lesions. Studies with vertical spore traps with sticky cello-tape (5 cm²) for over 12 months at TNAU, Coimbatore revealed the presence of *Nigrospora oryzae* in the air. The spores showed an increase from March and the maximum were trapped in August and then there was a decline (table 1). The reduction in the number of *Nigrospora* spores trapped during December to March may be attributed to the fact that blast susceptible varieties of rice crop are discouraged during this season in Tamil Nadu.

Repeated culturing of the leaf tissues with the blast lesions on oatagar yielded mostly *Nigrospora oryzae* around the colonies of *Pyricularia oryzae* in the petri plates. The fungus *Nigrospora* was found to grow and develop at room temperature (26° to 30°C) and is a fast growing fungus. *Pyricularia oryzae*, which occurs during the period of cold weather is a slow growing and sensitive fungus and fails to develop in the presence of other fungi. *Nigrospora oryzae* was grown in the same culture plate in which *Pyricularia oryzae* was inoculated 5 days earlier and after 10 days it was found that *Nigrospora* had grown around *P. oryzae*, thus limiting the growth and development of *P. oryzae*. This was similar to 'aversion phenomena' in respect of *Nigrospora* and *Pyricularia oryzae*. The pathogen *P. oryzae* is concentrated in the centre of the lesions on the neck and nodes and the margin of the lesions is occupied by *Nigrospora*, *Cladosporium* and *Fusarium* sp^{3,4}. These three fungi were obtained from the blast lesions from the leaf of IR 50, and the *Nigrospora* was predominant. A few micro-organisms can invade lesions and displace pathogens⁴.

Table 1 Spore trap record at Tamil Nadu
Agricultural University, Coimbatore

Month	Nigrospora spores trapped
February '86	74
March	144
April	418
May	472
June	640
July	1051
August	4580
September	1479
October	467
November	522
December	153
January '87	120