I was characterized by abnormal and unequal distribution of chromosomes to each poles (figure 3). The bivalents, while tended to divide, univalents always lagged behind and were seen lying at equatorial plane. Occasionally one or more lagging univalents were seen going under division. The chromatids of the divided univalents generally moved towards the opposite poles or both were included in the same nuclei. Anaphase II and telophase II were found abnormal and resulted in dyads, triads (figure 4), tetrads and polyoids in variable frequencies at sporad stage.

Plants of guayule var. USS-2x, that produced seeds were observed inspite of showing an extremely abnormal meiosis. Seeds were normal looking and their viability was higher.

This situation clearly indicates the USS-2x being a apomictic variety. Apomixis in guayule has been observed by several workers[2-7]. They had observed that reproductive behaviour in guayule, greatly varies in relation to its genetic structure or level of polyploidy (chromosome numbers). Plants having diploid ($2n=36$) chromosome numbers were found to produce seed sexually, while the tetraploids (or polyploids) were predominantly, a facultative apomict.

With a view to determining the degree of apomixis prevailing in different varieties of guayule grown at NBRI, Lucknow, the author made an experiment[8], as suggested by Tinnery and Aamodt[9]. The results indicated that USS-2x, which was a diploid exhibited 87.3% uniformity in its progenies with regard to their general appearance, growth rates, time of flowering, etc. and concluded that USS-2x was an apomictic variety.

The origin of USS-2x is not clear. However, there may be two possibilities. The first being a species-hybrid evolved from the natural crossing or hybridization with two dissimilar genomes. The other may arise from a haploid-parthenogenesis[10] in *Sisymbrium irio*.

Since meiotic behaviour of guayule USS-2x was highly abnormal and plants were producing seeds, regularly, the possibility of USS-2x being a hybrid had been ruled out, both on cytological and morphological grounds. On the other hand, the chances that USS-2x had originated from haploid-parthenogenesis[10] hold true, as most of the cytological parameters studied in this work are in favour of USS-2x being an apomict and evolved from the development of egg nucleus of a tetraploid plant without being fertilized.

Regeneration, is no problem in the case of guayule variety USS-2x. Being essentially an apomict, seeds are produced, simultaneously.

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1. Katayama, Y., *Jpn. J. Bot.*, 1935, **7**, 349.
2. Esau, K., *Proc. Nat. Acad. Sci. USA*, 1944, **30**, 352.
3. Esau, K., *Hilgardia*, 1946, **17**, 61.
4. Powers, L., *Genetics*, 1945, **30**, 323.
5. Powers, L. and Rollins, R., *J. Am. Soc. Agron.*, 1945, **37**, 184.
6. Rollins, R., *Am. J. Bot.*, 1945, **32**, 554.
7. Hammond, B. L. and Pothamus, L. G., *Tech. Bull. no. 1327, 1942–59, U.S. Dept. Agriculture*, 1965.
8. Narain, P. and Srivastava, G. S., *El Guayulero*, 1986, **8**, 1.
9. Tinnery, R. N. and Aamodt, G. S., *J. Hered.*, 1940, **31**, 457.
10. Khoshoo, T. N., *J. Hered.*, 1957, **48**, 239.

ADDITIONAL DNA HOMOLOGY BETWEEN *AGROBACTERIUM* AND *RHIZOBIUM*

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BOTH *Rhizobium* and *Agrobacterium* belong to the family Rhizobiaceae. The two bacteria are similar in properties of infection to dicotyledonous plants. On infection, species of *Rhizobium* form a specialized structure called root nodules on leguminous plants where symbiotic nitrogen fixation takes place. *Agrobacterium tumefaciens*, on the other hand, forms crowngall on many dicotyledonous plants after wound infection. Besides these similarities, relatedness between Ti plasmid of *Agrobacterium* and Sym plasmids of *Rhizobia* has been demonstrated[1]. DNA hybridization studies have shown that T-DNA homology of Ti plasmid is present in diverse species of *Rhizobium*[2]. Sym plasmids genes expression of *Rhizobia* in *Agrobacterium* and expression of Ti plasmid genes in *Rhizobium* has also been shown in many studies indicating close functional relationship[3-6]. In this communication, DNA hybridization experiments are described that demonstrate additional DNA homology between *Rhizobium meliloti*

and *A. tumefaciens* and the presence of *nif.* DNA in *A. tumefaciens* chromosome.

Rhizobium meliloti strain 102 F 34, *A. tumefaciens* C58 carrying pTiA$_6$NC, *Rhizobium trifolii* 162×68 and *Escherichia coli* HB101 (wild type) were used for total genomic DNA isolation. *A. tumefaciens* clones were CD-502 and CD-110. These clones carry chromosomal virulence (chv) genes of *Agrobacterium*[7]. *R. meliloti* clone was pRK-290.720; this clone carries *nif.* HDK region[8]. All clones were maintained in *E. coli* strain HB101 (pro, leu, thi, lacY, endoI, recA, hsd^R, hsd^M, str^R). *R. meliloti* gene bank used was constructed[9] in pRK-290.

Total genomic DNA from *Rhizobium*, *Agrobacterium* and *E. coli* was isolated and used for Southern blotting as described by Better *et al*[10]. Plasmid DNA was isolated by the procedure of Birnboim and Dolly[11] and nick translation was done following the procedure of Rigby *et al*[12]. DNA probes were hybridized overnight at 37°C in 5×SSC/45% formamide, 2 × denhardt solution, 200 μg/ml single stranded DNA carrier and 0.2% SDS for 16–20 h. Washing was done after hybridization in 2 × SSC (1 × SSC = 0.15 M sodium chloride/0.01—sodium citrate) and 0.1% sodium dodycyl sulphate.

DNA hybridization experiments were carried out using a 9.2 kb.KpnI fragment from CD-502 to fishout clones from *R. meliloti* gene bank constructed in pRK-290 by Ditta *et al*[9]. A clone designated as pRK 290.7 was identified besides the one described earlier[13]. Further studies on this clone revealed that 9.2 kb.KpnI fragment homology is contained in a 4.5 kb.XhoI fragment of pRK 290.7 (figure 1). In the next series of hybridization experiments, 4.5 kb XhoI fragment was used as probe and hybridized against the various digest of CD-110. The results showed that region of homology to 4.5 kb.XhoI fragment actually falls out side the *chv* region (figure 2). Further experiments, in which 4.5 kb.XhoI fragment was hybridized to different digest of pRK 290.720, revealed that this homology is contained in a 2.7 kb. Hind III plus Bgl II fragment of pRK 290.720 (figure 3). This region is covered by KDH genes of *R. meliloti*[8]. Thus the hybridization experiments demonstrate that (i) additional *nif* DNA homology is present in *R. meliloti* 102F34, (ii) this homology is also present in *A. tumefaciens* chromosome.

Hybridization studies using *nif* DNA from *Klebsiella penumoniae* had helped in identifying similar genes in *R. meliloti*[14] and it was shown that *Agrobacterium*

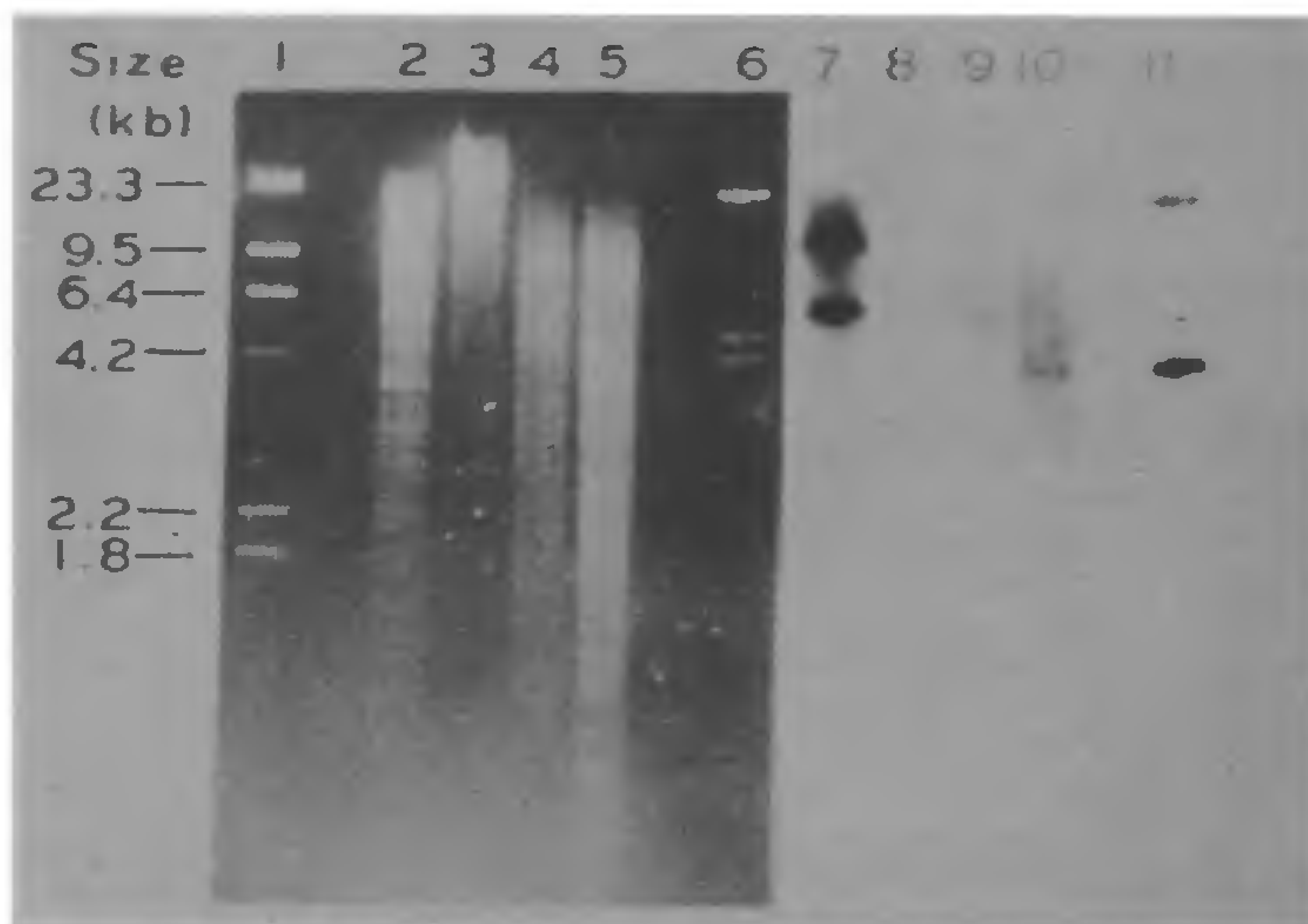

Figure 1. Hybridization of various DNAs to 9.2 kb.kpn I fragment of CD-502. **1.** Bacteriophage λ Hind III digest; **2–5.** XhoI digest of chromosomal DNA of *A. tumefaciens*, *E. coli*, *R. trifolii* and *R. meliloti* respectively; **6.** Xho I digest of pRK290.7; **7–11.** Autoradiogram of **2, 3, 4, 5** and **6** respectively.

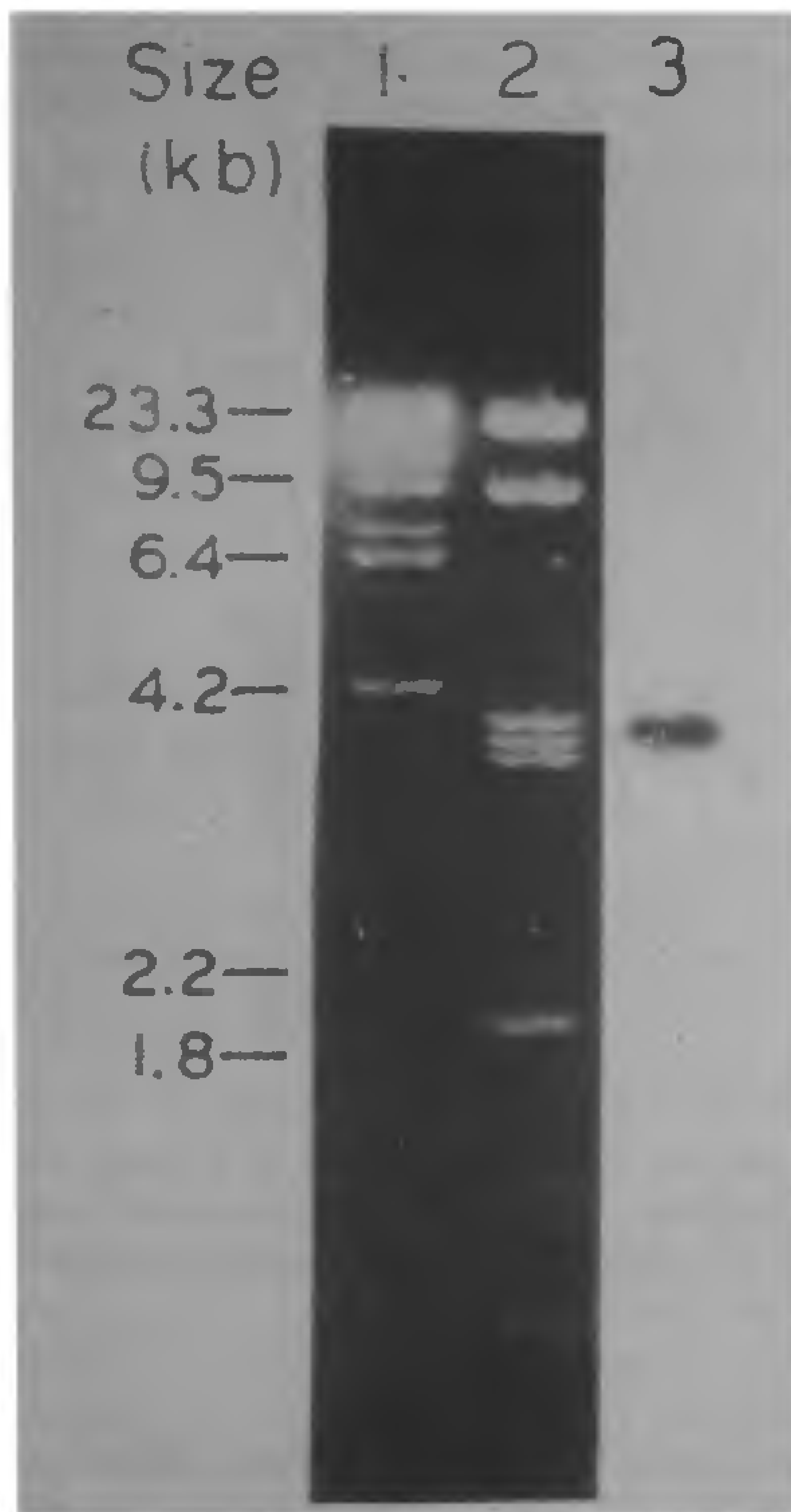

Figure 2. **1.** Bacteriophage λ Hind III digest; **2.** EcoRI digest of CD-110; **3.** Autoradiogram of 2 after southern transfer and hybridization to 4.5 kb. Xho I fragment of pRK 290.7.

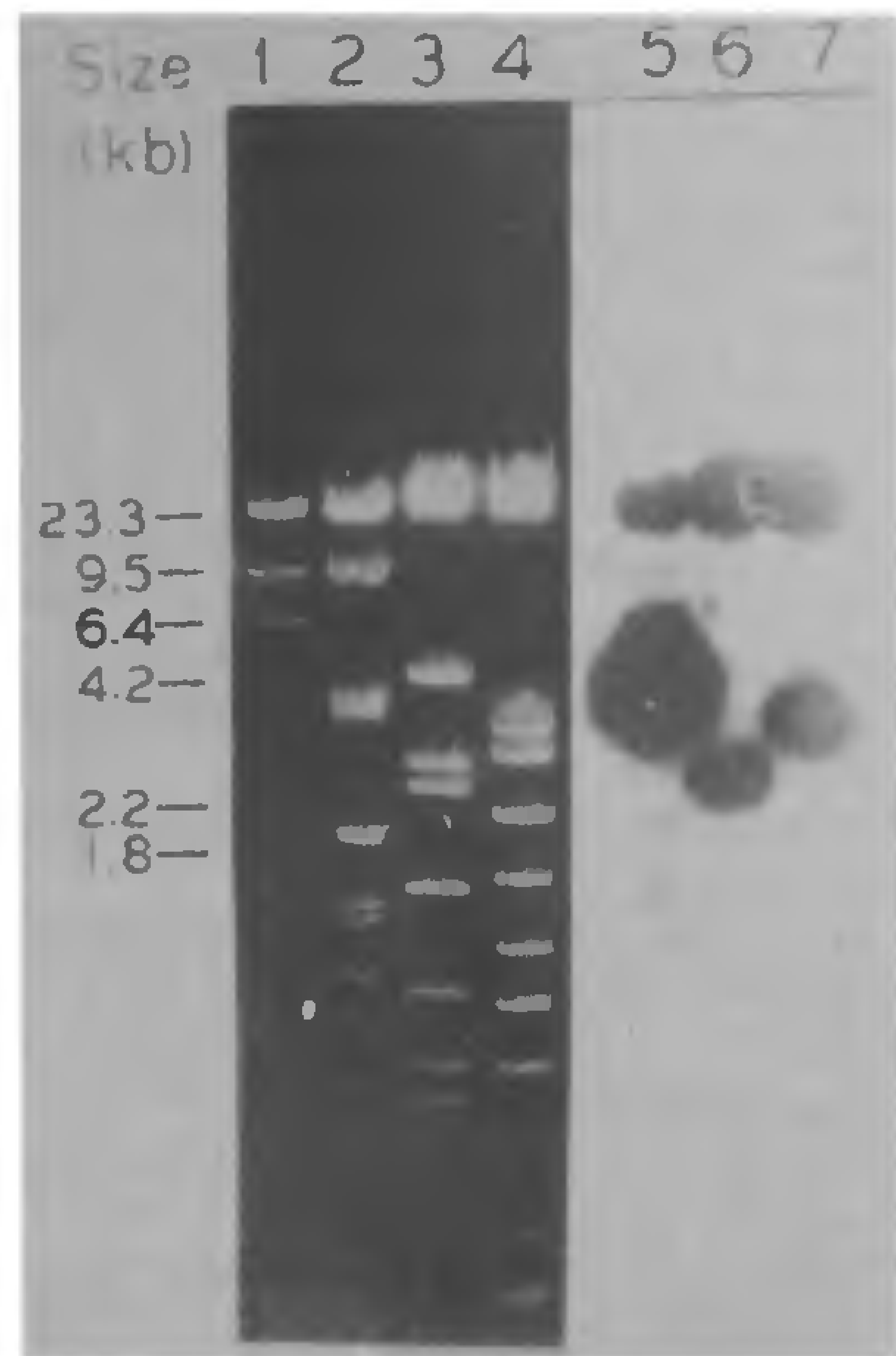

Figure 3. **1.** Bacteriophage λ Hind III digest; **2.** EcoRI digest of CD-110; **3.** Bgl II+Hind III digest of pRK 290.7?0; **4.** Bgl II+Xho I digest of pRK 290.720; **5–7.** Autoradiogram of **2, 3** and **4** after southern transfer and hybridization to 4.5 kb.Xho I fragment to pRK 290.7.

did not have *nif* KDH homologous DNA of *K. pneumoniae*. In the present study (figure 3) 4.5 kb.XhoI fragment of pRK 290.7 showed strong hybridization to 2.7 kb to 3.4 kb. fragments of pRK 290.720 (Lane 6 & 7 of figure 3) and to a 4 kb.EcoRI fragment of *Agrobacterium* (Lane 5) suggesting the presence of *nif* DNA homology in *Agrobacterium*. Vector hybridization to the probe in lane 5, 6 and 7 could be due to the impurity in the probe DNA.

The presence of additional *nif* DNA homology in *R. meliloti* (clone pRK 290.7) is interesting, though not surprising, since *R. phaseoli* is known[15] to have reiterated *nif* DNA and *R. meliloti* has shown gene product homology for nif N and *nif* K[16]. Whether the presence of additional *nif* DNA homology in *R. meliloti* is indicative of a new gene remains to be explored. Site specific mutagenesis in 4.5 kb.XhoI fragment of pRK 290.7 and its marker exchanging into *R. meliloti* chromosome will reveal its possible role in symbiotic process.

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1. Prakash, R. K. and Schilperoort, R. A., *J. Bacteriol.*, 1982, **149**, 1129.
2. Hadley, R. G. and Szalay, A. A., *Mol. Gen. Genet.*, 1982, **188**, 361.
3. Hooykaas, P. J. J., Klapwijk, P. M., Nuti, M. P., Schilperoort, R. A. and Rorsch, A., *J. Gen. Microbiol.*, 1977, **98**, 477.
4. Hooykaas, P. J. J., van Brussel, A. A. N., den Dulk-Ras, H., van Slogteren, G. M. S. and Schilperoort, R. A., *Nature (London)*, 1981, **291**, 351.

5. Hooykaas, P. J. J., den Dulk-Ras, H., Regensburg-Tuink, A. J. G., van Brussel, A. A. N. and Schilperoort, R. A., *Plasmid*, 1985, **14**, 47.
6. Martinez, E., Palacios, R. and Sanchez, F., *J. Bacteriol.*, 1987, **169**, 2828.
7. Douglas, C. J., Staneloni, R. J., Rubin, R. A. and Nester, E. W., *J. Bacteriol.*, 1985, **161**, 850.
8. Corbin, D., Ditta, G. and Helinski, D. R., *J. Bacteriol.*, 1982, **149**, 221.
9. Ditta, G., Stanfield, S., Corbin, D. and Helinski, D. R., *Proc. Natl. Acad. Sci. (USA)*, 1980, **77**, 7347.
10. Better, M., Lewis, B., Corbin, D., Ditta, G. and Helinski, D. R., *Cell*, 1983, **35**, 479.
11. Birnboim, H. C. and Dolly, J., *Nucleic Acids Res.*, 1979, **7**, 1513.
12. Rigby, P. W. J., Dieckman, M., Rhodes, C. and Berg, P., *J. Mol. Biol.*, 1977, **113**, 237.
13. Dylan, T. *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 1986, **83**, 4403.
14. Ruvkun, G. B. and Ausubel, F. M., *Proc. Natl. Acad. Sci. (USA)*, 1980, **77**, 191.
15. Martinez, E., Pardo, M. A., Palacios, R. and Cevallos, M. A., *J. Gen. Microbiol.*, 1985, **131**, 1779.
16. Mario Aguilar, O., Reilander, H., Arnold, W. and Puhler, A., *J. Bacteriol.*, 1987, **169**, 5393.

AMANITA PORPHYRIA—A NEW RECORD FOR INDIA

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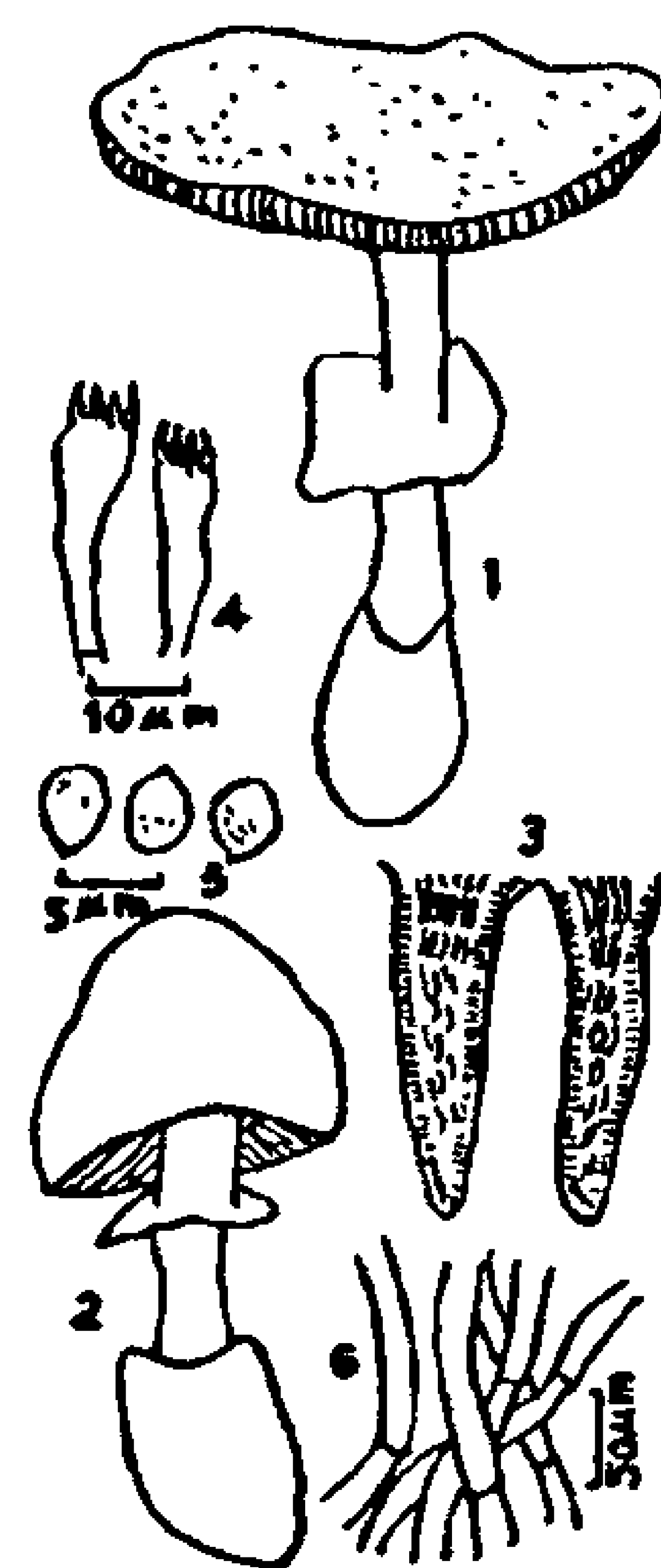
DURING the study on Agaricales of hills of Uttar Pradesh, many species of *Amanita* including poisonous forms have been recorded. Confusion with similar but edible fungi creates the danger of lethal mushroom poisoning. *Amanita porphyria* (Alb. & Schw) Fr. is suspected to be poisonous.[1] The fungus hitherto has remained unrecorded from India.[2-4]. The identification of the fungus was confirmed by the Commonwealth Mycological Institute, Kew, England (IMI 219653). The fungus is briefly described and illustrated here.

The young fungus in the initial stage is entirely covered by the universal veil and appears like a white egg. It is bell-shaped (figure 2) and on opening reaches a diameter of 12 cm. Pileus, 10–14 cm in height (figure 1), smoky brown, margin even, flesh thin, white. Gills, white, adnexed, close, medium in width, subventricose, thin (figure 3). Stipe, slender, 6–13 cm long, 1–2 cm diameter, soft, glabrous, hollow, whitish, with small bulb. Annulus, superior but distant, thin, membranous, white, pendent. Volva, flaccid, membranous, forming a thin cup, imbedded in the soil. Spores, spherical to ovate, 8–10 μm (figure 5), smooth, white. Basidia, clavate, four-spored, 20–30 × 6–8 μm (figure 4).



Figures 1–6. *Amanita porphyria*. **1.** Fruiting body; **2.** Unexpanded fruiting body; **3.** Section through gills; **4.** Basidia; **5.** Basidiospores, and **6.** Hymenophoral trama.

Habitat: Soil of Thalkedar Oak Forest, Pithoragarh, UP Hills, September 16, 1987.

The present collection shows close agreement with the description given by Kauffman[1] except for the somewhat larger size of stipe and of spores.

The authors thank Dr B. C. Sutton of CMI and Dr D. N. Pegler of Royal Botanic Gardens, Kew, England for confirming the identification.

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1. Kauffman, C. H. *The gilled mushrooms (Agaricaceae) of Michigan and the Great Lakes Region*, Dover