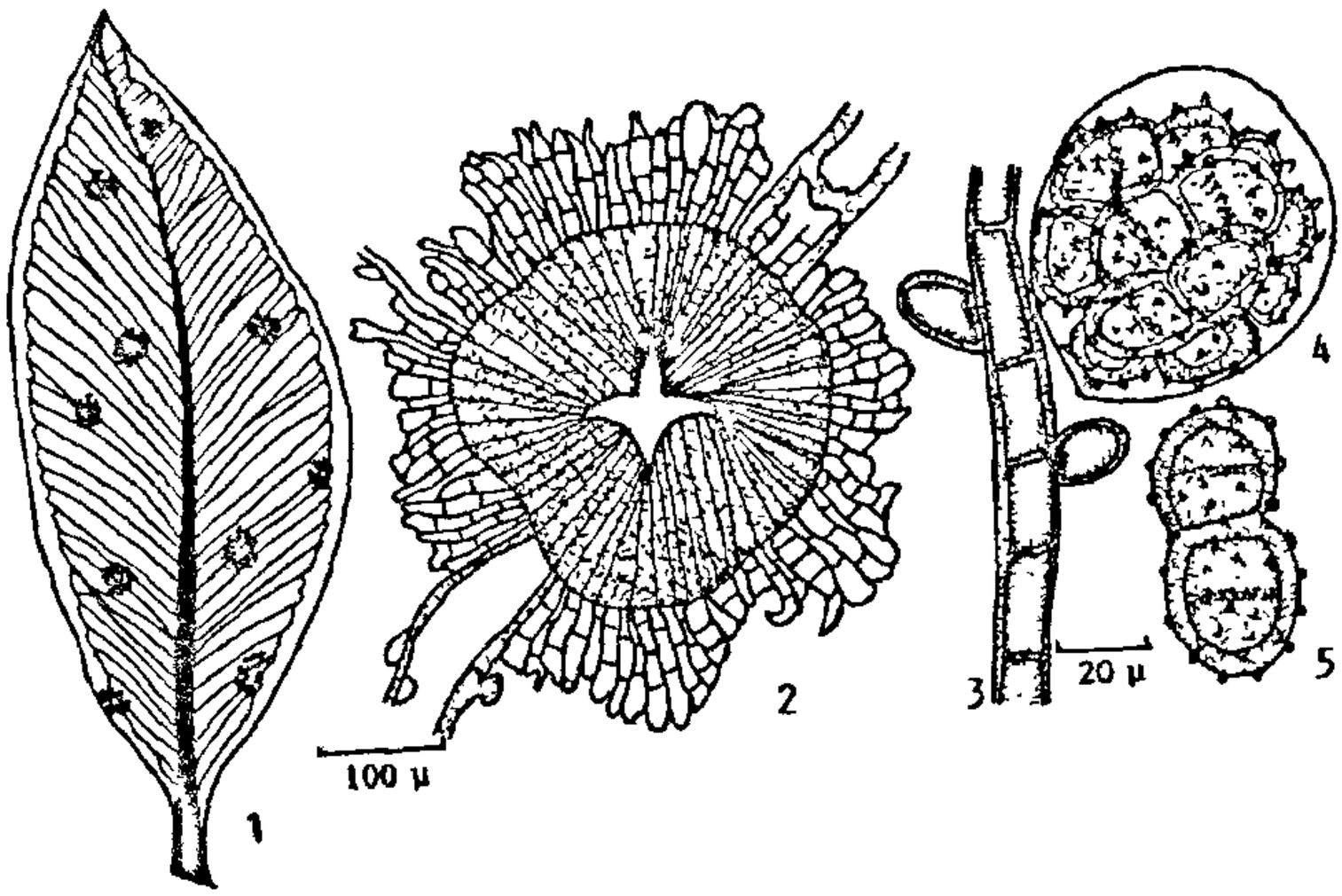

Figures 1–5. *Asterina gopalkrishnanii* sp. nov. **1.** *Syzygium cumini* leaf with infection; **2.** Thyrothecium; **3.** Hypha with capitate hyphopodia; **4.** Ascus with eight ascospores; **5.** An ascospore.

This species of *Asterina* differs from *Asterina fawcetti* Ryan[1] on the same host in having spinules and a dark band in the middle of each cell of the dark brown ascospores and hence is a new species reported from India[2].

The authors are indebted to Dr M. S. Balakrishnan, for encouragement.

9 April 1987

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A RAPID STAINING TECHNIQUE FOR STAGING OF MICROSPORES IN RICE (*ORYZA SATIVA* L.) AND RICE BEAN (*VIGNA UMBELLATA*)

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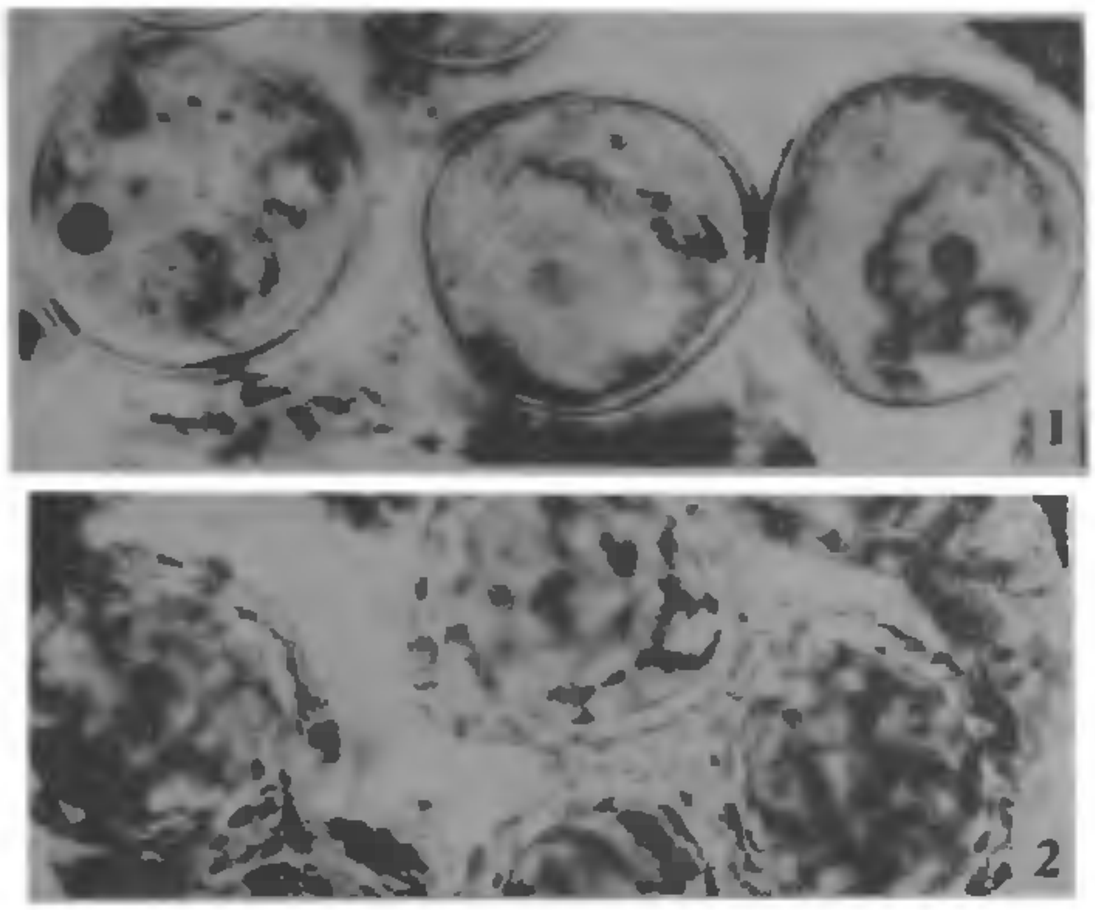
BREEDING through haploids reduces the time needed to reach homozygosity and allows expression of recessive genes in an early generation. In androgenic haploid production the stage of microspore at which the anthers are cultured is known to be crucial than the composition of the nutrient medium. There is a staging optimum for each species as has been reported in several cases. Anthers of many cereals respond better at the early uninucleate microspore stage[1] or on mid-uninucleate stage i.e. when the microspores are half-way through the uninucleate stage e.g. maize[2], wheat[3] and rice[4].

Microscopic staging of microspores for determining the mid uninucleate stage is desirable before plating of anthers, but some researchers have used external morphological features of the panicle to select microspores of this stage[5,6]. The use of such morphological features has been found to be erroneous in our laboratory, and also by Mercy *et al*[7]. Therefore, microscopic staging can only lead to the exact determination of the stage. The stain generally used in microscopic staging is 2% acetocarmine[8] which in our experience does not stain nucleus and cytoplasm differentially[9]. A modified acetocarmine staining was advocated by Genovesi and Magill[10], nevertheless, many researchers have found even this method as not very satisfactory. The present investigation reports an easy and rapid staining technique for the staging of microspores in rice.

Young panicles of rice[2], while enclosed in the boot leaf, were collected from field and stored in a



Figures 1 and 2. Nucleus of the microspores stained black by acetic acid-iron alum-haematoxylin. **1.** in *Oryza sativa* L. sp. *indica* (× 850), **2.** in *Vigna umbellata* (× 800).

BOD incubator maintained at 10 + 1°, until use. The anthers from fresh and cold-treated panicles were squashed in a drop of acetic acid-iron alum-haematoxylin stain. This was obtained by dissolving chloral hydrate (40%, wt./vol.) in a stock solution which was prepared by mixing 4 g haematoxylin and 1 g iron alum in 100 ml of 45% acetic acid[11].

Nucleus appeared deep grey to black coloured against colourless cytoplasm. Uninucleate (figure 1) as well as binucleate microspores were distinct. Using the same stain, microspore nucleus of rice bean (*Vigna umbellata*) was also seen clearly despite the presence of ornamentation of the wall (figure 2). However, when acetocarmine was employed microspore nuclei were neither visible in rice nor in rice bean even though various concentrations were used.

Iron alum has been widely used as a mordant in chromosome studies[12]. In the present study it is presumably adsorbed onto the nuclear material on which haematin gets deposited thus staining the nucleus distinctly. Haematin, after ferric mordanting, is known to possess a strong tendency to accumulate around densely stained material.

The author thanks Mr R. N. Bhuyan for technical help, and Mr B. K. Sarma and Mr Major Singh for providing flower buds of rice bean.

23 April 1987

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AMANITA FLAVOFLOCCOSA—AN ADDITION TO INDIAN AGARIC FLORA

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AMANITA FLAVOFLOCCOSA was originally described from Japan by Nagasawa and Hongo[1]. This is a very common species occurring in and around Madras and has been collected on several occasions by us. A description of the fungus is given below and this is the first report of this species outside Japan. The colour terminology used is that of Kornerup and Wanscher[2].

Amanita flavofloccosa Nagasawa and Hongo in Trans. Mycol. Soc. Japan **25**: 367 (1984), (figure 1a–d).

Pileus 3.5–11 cm in diam., conical becoming planoconvex; surface light yellow (4A5), orange (6B6)