

6.5 to 7. According to Sathianathan<sup>4</sup> once the pH is adjusted between 7 and 8, the fermenting mixture becomes well buffered and there is no change in pH even after the addition of acids or alkalies. This effect is clearly seen from the fermentation between 29 and 49 days where even though propionic and isovaleric acids are detected intermittently, the pH is steady at 7 when the environment became more congenial for methanogenesis.

The data thus show that the aquatic vegetative growth such as water hyacinth enhances the volatile fatty acids in the fermenting slurry which is a precursor of methane in the anaerobic fermentation and thus can profitably be used as additives in the gobar gas plant.

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1. *Methane Generation from Human, Animal and Agricultural Wastes*, National Academy of Science, Washington, D.C., 1977.
2. Guha, B. R., Bandopadhyay, T. K. and Chaudhary, K. K., *Khadigramodyog*, 1976, 22, 483.
3. Barker, H. A., *Bacterial Fermentation*, John Wiley and Sons, New York, 1956.
4. Sathianathan, M. A., *Bio-gas—Achievement and Challenges*. Association of Voluntary Agencies for Rural Development, New Delhi, 1975.

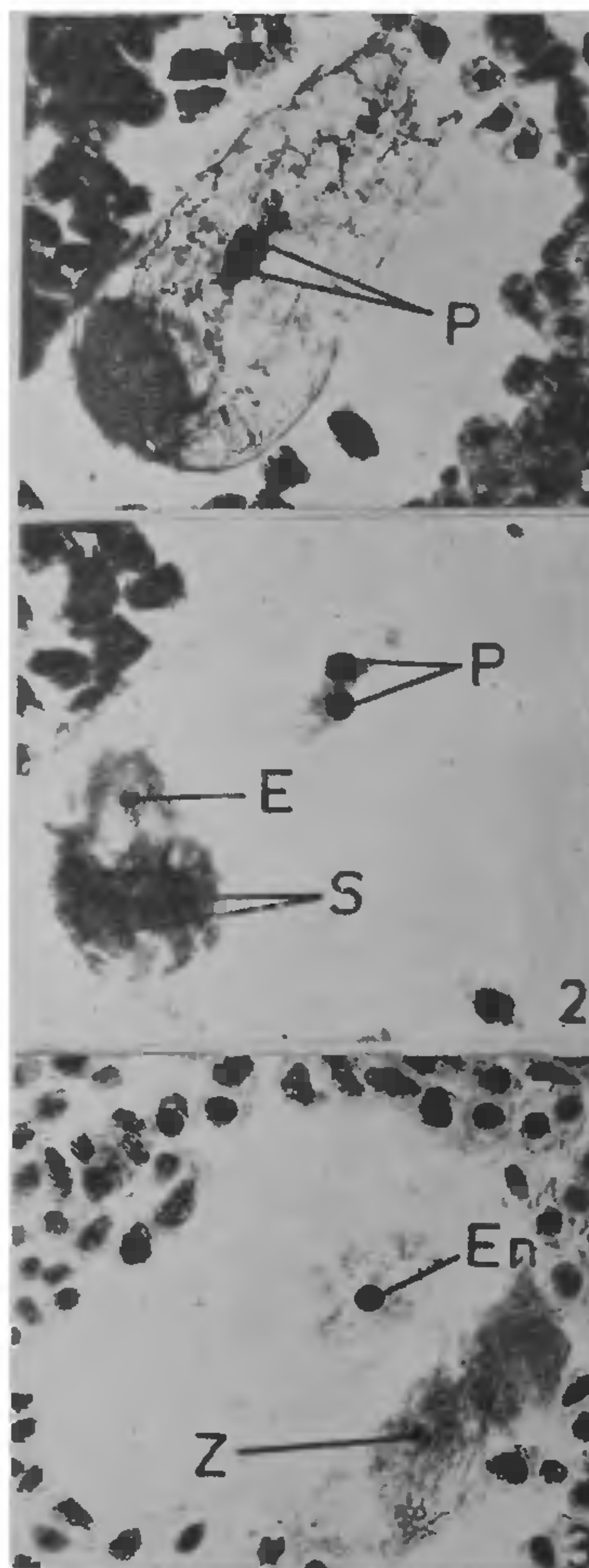
#### A RAPID METHOD FOR THE STUDY OF FERTILIZATION IN GROUNDNUT, *ARACHIS HYPOGAEA* L.

A STUDY of pre- and post-fertilization events in groundnut is of significance in attempts of interspecific gene transfer. Earlier studies on the details of fertilization in groundnut were based on conventional techniques using serial sections<sup>1-4</sup>. An attempt was made in the present study to find out the possibility of studying the pre- and post-fertilization events in the embryo sacs in iron acetocarmine squashes and the results are given in this report.

Flowers were emasculated on the evening preceding anthesis and pollinated at the time of anthesis. Ovaries were fixed at 2 h intervals in Carnoy's fluid for 48 h. The ovaries were mordanted in saturated ferric ammonium sulphate solution at 75°C for 5 minutes, washed twice in water at 75°C for 6 minutes, macerated in 50% HCl for 10 minutes and washed for 20 minutes

in running water. Ovules were dissected in a drop of water and squashed in acetocarmine.

Embryo sacs got popped out of the ovules by a little pressure on the coverglass. The structure of the embryo sacs was brought out very clearly in the squashes (Figs. 1 and 2). The embryo sacs were of the normal



FIGS. 1-3. Fig. 1. Embryo sac before fertilization. Fig. 2. The polar nuclei and the egg apparatus enlarged from Fig. 1, showing details. Fig. 3. Embryo sac after fertilization showing the primary endosperm nucleus and the zygote. (S, synergids; E, egg apparatus; P, polar nuclei; En, primary endosperm nucleus; Z, zygote.)



Polygonum type. The antipodals generally degenerated by anthesis. Fertilization of the egg and triple fusion were generally completed before 6 h after pollination (Fig. 3). There was considerable variation in different flowers with respect to the time of fertilization. This can be easily explained since groundnut flowers vary greatly in length. The egg and zygote had very dense cytoplasm.

Compared to conventional section cutting, study of acetocarmine squashes is faster and accurate, since embryo sacs could be observed in their totality. This method of studying fertilization in groundnut should be useful in studies of interspecific hybridization. The genus *Arachis* L. contains about 40 species which are valuable sources of disease and insect resistance. However, successful hybrids could be obtained between the cultivated and wild species only in a few cases<sup>5,6</sup>. The reasons for the failure of the crosses are not always clear. Using the acetocarmine squashes, it should be possible to study the entry of pollen tubes into the embryosacs, the presence or absence of fertilization and the mechanism of embryo and seed failure in interspecific crosses. More detailed studies on the cytology of reproduction in the different botanical varieties of groundnut and wild *Arachis* species are under progress.

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1. Banerji, I., *J. Bombay Nat. Hist. Soc.*, 1938, 40, 539.
2. Smith, B. W., *Am. J. Bot.*, 1956 a, 43, 81.
3. —, *Ibid.*, 1956 b, 43, 233.
4. Conagin, C. H. T. M., *São Paulo*, 1957, 16, 15.
5. Johansen, E. L. and Smith, B. W., *Am. J. Bot.*, 1956, 43, 250.
6. Gregory, W. C., Gregory, M. P., Krapovickas, A., Smith, B. W. and Yarbrough, J. A., In *Peanuts, Culture and Uses*, The Stone Printing Co., Va. USA, 1973.

#### CHARACTERIZATION OF RICE RAGGED STUNT VIRUS DISEASE IN INDIA

DURING November 1978, a few plants of Taichung (Native) 1 in the green house of All India Coordinated Rice Improvement Project (AICRIP), Rajendranagar, Hyderabad, developed typical symptoms of rice ragged stunt. The occurrence of ragged stunt virus disease

has been reported from Indonesia<sup>2</sup>, Philippines<sup>3</sup>, and Thailand<sup>4</sup>. Heinrichs and Khush<sup>1</sup> also reported the possible presence of rice ragged stunt virus disease in India and Sri Lanka on the basis of their observation of infected plants. The present investigation confirms the presence of rice ragged stunt virus disease in India as the first established record and deals with its transmission by *Nilaparvata lugens* (Stal).

The symptoms of infected plants with rice ragged stunt virus included stunting and ragged leaves (Fig. 1 a) which ranged from 1-4 per plant, the raggedness was mostly observed on one side of the leaf and seldom on both the sides. In addition to these symptoms, twisting of the tip of the leaves and vein swellings (Fig. 1 b, c), delay in flowering, production of nodal branches mostly at the time of panicle emergence, incomplete emergence of panicles which bore mostly unfilled grains were also observed.



FIG. 1 a-c. a. Taichung (Native) 1 infected with ragged stunt virus. b. Vein swelling on the infected leaf and leaf twisting. c. Leaf tip twisting and raggedness on the leaf margin.

Transmission studies were conducted with seedlings of Taichung (Native) 1. Sap, soil and seed transmission studies gave negative results. The disease could readily be transmitted only by brown plant hopper *N. lugens* but not by green leaf hopper and white-backed hopper, *Nephotettix* spp. and *Sogatella* spp. Under present conditions, Taichung (Native) 1 plants which were inoculated at 2-3 leaves stage by *N. lugens*, after giving an acquisition feeding of 3 days, on the diseased plants took 15-31 days to produce the typical symptoms of twisting of leaves followed by ragged leaf margins. The details of transmission by *N. lugens*, etc., will be published elsewhere.