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.

ACKNOWLEDGEMENTS. We thank Dr A. Bondyopadhyay, Director, CARI, Port Blair, for his keen interest, constant inspiration and facilities provided.

Received 2 April 1996; revised accepted 27 June 1996

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

A. P. Rao Arelli and D. M. Webb*

Department of Agronomy, University of Missouri, Columbia, MO 65211, USA

*Pioneer Hi-Bred International Inc., Johnston, IA 50130, USA

Contribution from the Missouri Agric. Exp. Stn Journal Series No. 12,435.

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.

Nelson, Curator, USDA-ARS, Soybean Germplasm Collection. These resistant accessions represent samples from five countries: Argentina, China, Japan, Russia and South Korea. Two susceptible controls, cv. Essex and cv. Hutcheson were also included in the study.

Near-homogeneous populations of *H. glycines* were developed for each of the five races 1, 2, 3, 5 and 14 based on the methods described previously[10]. Bioassays were performed for each of the five races based on the established procedures[3]. In brief, each soybean seedling was grown in a single polypropylene micropot (200 × 25 mm) filled with steam pasteurized Brosely fine sandy soil. Approximately 20 of these micropots were placed in a 20 cm diameter polypropylene container and maintained at 27 ± 1°C in thermoregulated waterbaths (Forma Scientific Inc., Marietta, OH, USA).

White and light yellow females of a given race of *H. glycines* were chosen selectively and were crushed to release eggs and larvae. Each seedling was inoculated with 1250 ± 25 eggs and larvae using an automatic pipetter (Brewer Automatic Pipetting Machine, Scientific Equipment Products, Baltimore, MD, USA). A total of 40 seedlings were inoculated for each race. The method of inoculation was already described[11].

Approximately 30 days after inoculation, plant roots were individually washed with a strong jet of water to dislodge *H. glycines* females and counted under a stereomicroscope. Resistance was determined for each soybean accession and for each of the five races, based on an index of parasitism.

Total genomic DNA was isolated from greenhouse-grown plants of each resistant accession and for each of the two susceptible controls. DNA extracts were obtained according to Keim and Shoemaker[12]. Purified DNA was quantified using a Beckman DU-65 spectrophotometer and UV quantitation of DNA method according to Sambrook *et al.*[13] Samples of genomic DNA were individually digested with restriction endonucleases *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Taq*I.

Established procedures were employed for gel electrophoresis, Southern blotting and hybridization with the exception that the hybridizations were conducted using 25–35 ml of hybridization solution at 65°C in glass tubes that were rotating on a rotisserie (Robbins Scientific Corporation, Sunnyvale, CA, USA).

The 32 probes used in the study were primarily single-copy DNA sequences and random clones from a genomic library[12] cloned into the *Pst*I site of the pBS^+ vector and were transformed into DH5 α strain of *E. coli*. The clones were prepared for radiolabelling by first amplifying the inserts via polymerase chain reaction (PCR) using oligonucleotides of the T_3 and T_7 promoter regions of the phagemid vector $pBS^{\pm}$ as amplification primers. The amplification was based on the established procedures[14]. The PCR amplified inserts were radiolabelled with ^{32}P using random priming reactions according to Feinberg and Vogelstein[15].

On a marker basis we have calculated polymorphism index $= 1 - \Sigma pi^2$, where p is the allele frequency for i alleles 1 to N. On a probe basis, the polymorphic index $= 1 - \Sigma\,\Sigma pi^2$ where different polymorphic loci are summed.

Only the fragments that were polymorphic among accessions and could be clearly scored were used in the data analysis. All bands having equivalent migration distance were given the same letter score. Genetic distances (GD_R) among all possible pairs of accessions were estimated from a modification of Nei's similarity equation[16] as used by Keim *et al.*[8] in soybean. The proportion of similar RFLP loci, Sxy, between pairs of PI lines was estimated as $2Nxy/(Nx + Ny)$, where Nxy is the number of RFLP loci for which PI lines X and Y possess the same allele, Nx is the number of alleles identified in line X and Ny is the number of alleles identified in line Y. GD_R was calculated as $1 - Sxy$. Based on the GD_R matrix (data not presented), a dendrogram was generated to graphically display the calculated distances between genotypes (Figure 1). Cluster diagrams were constructed using the average linkage

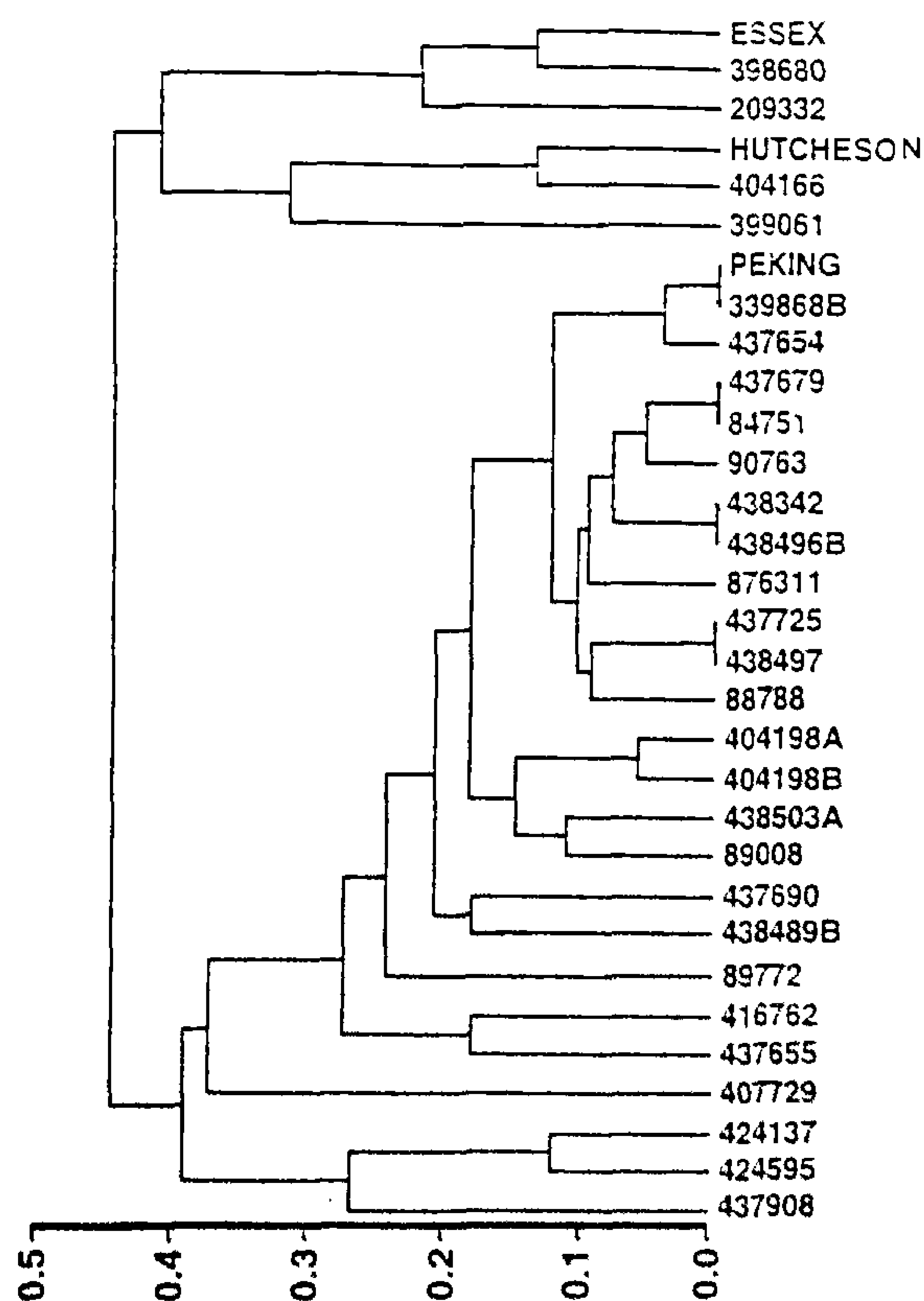

Figure 1. Dendrogram of soybean PIs and cultivars obtained from an analysis of 32 probes. Genetic distances calculated from RFLP values were analysed by average-linkage clustering. The scale on the dendrogram represents the degree of divergence

Table 1. Reaction* and index of parasitism** of soybean PI lines to *H. glycines* race isolates 1, 2, 3, 5 and 14

Accession	Race I	Race II	Race III	Race V	Race XIV
PI398680	MS (56.0)	S (78.6)	MR (26.2)	S (67.5)	MS (42.5)
PI209332	MS (47.0)	S (70.0)	R (4.0)	R (7.0)	R (5.0)
PI404166	R (1.0)	R (6.0)	R (0.6)	R (0.4)	MR (10.0)
PI399061	MS (38.0)	MS (41.7)	MR (33.3)	R (7.8)	S (63.7)
Peking	R (1.8)	MR (19.7)	R (1.5)	R (0.9)	MR (28.1)
PI339868B	R (2.0)	MR (20.0)	R (0.8)	R (1.7)	MR (12.6)
PI437654	R (0.2)	R (1.0)	R (0.3)	R (0.4)	R (0.3)
PI437679	MR (22.0)	MR (14.3)	R (4.0)	R (0.1)	R (2.2)
PI84751	R (3.2)	MR (28.0)	R (0.2)	R (1.4)	MR (14.0)
PI90763	R (7.9)	R (2.3)	R (0.4)	R (0.1)	MR (26.8)
PI438342	MS (38.0)	MR (21.6)	MS (41.7)	R (1.7)	MS (32.3)
PI438496B	MR (30.0)	S (96.0)	R (2.3)	MS (35.0)	MS (41.0)
PI876311	MS (38.0)	S (67.3)	R (9.0)	MR (16.6)	MR (10.9)
PI437725	R (0.6)	MR (18.7)	R (0.4)	R (2.4)	MS (45.7)
PI438497	R (5.0)	MR (17.0)	R (0.4)	R (2.2)	MR (29.0)
PI88788	MS (39.0)	S (77.2)	R (5.4)	MS (46.0)	R (2.4)
PI404198A	R (0.7)	R (8.5)	R (1.4)	R (1.0)	MS (54.6)
PI404198B	R (2.0)	MS (44.2)	R (1.7)	R (1.4)	MR (23.6)
PI438503A	MS (44.0)	MS (57.0)	R (2.9)	MR (22.1)	R (9.0)
PI89008	MS (35.0)	S (66.4)	MR (17.9)	MR (17.8)	MR (17.8)
PI437690	R (0.8)	R (7.1)	R (0.0)	R (1.7)	MR (25.0)
PI438489B	R (0.4)	R (4.5)	R (0.6)	R (1.1)	R (8.0)
PI89772	R (1.5)	R (3.5)	R (0.2)	R (0.8)	MR (13.6)
PI416762	MR (16.0)	MS (47.1)	R (6.5)	R (5.7)	R (8.1)
PI437655	R (1.0)	S (69.0)	R (0.6)	R (3.0)	MR (11.5)
PI407729	MR (11.0)	MS (46.5)	MR (14.5)	R (6.3)	R (4.5)
PI424137B	S (61.0)	S (88.0)	MR (34.9)	MR (11.8)	MR (27.5)
PI424595	MS (58.0)	MS (45.2)	S (95.2)	R (9.9)	S (64.9)
PI437908	S (74.0)	S (80.9)	S (95.2)	MS (54.8)	S (84.7)
cv. Hutcheson	S (100)	S (100)	S (100)	MS (100)	S (100)

*0–9% = Resistant; 10–30% = moderately resistant; 31–60% = moderately susceptible; ≥ 60% = susceptible.

$$**\text{Index of parasitism} = \frac{\text{Average number of females per PI line}}{\text{Average number of females per cv. Hutcheson}} \times 100.$$

cluster analysis (Statistical Analysis Systems, Cary, NC) on the distance matrices.

The principal component analysis (PCA) was done by first calculating a correlation matrix among the markers. Eigenvalues and eigenvectors were then obtained from the correlation matrix and these were used to calculate the coordinates of each accession. The accessions were then plotted on the basis of these coordinates (data not presented).

The reaction of PI lines to races of *H. glycines* is reported in Table 1. Results indicate that several PI lines with known resistance to some races were also found to be resistant to additional nematode races (Table 1). For example, PI438489B was resistant to races 3 and 5 and in this study it was found additionally resistant to *H. glycines* races 1, 2 and 14. Of the 32 probes examined, 13 were polymorphic producing two to nine restriction fragment bands. We found that 40% of the probes detected variation among the 29 resistant and two susceptible soybean genotypes.

Both cluster and principal component analyses separated the accessions and cultivars into several groups. Cluster analysis has grouped genotypes based on the proportion of marker alleles that accessions and cultivars have in common. Genotypes that have a high proportion of alleles in common included 'Peking' and PI339868B; PI437679 and PI84751; PI438342 and PI438496B; and PI437725 and PI438497 (Figure 1). These pairs are distinguishable but very closely related based on the RFLP data.

Based on both the cluster analysis and the principal component analysis (data not shown) most widely used genetic sources for *H. glycines* resistance have 'tight' grouping or clustering. These included Peking, PI88788, PI90763, and PI437654. A few of the resistance genes in both the accessions PI437654 and Peking have been recently mapped using RFLPs[17,18]. Genetic analyses have indicated that very few non-allelic genes exist among these sources of resistance[19], and their indiscriminate use in cultivar breeding appeared to have contributed to their genetic vulnerability. More virulent race populations of *H. glycines* are being reproduced which continue to infest the resistant cultivars.

Some PI lines with multiple race resistance that were genetically distant from the previously used sources were identified in this study (Table 1). These included

PI438489B, PI404198B, PI438503A, PI89772, PI404166, PI437908, PI209332 and PI437655. The resistance genes in these PIs will be mapped.

The results showed that greater diversity for markers existed among the PIs than the two cultivars used in this study. The two cultivars Essex and Hutcheson were also clustered away from the PIs in the dendrogram (Figure 1). This is expected, because the PIs are in general a diverse group of accessions collected in Asia and South America, whereas the cultivars were derived from a limited number of PIs introduced in USA at the turn of the century. In this study, no relationship was found in general, between geographical origin of PIs and the clusters obtained based on the limited number of probes used, but some degree of relationship was observed for spectrum of resistance. Both PI424595 and PI437908 susceptible to races 3 and 14 which clustered at the lower end of dendrogram have originated from South Korea.

Our study has demonstrated that an analysis of RFLP markers can be used to determine genetic relationships among PI lines of soybean. In the absence of typical pedigree information, DNA fingerprinting should be most useful in establishing their genetic relationship to develop appropriate populations for gene mapping studies.

1. Wrather, J. A., Chambers, A. J., Fox, J. A., Moore, W. F. and Sciumbata, G. L., *Plant Dis.*, 1995, **79**, 1076.
2. Riggs, R. D. and Schmitt, D. P., *J. Nematol.*, 1988, **20**, 392.
3. Rao Arelli, A. P. and Anand, S. C., *Crop Sci.*, 1988, **28**, 650.
4. Rao Arelli, A. P., Anand, S. C. and Wrather, J. A., *Am. Soc. Agron. Abst.*, 1991, 113.
5. Rao Arelli, A. P., Wrather, J. A. and Anand, S. C., *Plant Dis.*, 1992, **76**, 894.
6. Miller, J. C. and Tanksley, S. D., *Theor. Appl. Genet.*, 1990, **80**, 437.
7. Kesseli, R., Ochoa, O. and Michelmore, R., *Genome*, 1991, **34**, 430.
8. Keim, P., Beavis, W., Schupp, J. and Freestone, R., *Theor. Appl. Genet.*, 1992, **85**, 205.
9. Rao Arelli, A. P. and Webb, D. M., *Am. Soc. Agron. Abst.*, 1993, 182.
10. Anand, S. C. and Rao Arelli, A. P., *Crop Sci.*, 1989, **29**, 1181.
11. Rao Arelli, A. P., Matson, K. W. and Anand, S. C., *Plant Dis.*, 1991, **75**, 594.
12. Keim, P. and Shoemaker, R. C., *Soybean Genet. Newsl.*, 1988, **15**, 147.
13. Sambrook, J., Fritsch, E. F. and Maniatis, T. (eds), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY, 1989, pp. E.5.
14. Anonymous, Univ. of Missouri Maize RFLP Procedures Manual, 1995, p. 25.
15. Feinberg, A. P. and Vogelstein, B., *Anal. Biochem.*, 1983, **137**, 266.
16. Nei, M. and Li, W. H., *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5256.
17. Webb, D. M., Baltazar, B. M., Rao Arelli, A. P., Schupp, Jr., Clayton, K., Keim, P. and Beavis, W. D., *Theor. Appl. Genet.*, 1995, **91**, 574.
18. Qiu, B., Rao Arelli, A. P. and Sleper, D. A., *Am. Soc. Agron. Abst.*, 1995, 177.
19. Rao Arelli, A. P., Anand, S. C. and Wrather, J. A., *Crop Sci.*, 1992, **32**, 862.

ACKNOWLEDGEMENTS. We thank Dr R. C. Shoemaker, USDA-ARS, Ames, IA for providing DNA probes used in this study and Dr Pat Byrne, USDA-ARS, Columbia, MO for scientific review. This research was supported by grants from Missouri Soybean Merchandising Council 9510176-1 and USDA-CSRS-93-34113-8498.

Received 12 January 1996; revised accepted 3 July 1996

Aeolian deposition of Arabia and Somalia sediments on the southwestern continental margin of India

Onkar S. Chauhan

Geological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa 403 004, India

Kaolinite, smectite, illite and chlorite as major clay minerals and palygorskite and gibbsite in minor quantities have been recorded from the slope of southwestern continental margin of India. Contribution of kaolinite, smectite and gibbsite is from peninsular India through fluvial discharge. Since formation of palygorskite calls for an arid and hot climate and saline conditions, occurrence of this clay mineral in the sediments of the study area documents aeolian sediment contribution from Arabia and Somalia by the Arabian northwesterly winds.

STUDIES of marine clays are a significant tool to determine sources, sediment dynamics and environment of deposition[1–4]. The climate and geology of the source area[3] largely dictates the type of clay species supplied. By and large, characteristic clay minerals of different climatic and geological settings have been identified[1,3].

Studies of clay mineral variations in the western continental margin of India mainly suggest two important sources of the clays. Illite and chlorite are reported to be mostly contributed by the Indus River and the low salinity Bay of Bengal Waters (BBW), intruding into the southwestern continental margin during November–January[5-11]. Kaolinite, gibbsite and smectite (and minor amounts of illite) are produced due to intense chemical weathering of Indian subcontinent, and are contributed from the adjacent landmass[5-7]. Two contrasting opinions exist about the dispersal of these clay minerals. Ramaswamy and Nair[9] have reported a lack of cross-shelf sediment transport and have suggested an along shelf transport of the sediments brought by the major fluvial