Kanyerez, B. R., *Lancet*, 1966, **2**, 1210.
4. Wright, G. L., *Am. J. Clin. Pathol.*, 1972, **57**, 173.
5. O'Farrell, P. H. and O'Farrell, P. Z., *Methods in Cell Biology*, 1977, **16**, 407.
6. Anderson, N. L. and Anderson, N. G., *Proc. Natl. Acad. Sci. U.S.A.*, 1977, **74**, 5412.
7. Sai-Hung Yu., *Anal. Biochem.*, 1978, **85**, 287.
8. Drescher, D. G. and Lee, K. S., *Anal. Biochem.*, 1978, **84**, 559.

ISOLATION OF A MICRO-ORGANISM FROM *PHILOSAMIA RICINI*

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RECENTLY the presence of urease has been shown in *Antheraea mylitta*[1] and *Philosamia ricini* (S. Kumar, personal communication). The synthesise of ascorbic acid has also been reported[2] for *P. ricini*. The ascorbic acid does not synthesise in insects which do not harbour symbionts. Similarly the presence of urease is indicative of the presence of a micro-organism in *P. ricini*, since urease is usually not present in asymbiotic insects. The present note reports the presence of a symbiont in *P. ricini*.

P. ricini was reared in the laboratory as described earlier[3]. The micro-organism was isolated from the fat body of *P. ricini* in sterile condition, by making a sharp incision in the pupae and making streaks of fat body in blood agar plates. Minute colonies grow in blood agar plates in 5–7 days. Different growth media were then used for growing the micro-organism thus isolated.

The micro-organism isolated was non-motile, gram-negative, rod-shaped and fairly uniform in size (0.3 to 0.5 μ). The growth in blood agar and nutrient broth was very slow; in 199 medium, the growth was better whereas in potato slant the growth was profuse. The micro-organism was negative for all the sugars and biochemicals used *viz* sorbitol, glucose, maltose, sucrose, salicin, lactose, trehalose, adonitol, mannitol, arabinose, mannose, inulin, dulcitol, dextrin, indole, citrate, gelatin and nitrate. The reaction with litmus milk was acidic.

The micro-organism isolated resemble *Wolbachia melophagi*, an extra-cellular symbiont of sheep ked, *Melophagus ovinus*.

The presence of symbiont in silk worms has not been reported so far. Its isolation from *P. ricini* explains many anomalous results reported for *P. ricini*. Thus the reported results that cholesterol and ascorbic acid synthesis take place in *P. ricini*[2,4] can be explained. The presence of urease in *P. ricini* can also be similarly explained.

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1. Sharma, B., *Studies on insect biochemistry*, Ph.D. Thesis, Allahabad University, 1981.
2. Pant, R. and Pandey, K. N., *Indian J. Exp. Biol.*, 1978, **16**, 312.
3. Pant, R. and Lacy, P. S., *Indian J. Biochem.*, 1968, **5**, 13.
4. Nautiyal, G. C., *Studies on insect biochemistry*, Ph.D. Thesis, Allahabad University, 1972.

GREEN MOSAIC: A VIRUS DISEASE OF *HYOSCYAMUS MUTICUS* L. IN INDIA

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DURING 1981–82 *Hyoscyamus muticus* L. (Egyptian henbane), one of the important medicinal crops was severely affected by mosaic disease in the experimental farm of CIMAP at Lucknow. The diseased plants showed severe stunting and bushy appearance. The leaves were reduced in size (figure 1-A) and sometimes narrowing of leaves into shoe lace was also recorded. In general, mosaic mottling symptoms were more pronounced in young leaves. Often the flowers became discoloured and seed setting was poor. A systematic study of this new disease was made in terms of transmission, host range, virus purification and some properties of the virus *in vitro*.

Mechanical transmission studies conducted in the glass-house (15–35°) revealed that the disease was readily transmissible through sap. The sap was extracted from the mosaic infected leaves in 0.1 M

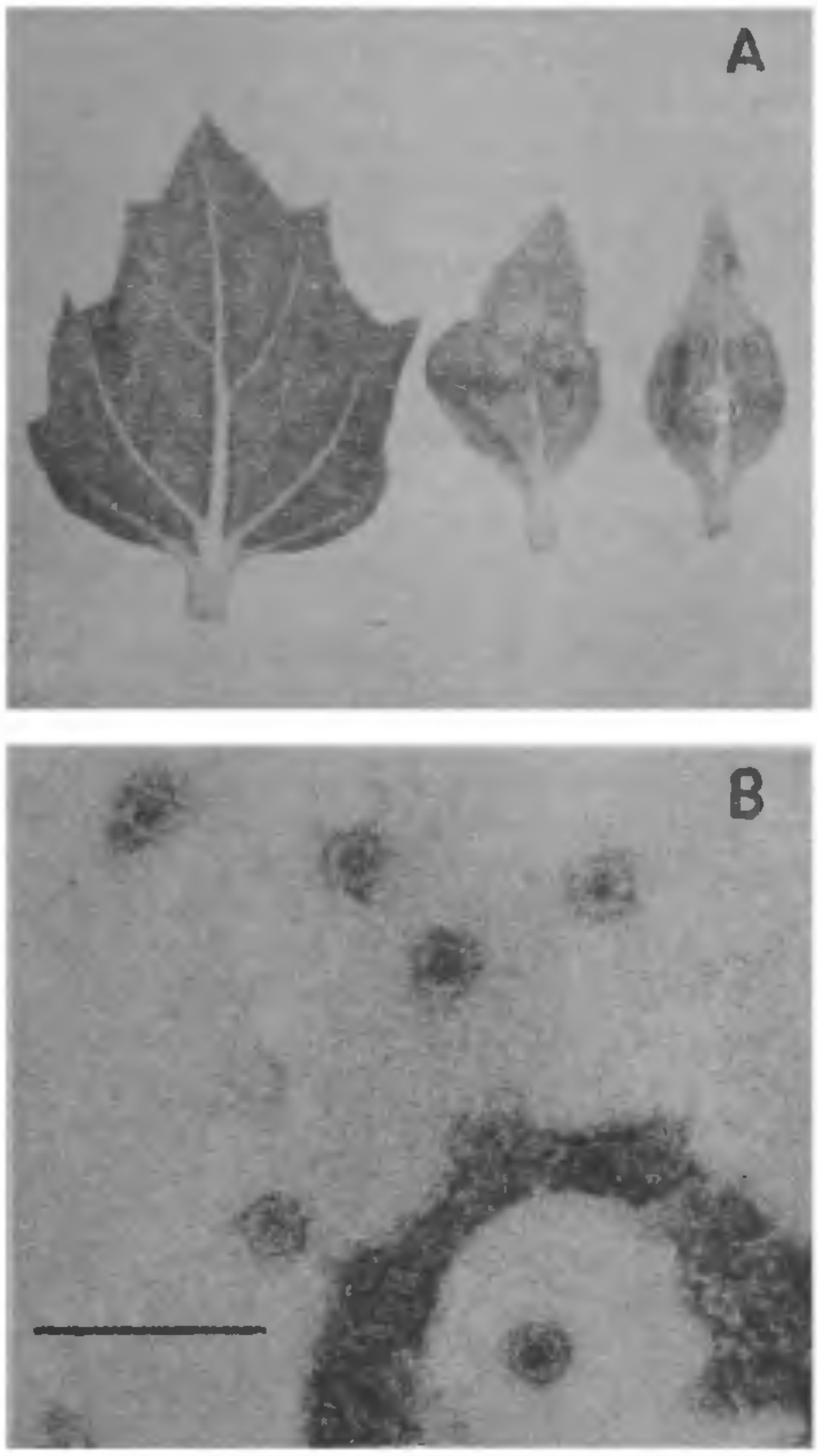

Figure 1A. *Hyoscyamus muticus* leaves (left), healthy; (right), infected., **B.** Virus particles, bar represents 100 nm.

phosphate buffer at pH 7 containing 0.1% 2-mercaptoethanol and rubbed on leaves of healthy *H. muticus* and other hosts which were previously dusted with carborundum (600 mesh). The inoculated *H. muticus* plants showed typical mosaic and stunting symptoms after 13–15 days. In seed transmission tests, the germination of the seed collected from the infected plants was very poor. Ten percent of the plants that have germinated and grown well, showed no symptoms. Back inoculations from these plants were negative. No positive results were obtained with soil transmission. Aphid (*Myzus persicae* Sulz.) was used in the transmission studies. Virus-free alate *M. persicae* were fed on infected *H. muticus* for 24 hr. After fasting for 4 hr, the aphids were transmitted to two sets of 50 plants each. One set was sprayed with 0.03% rogor to kill the aphids after 24 hr. On the other set, the aphids were allowed to feed for 24 hr and then transferred to another set of healthy plants daily for 20 days to test for nonpersistant or persistant virus transmission. Only 20% of the inoculated plants showed symptoms and the virus was transmitted in a nonpersistant manner.

In the host range studies, *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste & Reyn, *C. quinova* Willd, *Vigna unguiculata* (L.) Walp cv. 152 and *Beta vulgaris* L. produced necrotic lesions 2–3 days after inoculation. Only chlorotic rings were recorded on *Helianthus annuus* L. whereas on the following hosts, *Zinnia elegans* Jacq., *Citrullus vulgaris* Schrad., *Cucumis sativus* L., *Lycopersicon esculentum* Mill., *Hyoscyamus niger* L., *H. albus*, *Nicotiana glutinosa*, *N. rustica* L., *N. tabacum* cvs. Samsun, Samsun NN, White burley, Xanthi, and *Scopolia sinansis* L., systemic symptoms with varied degrees of mosaic mottling symptoms were produced 10–15 days after inoculation.

Other hosts namely *Arachis hypogaea* L. cv. TMV-2, *Brassica alba* L., *Phaseolus vulgaris* L. cvs. Bountiful, Redkidney, Topcrop., *Cajanus cajan* Milsp. cvs. BDN-1, ICP-2376, *Mentha spicata* L., *Raphanus sativus* L. gave negative results. No virus was recovered when back inoculated on *C. amaranticolor*. *In vitro* (LIV) the virus under study retained its pathogenecity up to 2 days at 25°C and observed to be infective up to a dilution (DEP) of 10^{-4}. The thermal inactivation point (TIP) of the virus was found to be 60–65°C. The virus was purified from fresh leaves of *Nicotiana rustica* and *Hyoscyamus muticus* inoculated 20 days earlier by homogenising (g/4 ml) in cold 0.1 M phosphate buffer containing 1% 2-mercaptoethanol. Initial purification was done largely according to the method of Waterworth and Povish[1]. The clarified extract was further purified by 4% polyethelene glycol (PEG) and 1% NaCl precipitation. The virus suspension obtained from PEG precipitates was subjected to one cycle of differential centrifugation (90 min at 35,000 rpm; 10 min at 10,000 rpm). Further purification was by sucrose gradient centrifugation. The gradients (10–40%) were centrifuged at 24,000 rpm for 90 min in a Beckman SW 27 rotor. The virus zones, were collected, diluted with buffer and centrifuged for 1 hr at 35,000 rpm. The resultant pellet was suspended in 0.01 M phosphate buffer, pH 7.

The UV absorption spectrum of the purified virus was typical of the virus with the maximum at 256 nm and minimum at 240 nm. The A240/260 ratio was 0.86 and A260/280 was 1.64. Electron microscopic studies of the purified virus preparation showed spherical particles of 26 nm diameter (figure 1B).

Based on the information obtained from the host range, physical properties and particle morphology[2,3] the virus under study appeared to be identical to cucumber mosaic virus (CMV). However, this assumption could only be confirmed by the serological studies which are in progress. A perusal of the literature on plant virus diseases[4] indicates that there is no record of any virus disease on *H. muticus* from India.

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1. Waterworth, H. E. and Povish, W. R., *Phytopathology*, 1975, **65**, 728.
2. Orellana, R. G. and Antonio Quacquarelli, *Phytopathology*, 1968, **58**, 1439.
3. Kaper, J. M. and Waterworth, H. E., In *Hand book of plant virus infections and comparative diagnosis* (ed.) E. Kurstak, 1981, p. 258.
4. Sastry, K. S., *Plant virus and mycoplasmal diseases in India: A bibliography*. Bharati Publications, Delhi, 1980, p. 292.

INDUCED AUTOTETRAPLOIDY IN *CATHARANTHUS ROSEUS*— A PRELIMINARY REPORT

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CATHARANTHUS ROSEUS, also known as *Vinca rosea*, derives its importance from the anti-cancer alkaloids, vincristine and vinblastine, found in its leaves. Its roots contain ajmalicine and serpentine alkaloids which are used in the treatment of hypertension. Several reports on the increase in chemical constituents of plants through polyploidy indicate the possibility of increasing the alkaloid content of *C. roseus* through autopolyploidy[1-3]. There are quite a few reports on induced autotetraploidy in *C. roseus*[4-10]. However, the effect of autotetraploidy on the alkaloid content in different parts of the plant and also, its effects on economically important characters such as, leaf and root yield have not been reported. Information on these aspects is necessary to evaluate the possibility of developing tetraploid varieties in *C. roseus*.

Tetraploids in *C. roseus* were induced by immersing the apical buds of seven-day old seedlings in 0.5% colchicine solution for 19 hr. Since no pure-lines were available, the treated seedlings were expected to be genetically heterogenous. The variability in the treated material would result in variability in the induced tetraploids which would, in turn, be useful for further improvement of tetraploids.

Several tetraploids were obtained through colchicine treatment of seedlings. Seeds of individual tetraploids were sown to raise C_2 generation. Seven diploid seedlings were obtained in the progeny of one tetraploid plant. These diploid seedlings and 10 tetraploid seedlings of the same plant were individually randomized in the field (in a plot of size 8.5 × 3.0 m.) along with the progeny of other tetraploids. When the plants were 9 months old, five diploid and five tetraploid plants (*i.e.* progeny of the same plant) were harvested and observations were recorded on different characters. (It was observed that defoliation due to senescence started at about this stage in the diploids). Rest of the plants were left for seed multiplication. The content of total alkaloids in different parts of the plant were estimated[12].

The differences between diploids and tetraploids were significant (at 5% level of significance) for only 4 of the characters studied, *viz* total number of branches per plant, average leaf weight, seeds per follicle and 50-seeds weight (table 1). Large standard errors associated with the mean of a majority of the characters studied appear to have rendered the differences between diploids and tetraploids non-significant. Comparisons based on replicated trials could be expected to reveal more significant differences between diploids and tetraploids.

The differences between diploids and tetraploids for other characters (other than the four mentioned above) were tested at 10% level of significance assuming that differences for the characters found to be significant at 10% level of significance would be likely to be found significant at higher levels of significance when comparisons are made from data obtained from replicated experiments. It was found that tetraploids had significantly larger leaf yield/plant yield ratio than the diploids. The diploids had significantly more number