patula. The *Drosera* scheme (12 II + 12 I) and total sterility in the former, is not consistent with 36 II and reasonable fertility in the latter. Thus A genomes in *T. erecta* and *T. patula* do not appear to correspond exactly as is also clear from the total sterility in F_1 triploid hybrid. Obviously, the genomic formulae, are only approximations and do not indicate homology/non-homology in absolute terms[4]. It cannot be said with certainty if the divergence in the prototype *T. patula* took place subsequent to its origin or that some closely related species to *T. erecta* has been the source of its A genome. Thus, out of the three genomes of hexaploid (A_1A_1 ApAp BpBp), two, though related, are, however, not able to work harmoniously, and this amphiploid is a typical segmental allohexaploid in character[5] in that it possesses, apart from bivalents, a low multivalent frequency, partial fertility (Table I) and segregates genetically for parental characters due to intergenomal pairing. Obviously, such a condition is not stable and must segregate in the direction of auto- or allo- or stable segmental alloploidy. Thus, it must undergo a period of rigorous selection for fertility and stability of desired morphological attributes. This is possible only when heterogenetic associations get restricted due to loss of large duplicated loci. The recovery of about 17% diploids from the progeny of amphiploid *T. erecta-patula* is not an indication of the phenomenon of depolyploidy[2] as it is not a case of enbloc segregation of A_1 or Ap genomes, because the diploid progeny does not resemble diploid parental species in morphology or fertility.

The present results tend to indicate that hexaploidy may not be successful in marigolds. The highest level of ploidy reported in about 50% of the species[6] of the genus *Tagetes* is tetraploidy. Furthermore, the long course of domestication has not been able to establish hexaploidy in *T. erecta* and *T. patula* complex, although there must have been ample opportunities for the same during the course of domestication extending for over 400 years.

Our thanks are due to Mr. A. K. Sengupta and Mr. T. K. Sharma for illustrations.

1. Bolz, G., *Zeit. Pflanzenzucht.*, 1961, 46, 169.
2. Carlbom, C., *Hereditas*, 1969, 62, 437.
3. Kaplan, L., *Econ. Bot.*, 1960, 14, 200.
4. Khoshoo, T. N. and Arora, O. P., *Chromosoma*, 1969, 26, 259.
5. Stebbins, G. L., *Variation and Evolution in Plants*, Columbia Univ. Press, New York, 1950.
6. Towner, J. W., *Am. J. Bot.*, 1961, 48, 743.
7. Watt, G., *A Dictionary of the Economic Products of India*, London, 1893, 4 (3), 403.

SEROLOGICAL GROUPING OF THE INDIAN BACTERIOPHAGES OF *XANTHOMONAS ORYZAE* (UYEDA AND ISHIYAMA) DOWSON

ROHINI IYER
Division of Mycology and Plant Pathology, I.A.R.I., New Delhi
D. N. SRIVASTAVA
Indian Council of Agricultural Research, New Delhi
AND
B. M. GUPTA
Virology Division, C.D.R.I., Lucknow

THE importance of serological relationships in characterization and classification of bacteriophages including those affecting *Xanthomonas oryzae* has been emphasized by earlier workers[1-5]. In India, Rao[6] isolated phages for seventy-two isolates of *X. oryzae* collected from various parts of the country and grouped them into fourteen distinct strains based only on their physical properties and host range. The present work, therefore, was undertaken to study the serological behaviour of these phages.

Materials

Two phages, *viz.*, XOP_{11}[6], polyphagous bacteriophage of Indian origin and OP_2, a well-studied phage established by Japanese workers were used. Both these phages were grown on S_4 strain of *X. oryzae* (Cultures of the bacterium and phages were obtained from Dr. Y. P. Rao, I.A.R.I., New Delhi).

Preparation of High Titred XOP_{11} and OP_2 Phage Stocks

X. oryzae strain 4 was allowed to grow in P.G.S. broth having Peptone (10 g), Glutamic acid (0·5 g) and Sucrose (10 g) under aerobic conditions in a water bath (27°–30° C) provided with reciprocal shaker arrangements. When the population reached 5×10^7 cells/ml, approximately 500 phage particles each of XOP_{11} and OP_2 were added separately to each ml of the bacterial culture. The number of

plaque forming particles/ml was assayed by the double layer technique[7]. The turbidity of the bacterial culture was found to decline gradually reaching the maximum after 18 hr of incubation. The lysed culture (lysate) was centrifuged once at 1,500 rpm for 15 minutes and the supernatant was collected and kept at 40° C for a week. This was centrifuged again at 500 rpm for 5 minutes and the supernatant was stored in a deep freeze after checking the number of plaque forming units/ml. The antigens thus prepared were used for the preparation of antiserum.

Preparation of Antisera

Three rabbits (1·3 kg each) were inoculated by each of the two phages (XOP_{14} and OP_2) intravenously in ear veins at the rate of 2·5 ml of phage (10^{10} plaque forming units/ml). Seven injections were given on alternate days. Four days after the last injection, five ml of blood were collected from ear-marginal vein and heart of each rabbit. The blood samples, in petrolatum-lined centrifuge tubes, were left to clot at 37° C and then in a refrigerator overnight. The remaining fluid was centrifuged at 500 rpm for 5 minutes, the supernatant was held at 56° C for 10 minutes to remove non-specific inhibitors. This was then filtered through sterile sintered glass filters. The antiserum thus obtained was stored in screwcap vials at 4° C for further studies.

Spot Neutralization Test

Employing the antisera of XOP_{14} and OP_2, the serological relationship between these and five others, *viz.*, P_3, P_5, P_6, P_{11} and P_{12} (having narrow and wide host range) were tested. A mixture of 0·1 ml of phage suspension (250–300 particles/ml) and 1·0 ml of the host-bacterial suspension was added to 5 ml of molten PGSA medium (containing 1·5% agar) and poured over a primary layer of 2·0% plain agar in petriplates. After the top layer solidified, drops of specific antisera were placed, by means of a pipette, on previously marked places. In homologous systems, plaques failed to appear in the areas covered by the antiserum and instead, the bacterial growth occurred. The results are given in Table I.

From the results, it is apparent that all the six Indian phages tested were found to be serologically related to the OP_2 phage which is of Japanese origin. It is interesting to note that all the Indian phages, tested here, included strains with wide as well as narrow host-range. Yet it is difficult to exclude the possibility, of serotypes of OP_1, OP_1h and $OP_1\ h_2$ or any other hitherto unidentified phage of *X. oryzae* not occurring in India unless a large number of Indian isolates are examined.

Table I

Serological relationship amongst strains of phages of Xanthomonas oryzae

Phage strains	Reactions		
	Normal/ serum	OP_2 antiserum	XOP_{14} antiserum
*XOP_3	—	+	+
XOP_6	—	+	+
XOP_{10}	—	+	+
XOP_{11}	—	+	+
XOP_{12}	—	+	+
XOP_{14}	—	+	+
OP_2	—	+	+

+ Plaques failed to appear indicating +ve neutralization, — Plaques appeared indicating —ve reaction.

*XOP_3 was grown on S_3 strain of *X. oryzae* while the others were grown on S_4 strains in which both XOP_{14} and OP_2 phages produced smaller and solid plaques.

Another important information available from this study is that serological reactions of the phages are not related to their host-range. Between XOP_3 and XOP_{14} which are serologically alike, the former was specific to a single strains of *X. oryzae* whereas the latter had a very wide host-range, typing all the ten strains of the bacterium and even other species of *Xanthomonas*. Thus, serological typing of phages of *X. oryzae* alone would offer little prospect of tracing the source, origin and movement of the Indian strains of the bacterium from one rice locality to another or identify the distributional pattern of the bacterial disease in an epidemic year within the country. Lack of correlation between serological reactions and host-range specificity among wild type related even phages of *Escherichia coli* has been reported by Yadava, Chandra and Gupta[8].

The authors are grateful to Director, Central Drug Research Institute, and Head, Division of Mycology and Plant Pathology, for providing the necessary facilities. During the period of study, one of the authors (R. I.) was supported by a Fellowship of the C.S.I.R.

1. Wakimoto, S., *Sci. Bull. Fac. Agric. Kyushu, Univ.*, 1954, 14, 485.
2. — and Yoshii, H., *Ibid.*, 1955, 15, 161.
3. Goto, M., *Ann. Phytopath. Soc., Japan,* 1965, 30, 253.
4. Kuo, T. T., Huang, T. C., Wu, R. Y. and Yang, C. M., *Bot. Bull. Acad. Sinica,* 1967, 8, 216.
5. Singh, K. G., Uematsu and Wakimoto, S., *Ann. Phytopath. Soc., Japan,* 1970, 36, 56.
6. Rao, Y. P., *Ph.D. Thesis,* I.A.R.I., 1970.
7. Adams, M. H., *Bacteriophages,* Interscience Publishers, Inc., New York and London, 1959.
8. Yadava, J. N. S., Chandra, K. and Gupta, B. M., *Indian Journal of Experimental Biology,* 1970, 8, 201.