16. Subrahmanyam, B., Muller, T. and Rembold, H., *J. Insect Physiol.*, 1989, **35**, 493–500.
17. Rao, P. J. and Subrahmanyam, B., *J. Entomol. Res.*, 1987, **11**, 166.
18. Ayyangar, G. S. G. and Rao, P. J., *Indian J. Entomol.*, 1989, **51**, 373.
19. Ayyangar, G. S. G. and Rao, P. J., *Indian J. Entomol.*, 1990, **52**, 69.
20. Shashi Gupta and Rao, P. J., *Indian J. Entomol.*, 1990, **52**, 589.
21. Borst, D. W. and O'Connor, J. D., *Science*, 1972, **178**, 418–419.
22. Chang, E. S. and O'Connor, J. D., in *Methods of Hormone Radioimmunoassay* (eds Jaffe, B. M. and Behrman, H. R.), Academic Press, New York, 1977, pp. 797–812.
23. Kranti, K. R., Ph D thesis, P.G. School, IARI, New Delhi, 1991, p. 80.
24. Smith, S. L. and Mitchell, M. J., *Biochem. Biophy. Res. Commun.*, 1988, **154**, 559–563.
25. Zhu, X. X., *Contrib. Shangui Inst. Entomol.*, 1986, **4**, 65–69.
26. Tojo, S., Morito, M., Agni, N. and Hiruma, K., *J. Insect Physiol.*, 1985, **31**, 283–292.
27. Bidmon, H. J., Stumpf, W. E. and Granger, N. A., *Cell Tissue Res.*, 1991, **263**, 183–194.
28. Dominick, O. S. and Truman, J. W., *J. Expt. Biol.*, 1985, **177**, 45–68.

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Isozyme polymorphism in diploid and heat shock-induced tetraploid Indian major carp, *Labeo rohita* (Hamilton)

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Heat shock-induced tetraploids of *Labeo rohita*, an Indian major carp, were studied for esterase, glucose-6-phosphate dehydrogenase (G6PD) and xanthine dehydrogenase polymorphism involving different tissues. Polymorphic loci were identified in eye lens, kidney and skeletal muscle for G6PD and only in kidney for esterase of tetraploids. In general, intensification of isozyme bands was observed in tetraploids. The polymorphism in G6PD-1 and 2 locus in eye lens, G6PD-2 in kidney and G6PD-1 in skeletal muscle and EST-1 locus in kidney could be used as reliable marker in identifying tetraploid stocks from diploids.

Isozymes offer a potentially powerful and reliable tool in resolving genetic relatedness/divergence employing the degree of polymorphism of diverse alleles at different loci involved in translating specific enzymes and their varied multiple molecular forms, i.e. isomers. They have amply been used as molecular tags in genetic, phylogenetic, taxonomic, evolutionary studies and in strain or type identification[1–5].

Enzymes are mostly primary products of transcriptionally active genes and it is assumed that specific enzyme profile is the reflection of the genetic make-up of a given species and may be used as 'finger print' considering all other variables as constant. The present study was aimed at identifying any induced variation due to increased ploidy level (diploid to tetraploid) in three different enzyme profiles across diverse tissues and to assessing the feasibility of employing such altered profile (as marker) in identification of tetraploid fishes. Confirmation of tetraploid status has largely been based on chromosome counting[6], cellular volume measurement[7], DNA content[8] and protein electrophoresis[9].

Ploidy manipulation was found as a novel approach in altering diverse traits in fish system[8,10] and promising results in many cases encouraged us to undertake the same with *Labeo rohita* in the Central Agricultural Research Institute, Port Blair during 1993. Thermal shock-induced tetraploid stocks[11] were developed and reared under in house hatchery and in natural pond condition for evaluation and characterization. Specimens were collected from the polycraft pool and pond after eight months of rearing. After having tetraploid status confirmed (through chromosomal count following standard technique[12]), liver, kidney, eye lens and skeletal muscle were dissected out immediately on sacrificing the experimental fishes. The cell lysate was prepared in tissue homogenizing buffer[13]. Homogenates were loaded and electrophoresed following standard procedure[5] in a 5% native PAGE using discontinuous buffer system. Isozymes were detected through specific histochemical staining following Shaw and Prasad[14] for G6PD and Paul *et al.*[15] for esterase (EST) and xanthine dehydrogenase[10] (XDH). The mobility of individual band was calculated through the measurement of R_m values.

The profiles of all the three isozymes are presented in Figures 1–3 along with corresponding zymograms. The allellic expression of G6PD (E.C.1.1.1.49) could be distinctly grouped into two regions in liver and three regions in kidney of tetraploids whereas only two activity zones were found in diploids (Figure 1). The anodal band (locus-3) was a homozygous locus and represented by a single intensely stained band in tetraploids with slow mobility. In diploid, this locus was represented by light-stained band coupled with faster mobility. To the contrary, the presence of an additional zone of activity (locus-2) in the kidney was found to be unique in tetraploids. In the eye lens, G6PD was expressed in three distinct loci (locus-1, 2, 3) of tetraploids (Figure 1, lanes 10 & 11). Three alleles of heterozygous nature

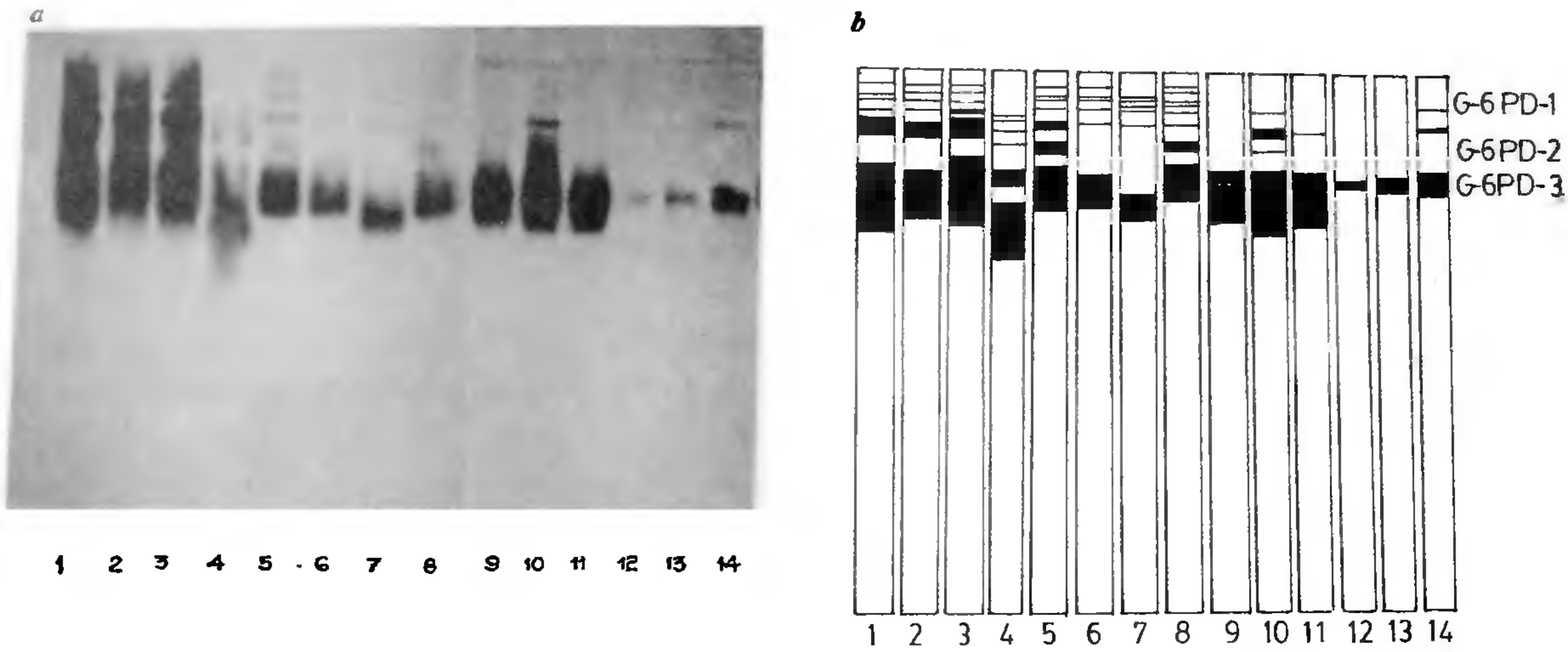

Figure 1. Glucose-6-phosphate dehydrogenase polymorphism in *L. rohita* (diploid and tetraploid). *a*, photograph; *b*, zymogram. Liver: lanes 1–3 (tetraploid), lane 4 (diploid); kidney: lanes 5, 6 & 8 (tetraploid), lane 7 (diploid); eye lens: lanes 10 & 11 (tetraploid), lane 9 (diploid); skeletal muscle: lanes 13 & 14 (tetraploid), lane 12 (diploid).

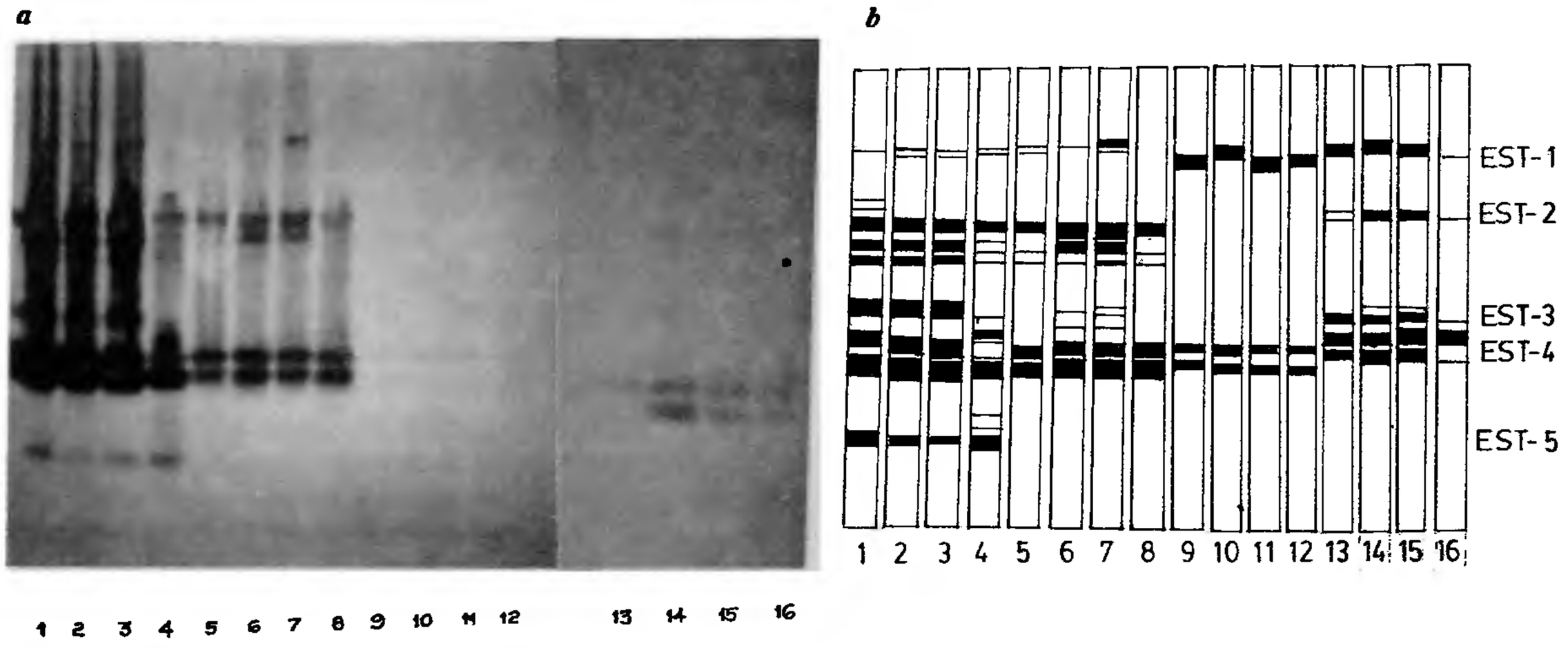

Figure 2. Esterase polymorphism in *L. rohita* (diploid and tetraploid). *a*, photograph; *b*, zymogram. Liver: lanes 1–3 (tetraploid), lane 4 (diploid); kidney: lanes 5–7 (tetraploid), lane 8 (diploid); eye lens: lanes 9–11 (tetraploid), lane 12 (diploid); skeletal muscle; lanes 13–15 (tetraploid), lane 16 (diploid).

characterized by one intensely stained band and two light bands (R_m 0.081–0.1) were found in the cathodic zone. This locus (G6PD-2) was specific to tetraploids. The anodal region, however, in both the diploids and tetraploids was represented by a single intensely stained band with R_m values of 0.298 and 0.317 respectively (Figure 1, lanes 9–11). In skeletal muscle, G6PD was expressed in one locus (locus 3) as a light band encoded by a single homozygous allele in diploids whereas in tetraploids an additional band in locus-1 (R_m values 0.08 and 0.093) having two alleles was expressed.

Information on G6PD polymorphism in fishes is scanty[17]. Involvement of one locus in G6PD expression in trout[18,19] has been reported earlier. Five bands of this isozyme have been observed in the brain and eye tissue of *Heteropneustes fossilis* with identical electrophoretic mobility[16]. In the present study, three loci could be identified for G6PD which expressed across different tissues. G6PD activity in the liver, kidney, eye lens and skeletal muscles of *L. rohita* diploids and tetraploids was found variable and distinctly a tissue-specific pattern was observed.

The esterase (E.C.3.1.1.1) activity at different loci in

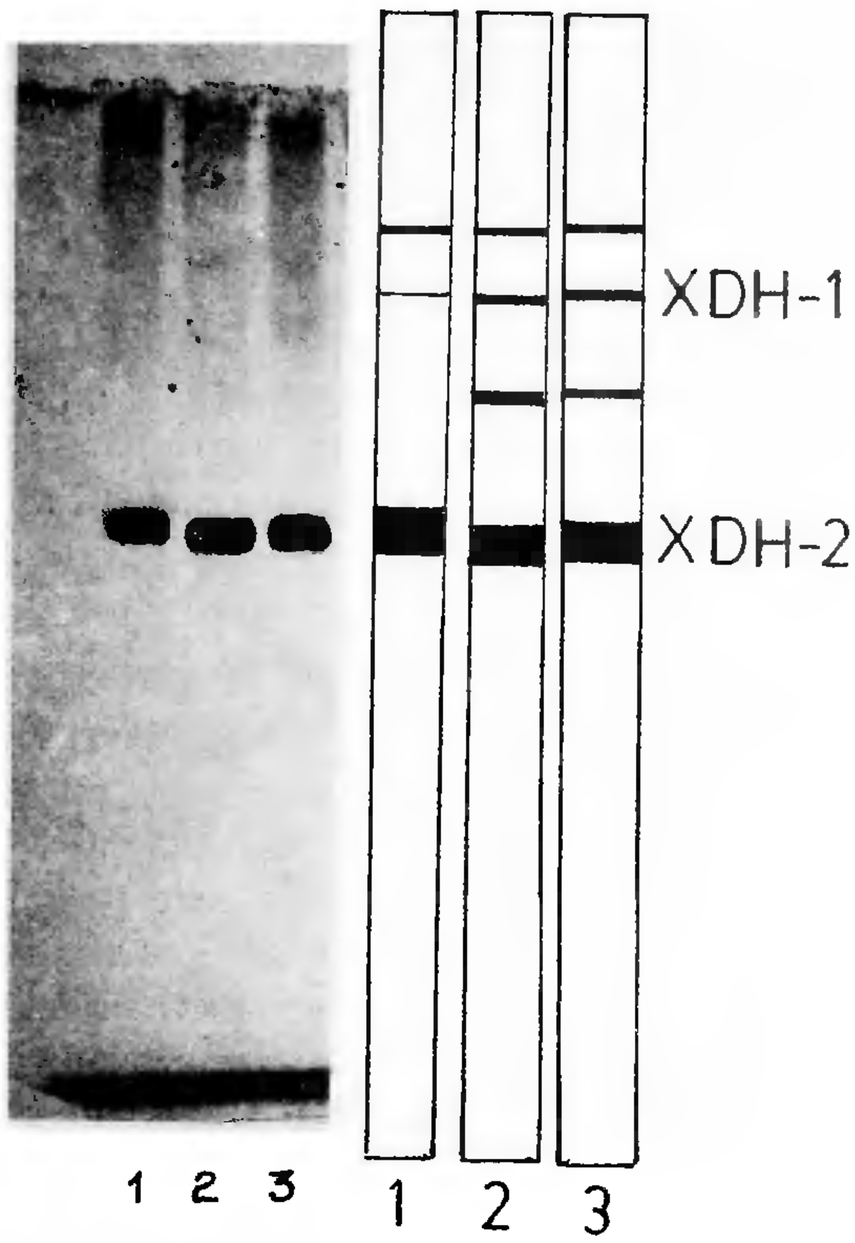

Figure 3. Xanthine dehydrogenase polymorphism in *L. rohita* (diploid and tetraploid). Liver: lanes 2 & 3 (tetraploid), lane 1 (diploid).

liver is well-recognized with diverse R_m values (0.17–0.84), e.g. five zones in liver, four zones in kidney, two zones in eye lens and three zones in skeletal muscles (Figure 2). Unlike diploids, the esterase activity in liver of tetraploids had five zones with intense staining (R_m 0.18, 0.3–0.43, 0.53, 0.60–0.67 and 0.83). Est-1 was found expressed through two alleles in all except in one specimen. In all the tetraploids, Est-3 was represented by three intensely stained bands and Est-4 (R_m value 0.4) was found to be unique for tetraploids coded by a single allele with high enzyme expression. The anodal Est-5 of these specimens were found to be highly active zones compared to diploids (Figure 2, lanes 1, 2 & 3). In kidney, the enzyme activity which was faintly visible at Est-1 and Est-3 loci of diploids (Figure 3, lane 8) was found to be more or less clearly visible zones (still less active regions) in tetraploids, maybe coded by alleles translating less amount of enzyme activity. The isozyme in eye lens of tetraploids and diploids did not show any variation as represented by light bands at Est-1 and Est-5 (Figure 2, lanes 9–12). In the skeletal muscle, esterase activity was expressed in three loci (Est-1, 3 and 5). The allelic expression in tetraploids was characterized with three alleles and one additional less active region (Figure 2, lanes 14 & 15) at the anodal locus (Est-5). Whereas in diploids extremely low activity was observed at Est-1 and 2 (Figure 3, lane 16).

Esterase in all the tissues of tetraploids was found to express intensely stained bands when compared to the diploids. In locus-3 and locus-5 of liver and kidney, the activity in tetraploids was well marked, being coded by three and two alleles respectively, whereas the activity was very much low for diploids. The presence of highly active Est-4 in liver and Est-1 in muscle could be the characteristic expression of enzyme in tetraploids.

XDH (E.C.1.1.1.204) was expressed at two loci in both diploids and tetraploids. The intense anodal bands of homozygous nature were clearly marked in all individuals (R_m 0.44, 0.45) but the migration of these bands in tetraploids was comparatively faster. The cathodal XDH-1, characterized with light bands (R_m 0.197–0.296), was heterozygous and less expressive in nature. The activity of XDH-1 locus in diploid specimens (lane 1) was almost negligible in comparison to tetraploids (lanes 2 & 3). The phenotypic expression of XDH with a single intensely stained band in liver corroborated the previous observation[20] and the presence of three faintly stained bands in tetraploid liver tissue, reported here for the first time, could be used as a marker in identifying tetraploids.

The present study was undertaken to assess the possibility of using altered isoenzyme profile as a genetic marker in identifying tetraploid fishes. G6PD-1 and G6PD-2 of eye lens (R_m values of 0.081 and 0.096), G6PD-1 of skeletal muscle (R_m values 0.08 and 0.94) and G6PD-2 of kidney (R_m value 0.176) were found as distinct marker bands in tetraploids.

In esterase, more intensely stained bands in different loci of tetraploids perhaps indicative of increased gene dose due to gene duplication[13] and absence of some less active region, might be an epigenetic modification or post-translational modifications. Similar observations were also reported for malate dehydrogenase in herring[13]. XDH in tetraploids was characterized mostly through fast mobility of the intensely stained single band (locus-2).

Appearance of additional bands in tetraploids as against diploids may be assumed due to gene duplication and thereby enhanced enzyme expression in stainable and delectable quantum which probably could have failed to be stained due to negligible amount in diploids. Formation of heteropolymeric isozyme due to hybridization of protein products of separate loci may be another plausible explanation for development of new bands with entirely different R_m values. It is maintainable that hybrid polymorphs have been reported in many fish species and in polyploid organisms that have undergone extensive gene duplication[5]. The chromosomal duplication achieved through ploidy manipulation was well-expressed in manifesting the structurally polymorphic isozymes in fish.

In conclusion, G6PD-1 and 2 in eye lens, G6PD-1 in skeletal muscle, G6PD-2, Est-1 in kidney, Est-4 and

XDH-1 and 2 in liver could be used as reliable marker with ease and confidence in identifying tetraploid stock of *L. rohita*.

1. Moss, D. W., *Isoenzymes*, Chapman and Hall, London, 1982, pp. 204.
2. Callegarini, C. and Basaglia, F., *Bull. Zool.*, 1978, **45**, 35–40.
3. Basaglia, F. and Callegarini, C., *Comp. Biochem. Physiol.*, 1988, **B89**, 731–736.
4. Mc Andrew, B. J. and Mazumder, K. C., *Aquaculture*, 1983, **30**, 249–261.
5. Richardson, B. J., Baverstock, P. R. and Adams, M., *Allozyme Electrophoresis*, Academic Press, New York, 1986.
6. Stanley, J. G., *Rapp. P. V. Reun. Cons. Int. Explr. Mer.*, 1981, **178**, 485–491.
7. Allen, S. K. Sr. and Stanley, J. G., *Trans. Am. Fish Soc.*, 1979, **108**(5), 462–466.
8. Thargaard, G. H., *Fish Physiology* (eds Hoar, W. S. Randall, D. J. and Donaldson, E. M.), Academic Press, New York, 1983, pp. 405–434.
9. Allen, S. K. Jr. and Stanley, J. G., *Coop. Res. Rep. Int. Conne. Explor. Sea Ser.*, 1981, **B28**, 1–28.
10. Chourrout, D., *Genetic Manipulation in Fish*, Tiews K. Bundersforschungsanct Tisch Hamburg, 1978, pp. 2111–2127.
11. Sarangi, N. and Mandal, A. B., *The Nucleus*, 1994, **87**(122), 62–66.
12. Reddy, P. V. G. K. and John, G., *Aquacult. Hurg.*, 1986, **5**, 31–36.
13. Pasteur, N., Pasteur, G., Benhorame, F., Catalan, J. and Britton-Davidian, J., *Practical Isozyme Genetics*, 1988, pp. 1–53.
14. Shaw, C. R. and Prasad, R. *Biochem. Genet.*, 1970, **4**, 297–320.
15. Paul, B. A., Gary, A. W., David, J. T., George, B. M. and Tred, M. U., NOAA Technical Report, 1987, NMFS 61.
16. Chatterjee, K., *Threatened Fishes of India*, 1994, **4**, 327–335.
17. School, A., *Genetics and Mutagenetics in Fishes* (eds Schroeden, H.), Springer, Berlin, 1973, pp. 277–335.
18. Yamauchi, T. and Goldberg, E., *Biochemistry*, 1973, **10**, 123–299.
19. Deibig, E., Meyer, J. N. and Glodek, P., *Biochem. Genet.*, 1979, **10**, 165–174.
20. Padhi, B. K. and Khuda Bukhush, A. R., *Biochem. System. Ecol.*, 1990, **18**(5), 381–386.

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Molecular genetic diversity among soybean plant introductions with resistance to *Heterodera glycines*

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Restriction fragment length polymorphisms were used to estimate the genetic diversity among 29 soybean (*Glycine max*) accessions with resistance to cyst nematode (*Heterodera glycines*). Based on the common marker alleles, both the analyses cluster and principal component have separated the resistant soybean accessions into several groups. Several accessions with known resistance to some races were also found to be resistant to additional nematode races in this research.

In USA, *Heterodera glycines* Ichinohe (soybean cyst nematode) parasitism is a major limiting factor of soybean (*Glycine max* L. Merr.) production. This nematode was first discovered in USA in 1954 and has since been found in 27 states. In 1994 the estimated soybean yield losses were valued at 115 million dollars for 16 southern states[1].

The management of *H. glycines* primarily relies on the use of resistant cultivars of soybean. Most cultivars are resistant to one or two nematode race isolates and the current classification system includes 16 different race isolates[2]. The modern soybean gene pool for resistance is generally regarded to be genetically very narrow, mainly because the introgression of resistance genes from the available sources is restricted to either cv. Peking or PI88788 or both. The narrow genetic base of the resistance sources used in cultivars has been causing shifts in *H. glycines* populations favouring development of more aggressive races and the resistance has not been durable. Use of non-allelic genes, i.e. genes located at different loci for resistance will produce more durable resistance in soybean cultivars.

Presently, 118 soybean accessions are available, which are resistant to *H. glycines*. Some of them were reported to carry non-allelic genes for resistance[3,4], but they have not been utilized in breeding programmes, except for a few resistance genes from PI437654 soybean. This line is resistant to race isolates[5] 1, 2, 3, 5, 6, 9 and 14. Prior knowledge of the genetic relationships among the accessions would facilitate development of resistance genes to improve genetic diversity and gene pyramiding. Traditional techniques do not offer adequate tools for establishing these relationships.

Restriction fragment length polymorphism (RFLP) markers has been widely accepted for genetic analysis and varietal identification by DNA 'fingerprinting'. Genetic relationship on the basis of single-copy RFLP markers has been reported for several crop species[6,7], including soybean[8]. All of these investigations pertain to crop cultivars with known pedigrees.

We are not aware of any report in the literature on the evaluation of genetic relationships among soybean accessions with unknown pedigrees having resistance to *H. glycines*. In the study described here, we surveyed 29 resistant accessions and two susceptible cultivars of soybeans using RFLPs to obtain information on their genetic diversity and relationships. A brief summary of this research has been reported in the *Agronomy Abstracts*[9].

For this study, seeds from 29 soybean accessions with resistance to *H. glycines* were obtained from R. L.