Jacq. as stock cultures. Viable spores were individually collected without organic debris and were surface-sterilized by chloramine T (2%) and streptomycin (0.2%) with a little of tween-80. Samples were put into individual Thunberg tubes to which 3 ml of phosphate buffer (pH 7.0), 1 ml of 0.1 M neutral succinic acid and 1 ml of 0.1 M KNO_3 were added. One ml of distilled water was added instead of KNO_3 to another set which served as control. Air was evacuated from the tubes and was sealed airtight. The tubes were kept in waterbath at 35°C in dark. Samples were taken at 2nd, 4th, 6th, 12th and 24th h and 2 ml of each were withdrawn to which 1 ml of 0.02% N-(1-napthyl)-ethylenediamine dihydrochloride plus 1 ml of 0.1% sulphanilamide were added. The amount of nitrite present in the sample was calculated colorimetrically with Baush and Lomb Spectronic 20 at 540 nm, using distilled water as blank. There were three replications for each treatment.

Table 2 shows the nitrate reduction of different VAM isolates. There were no reduction in controls whereas all the twelve VAM isolates reduced the nitrate to nitrite. The efficiency of reduction was maximum in three isolates viz. MKU7, KK2 and *G. fasciculatum;* moderate in three isolates designated as MKU4, MKU6 and *G. aggregatum* less in three isolates viz. CK2, CK3 and CK4 while isolates Tm2. Tm6 and Tm8 showed the least activity. The results showed the nitrate-reducing capacity of VAM fungi isolated from various areas. With the ability of reducing nitrate these fungi can have efficient symbiosis with the host by the assimilation and translocation of nitrogen. The earlier experiment on the screening of their efficiency in improving the nutrition of cowpea plants[6] also showed considerable increase in nitrogen uptake by the two VAM fungi (MKU7 and *G. fasciculatum*) which were shown to have efficient nitrate reducing capacity.

Table 2 *Nitrate reduction of various isolates of VAM fungi. Nitrite (μmol) formation after the treatment of KNO_3 at different incubation periods*

	Incubation hours				
VAM isolates	2	4	6	12	24
KK2	0.035	0.108	0.267	1.490	3.124
CK2	0.014	0.076	0.101	0.762	2.003
CK3	0.017	0.079	0.109	0.890	2.084
CK4	0.009	0.034	0.093	0.546	1.984
MKU4	0.024	0.096	0 173	0.986	2.701
MKU 6	0.024	0.088	0.156	0.998	2.563
MKU7	0.042	0.196	0.259	1.560	3.172
Tm2	0.003	0.056	0.093	0.634	1.224
Tm6	0.025	0.038	0.102	0.594	1.553
Tm8	0.034	0.045	0.125	0.532	1.758
G. fasciculatum	0.033	0.152	0.201	0.917	2.974
G. aggregatum	0.021	0.073	0.167	0.803	2.168

All the values reported here are means of three samples, and should be multiplied by 10^{-2}.

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MALE AND FEMALE GAMETOPHYTES OF *RAUVOLFIA SUMATRANA* JACK.

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THE genus *Rauvolfia* has attracted global attention due to its alkaloid-reserpine which is widely employed in the treatment of hypertension and mental disorders. *Rauvolfia serpentina* (India's wonder drug plant) is now extensively cultivated throughout India on account of its great export potential. Intensive search is going now, on the species of *Rauvolfia*, other than the *serpentina* to find out their alkaloid potential and to ascertain their medicinal values. *Rauvolfia sumatrana* Jack. is one such plant which was introduced into the Indian Botanic Gardens, Howrah. The present investigation focuses our attention on the development of male and female gametophytes of *R. sumatrana* Jack. and explains the causes of sterility in this interesting plant.

The development of the anther wall is of the Dicotyledonous type (figure 1A, B) and at maturity it consists of an epidermis, fibrous endothecium, a middle layer and an uniseriate secretory tapetum

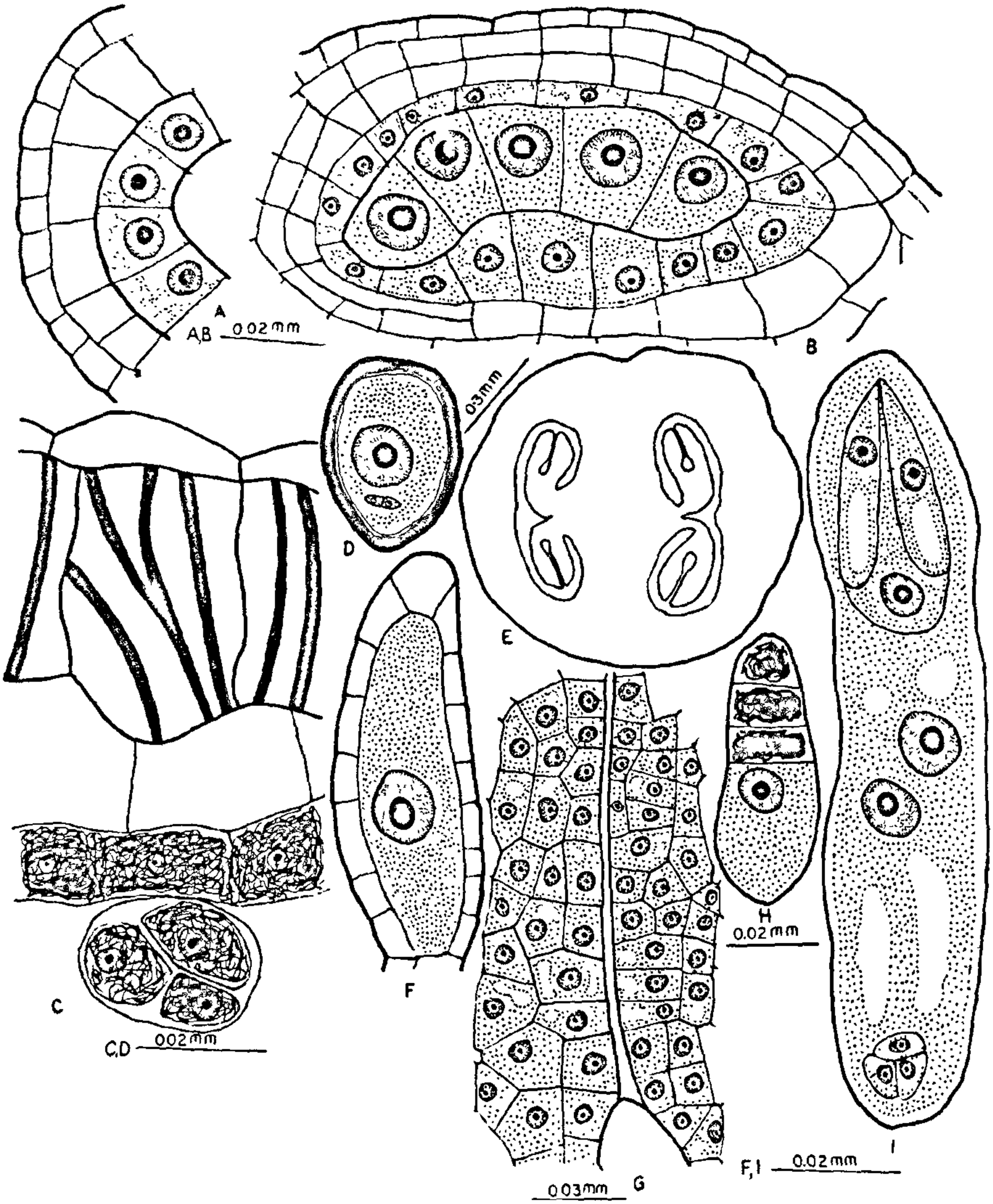

Figure 1A–I. T. s. part of anther showing primary parietal and primary sporogenous layers; **B.** T. s. of anther showing wall layers, tapetum and pollen mother cells. Note the connective tapetum; **C.** T. s. part of anther showing fibrous endothecium and degenerating tapetum and pollen tetrad. Note persistent middle layer; **D.** 3-celled pollen grain; **E.** T. s. of ovary; **F.** Megaspore mother cell; **G.** Upper part of the ovule showing integumentary tapetum and micropyle; **H.** Megaspore tetrad; **I.** Mature embryo sac.

with uninucleate cells. The connective tapetum is also differentiated (figure 1B). The tapetum degenerates at the pollen tetrad stage but the middle layer remains persistent (figure 1C). Simultaneous divisions in the microspore mother cells result in the formation of isobilateral and tetrahedral tetrads. Cytokinesis is by furrowing. Pollen grains are tri-aperturate and 3-celled at shedding (figure 1D). Degeneration of pollen occurs quite frequently from the microspore mother cell stage onwards.

The gynoecium is superior and the ovary is bicarpellary, syncarpous and bilocular with two orthotropous, unitegmic and tenuinucellate ovules in each locule on axile placentation (figure 1E). Integumentary tapetum is differentiated only on the micropylar region of the ovule and is multiseriate with uninucleate cells (figure 1G). A rudimentary aril is found on the funicular region of the ovule.

The hypodermal archesporial cell directly functions as the megaspore mother cell (figure 1F) which undergoes the usual meiotic divisions and gives rise to a linear tetrad of megaspores (figure 1H). The chalazal megaspore develops into an 8-nucleate embryo sac of the Polygonum type (figure 1I). Mature embryo sac is cylindrical in shape. Egg apparatus consists of an egg and two flask-shaped synergids. Polars fuse before fertilization. Antipodals are three in number and uninucleate. In rare instances, multiple embryo sacs are observed but only one reaches maturity. Pollen sterility was very high and this may be due to the early degeneration of the nutritive layer i.e. tapetum. Though a large number of flowers were studied not even a single case of fertilization of embryo sac was observed.

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VARIABILITY OF ACETYLENE REDUCTION ASSAY IN THE ESTIMATION OF NITROGEN FIXATION IN TROPICAL FOREST SOILS

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Burns and Hardy[1] estimated that 4100×10^6 ha of earth's forests and woodlands account for 10 kg/ha/year of biological nitrogen fixation, which is about 28% of the total, estimated to occur in terrestrial ecosystems. Because of inadequate data from natural tropical and managed forests[2], the current estimates of nitrogen fixation are not reliable[3,4]. Therefore, we have been monitoring nitrogen fixation in Kalakad Reserve Forest, a tropical forest, located in the Western Ghats in Tamil Nadu, India. In the first instance, it was found that acetylene reduction assay (ARA) was highly variable and was influenced by soils, moisture, temperature and anaerobic conditions.

Soil samples from 10 places, from surface up to 5 cm depth, were collected from a 50 × 50 m plot in evergreen forest located at an altitude of 940 m and pooled. They were transferred to sterile polythene bags, sealed and assayed for acetylene reduction within 16 h of collection.

The soil sample (1 g) was transferred to a 250 ml Erlenmeyer flask fitted with 'Suba' seal stopper and flushed with argon for 30 sec. Immediately, 20 ml of the gas were removed from each flask by a hypodermic syringe and the same amount of acetylene was injected into it. All the experiments were duplicated.

Acetylene reduction was measured by withdrawing 0.5 ml of the gas from each flask by a gas tight plastic disposable syringe at prefixed intervals and injected into a Hewlett-Packard gas chromatograph (model 5840 A) fitted with Porapak-N column and hydrogen flame ionization detector. The temperatures of column, injection port and flame ionization detector were set at 60°, 100° and 100°C respectively. The carrier gas was nitrogen and its flow rate was maintained at 40 ml/min.

Since the temperature in the forest ranged from 22°C to 40°C, the effect of temperature on the nitrogenase activity was measured. The moisture level in the forest fluctuated from saturated condition to near dry status and the effect of moisture on ARA was also determined.

Flasks with 10 g of the soil with different moisture levels were flushed with argon and analysed for acetylene reduction.

The influence of nitrogen-free Jensen's modified medium[5] on nitrogenase activity of soil was ascertained. One set of 250 ml Erlenmeyer flask containing 1 g soil suspended in 100 ml medium and another set without the medium were used.

Nitrogen-fixing activity as measured by ARA was high at 24°C and higher temperatures reduced it (figure 1). Hardy *et al*[6] observed that the nitrogenase activity increased at incubation temperature from 10 to 20°C, reached a maximum between 20 and 30° C and declined thereafter. Akkermans[7] and Wheeler[8] found a maximum nitrogenase activity in soils between 20 and 25° C followed by a sharp