Journal of Tropical Agriculture 38 (2000) : 9-14

ISOENZYME VARIATION IN *PIPER NIGRUM* L.

Abraham Sebastian, V. S. Sujatha, E.V. Nybe, G. Sreekandan Nair and V. K. Mallika
College of Horticulture, Trichur 680 656. Kerala, India

Abstract: Isoenzyme patterns of 26 varieties of *P. nigrum* were compared for three enzymes viz., esterase, peroxidase and glutamate oxaloacetate transaminase. Similarity among the varieties ranged from 0.40 - 1.00. Wide variation was observed among the cultivated types of *P. nigrum* for the three enzymes analysed.

Key words: Isoenzymes, *Piper nigrum*, similarity index

INTRODUCTION

Black pepper, *Piper nigrum* is the most important spice crop of India. It originated in the Western Ghats. A large number of varieties / types of *Piper nigrum* are being cultivated. High degree of variability is reported in morphological characters among the cultivated types of *Piper nigrum* (Kanakamani, 1985; Ravindran and Babu, 1994). Isoenzymes have been used for characterization and evaluation of variability in many crop plants (Feldmann, 1985; Valizadeh, 1977; Sujatha *el al.*, 1991). Studies on isoenzyme variation have not been attempted among *Piper nigrum* cultivars so far. The present study aims to find out the variability within *P. nigrum* at isoenzyme level.

Table 1. *P. nigrum* cultivars / varieties included in the study

Sl.No.	Name of variety / cultivar	Place of collection	Remarks
1	Kalluvally Type 1	Thaliparamba	
2	Kottanadan	PRS, Panniyur	
3	Karimkotta	Vilakode	
4	Neelamundi Type 2	PRS, Panniyur	
5	Perumunda	Travancore	
6	Arakkulamunda	Kottayam	
7	Veluthanamban	Thodupuzha	
8	TMB-2	Thaliparamba	
9	Doddiga	Mysore	
10	Shimoga	Mysore	
11	Malligesera	Mysore	
12	Ceylon	Sri Lanka	
13	Sullia	Mysore	
14	Nilgiri-4	Nilgiris	
15	Cheriyakaniyakkadan	Kottayam	
16	Panniyur-1	PRS. Panniyur	Hybrid of Uthirankotta x Cheriyakaniyakkadan
17	Panniyur-3	PRS. Panniyur	Hybrid of Uthrankotta x Cheriyakaniyakkadan
18	Uthirankotta	Thaliparamba	
19	Panniyur-2	PRS, Panniyur	Selection from Balankotta
20	Panniyur-4	PRS, Panniyur	Selection from Kuthiravally Type 2
21	Panniyur-5	PRS. Panniyur	Selection from Perumkodi
22	Narayakodi Type 1	Travancore	
23	Sreekara	HSR, Calicut	Selection from Karimunda
24	Subhakara	IISR, Calicut	Selection from Karimunda
25	Panchami	IISR, Calicut	Selection from Aimpiriyan
26	TMB-5	Thaliparamba	

MATERIALS AND METHODS

Twenty-six varieties / cultivars of *P. nigrum* with wide genetic base representing different geographical areas were subjected to isoenzyme studies at the College of Horticulture, Trichur 680 656, Kerala, India during the period 1992-94. The list of germplasm collection used in the study is furnished in Table 1.

Polyacrylamide gel electrophoresis (PAGE) was conducted using vertical slab gel unit of M/s. Biotech.

Samples were homogenized in *0AM* tris chloride of pH 7.6 and centrifuged at 15000 rpm at 4°C. Supernatant was used for the analysis. A sample buffer ratio of 4:5 was found ideal to get sufficient volume of extract.

Electrophoresis was carried out at 5°C. A constant current of 25 mA per slab was maintained throughout the run. Bromophenol blue in imidazole buffer was used as tracer dye.

Three enzymes namely, peroxidase (PRX), esterase (EST) and glutamate oxaloacetate transaminase (GOT) were assayed. Numbering of the isoenzymes was done by pooling the isoenzymes of all the species studied (Sebastian *et al.*, 1996). The enzymes were serially numbered starting from the fastest moving anodal band (e.g. PRX 1-31). Staining techniques as per Shaw and Koen (1968) were adopted.

Measurement of similarity: Similarity was calculated by making pair-wise comparison of genotypes following the method outlined by Sokel and Sneath (1963).

RESULTS AND DISCUSSION

Peroxidase (PRX)

Four variant isoenzymes were observed in the varieties studied. Ten bands were found common in all the varieties. They were PRX-4, 6, 10, 13, 14, 17, 18, 19, 20 and 21 (Fig 1). PRX-22 was a common variant additionally observed which was present in 11 varieties (Table 2). PRX-23 was found in five varieties. PRX-25 was observed in three varieties, namely, Arakkulamunda, TMB-2 and Doddiga. Another variant PRX-24 was less frequent and was observed only in the variety Shimoga among the 20 varieties studied.

Table 2. Groups of *Piper nigrum* varieties having similarity index 'one' among the members for peroxidase zymogram

Group 1	Group 2	Group 3
Kalluvally Type 1	Kottanadan	Arakkulamunda
Neelamundi Type 2	Karimkotta	TMB-2
Perumunda	Ceylon	Doddiga
Veluthanamban	Sullia	
Malligesera	Cheriyakaniyakkadan	
Nilgiri-4	Narayakodi Type 1	
Panniyur-1	Sreekara	
Panniyur-3		
Uthirankotta		
Panniyur-2		
Panniyur- 4		
Panniyur-5		
Subhakara		
Panchami		

Table 3. Grouping of *Piper nigrum* varieties based on presence of EST-2 or EST-4

Varieties with EST-2	Varieties with EST-4
Kalluvally Type 1	Karimkotta
Kottanadan	Neelamundi Type 2
Neelamundi Type 2	Perumunda
Arakkulamunda	Shimoga
Veluthanamban	Malligesera
TMB-2	Nilgiri-4
Doddiga	Cheriyakaniyakkadan
Sullia	Panniyur-1
Uthirankotta	Panniyur-3
Panniyur-4	Utirankotta
Sreekara	Panniyur-2
Ceylon	Panniyur-5
	Narayakodi Type 1
	Sreekara
	Shubhakara
	Panchami
	TMB-5

The rarer isoenzymes observed viz., PRX-23, 24 and 25 had no geographical isolation. Varieties from northern and southern Kerala, and Karnataka had the variant isoenzymes distributed in them.

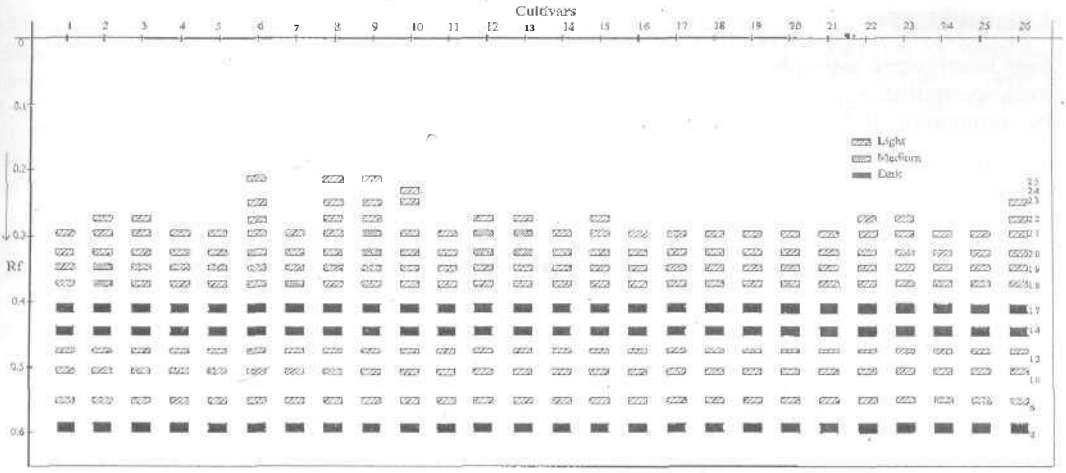

Fig 1. Zymogram of peroxidase in *Piper nigrum* varieties

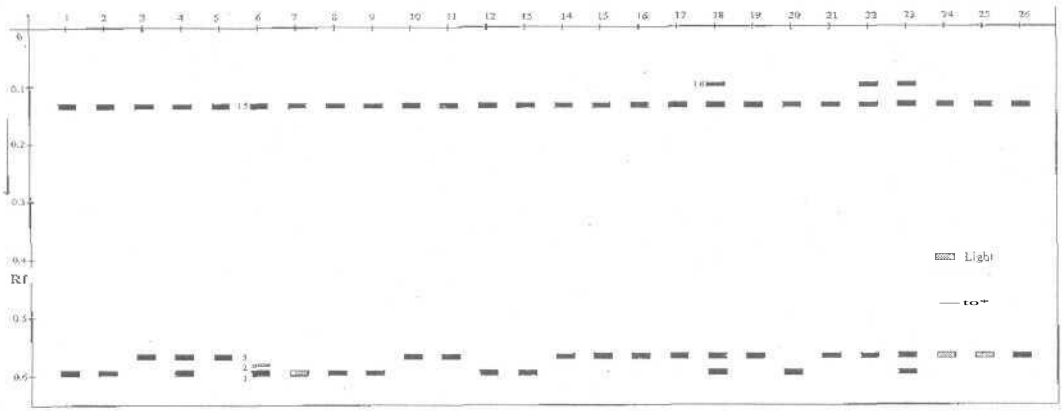

Fig 2. Zymogram of esterase in *Piper nigrum* varieties

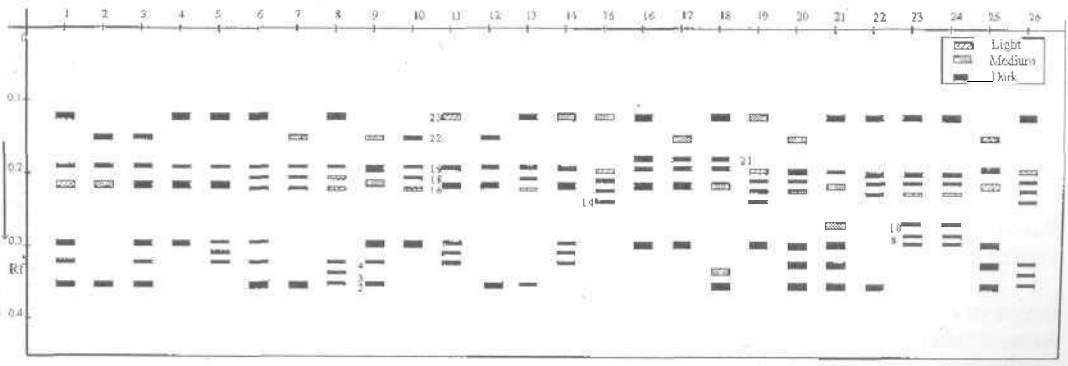

Fig 3. Zymogram of GOT in *Piper nigrum* varieties

Esterase (EST)

Five isoenzymes were observed in the 26 collections studied (Fig 2). EST-15 was found to be common in all the 26 types.

Varieties could be grouped into two based on the presence of either EST-2 or EST-4 in them (Table 3). These could be alleles of the same locus, which only a genetic analysis can confirm. EST-3 was a rare variant observed in the variety Arakkulamunda that appeared as a narrow band adjacent to EST-2. EST-16 was observed in Uthirankotta, Narayakodi Type 1 and Sreekara.

Table 4. Grouping of *Piper nigrum* varieties based on isoenzyme banding pattern of GOT

Varieties with only GOT-2	Varieties with only GOT-7	Varieties with GOT-3. 4 and 7	Varieties with GOT-4. 6 and 7
Kottanadan Veluthanamban Ceylon Cheriyakaniyakkadan Narayakodi Type 1	Neelamundi Type 2 Shimoga Panniyur-1 Panniyur-2 Panniyur-3	Kalluvally Type-1 Karimkotta Arakkulamuna Doddiga Panniyur-4 Panchami	Perumunda Malligesera Nilgiri-4

Table 4 continued

Varieties with only GOT-2, 3 and 4	Varieties with GOT-2. and 4	Varieties with GOT-7, 8 and 10	Varieties with GOT-2, 4, 7 and 10
TMB-2 TMB-5	Uthirankotta	Sreekara Shubhakara	Panniyur-5

Glutamate oxaloacetate transaminase (GOT)

As it can be seen from Fig 3, the varieties showed considerable variation in the banding pattern of GOT enzyme. A total of 15 isoenzymes were observed in the 26 varieties of *Piper nigrum* analysed. Pooling them with general isoenzymes of *Piper* spp. studied, the isoenzymes present in *Piper nigrum* were numbered as GOT-2, 3, 4, 6, 7, 8, 10, 14. 16, 17, 18, 19, 21, 22 and 23. GOT-3 was observed in two varieties viz., TMB-2 and TMB-5. GOT-6 was present in Perumunda, Malligesera and Nilgiri-4. GOT-8 was visible in Sreekara and Subhakara, which were selections from Karimunda. Cheriyakaniakkadan, Panniyur-2 and TMB-5 possessed GOT-14. GOT-10 was found in Panniyur-5, Sreekara and Subhakara.

Based on this complex banding pattern, the varieties were grouped into eight (Table 4).

Isoenzyme pattern for GOT in *Piper nigrum* gave a very complicated picture with 15 isoenzymes in different combinations in different varieties. GOT is a dimeric protein and such complicated pattern could be expected in a highly heterozygous population. A genetic analysis would have much simplified the existing situation.

Similarity index

Similarity index among *Piper nigrum* for the three enzymes ranged from 0.40 to 1.0 (Table 5). Maximum similarity among the 26 varieties studied was between Kottanadan and Ceylon as one group and Perumunda, Malligesera and Nilgiri-4 as the second group. The least similarity was between TMB-2 and Panniyur-3 (SI = 0.40).

The wide variation among the cultivated *P. nigrum* is in conformity with the earlier observations made by Kanakamani *et al.* (1985). On the basis of the morphological studies, they got 40 groups out of the 45 types studied. There was considerable variation in different morphological characters among the varieties *of Piper nigrum.*

Even though Gottlieb (1981) reported a high level of similarity (SI - 0.95) among the conspecific taxa at electrophoretic loci, high amount of variation within the species has been observed in the present study. This could be due to vegetative propagation in the species where variability once created gets

fixed. Such variation in isoenzyme banding pattern is observed in other vegetatively propagated crop species like banana (Bhat *et. al.*, 1992a and 1992b), pineapple (De Wald *et al.*, 1988), sugarcane (Feldmann, 1985) and grapes (Parfitt and Arulsekar, 1989; Weeden *et al.*, 1988).

Isoenzyme analysis is of considerable use in cultivar identification using isoenzyme fingerprinting. In the present study, PRX-24 was a cultivar specific isoenzyme in variety Shimoga that was not found in any other variety. However, there is no doubt that for the proper isoenzyme fingerprinting, the entire germplasm should be analysed for more enzymes. Presence of wide variability in isoenzyme banding pattern of *P. nigrum* studied shows that the task may be easy to solve in *P. nigrum.*

Table 5. Average similarity among *Piper nigrum* L. varieties*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1																									
2	.78	1																								
3	.65	.67	1																							
4	.78	.64	.67	1																						
5	.68	.50	.80	.78	1																					
6	.69	.58	.51	.55	.49	1																				
7	.74	.80	.54	.60	.78	.65	1																			
8	.74	.69	.49	.55	.47	.81	.76	1																		
9	.83	.84	.73	.62	.53	.70	.71	.77	1																	
10	.46	.46	.71	.60	.70	.65	.61	.48	.50	1																
11	.68	.50	.80	.78	1	.49	.48	.47	.53	.69	1															
12	.78	1	.67	.64	.50	.44	.80	.70	.84	.46	.50	1														
13	.76	.76	.52	.62	.49	.74	.86	.85	.69	.51	.49	.76	1													
14	.68	.50	.80	.78	1	.49	.78	.47	.53	.69	1	.50	.49	1												
15	.53	.53	.73	.61	.70	.57	.60	.60	.46	.71	.70	.53	.72	.70	1											
16	.63	.51	.76	.82	.86	.45	.48	.43	.49	.69	.86	.51	.50	.86	.74	1										
17	.57	.58	.83	.72	.79	.41	.53	.4	.56	.75	.79	.58	.45	.79	.70	.89	1									
18	.74	.61	.64	.73	.67	.54	.57	.57	.59	.48	.67	.61	.59	.67	.61	.69	.63	1								
19	.67	.56	.70	.70	.78	.55	.68	.63	.55	.80	.78	.56	.72	.77	.90	.79	.74	.57	1							
20	.81	.76	.62	.63	.54	.73	.90	.77	.80	.63	.54	.76	.80	.54	.59	.51	.56	.60	.59	1						
21	.73	.54	.84	.70	.88	.52	.51	.50	.58	.68	.88	.54	.53	.88	.77	.83	.78	.71	.77	.58	1					
22	.51	.51	.63	.56	.60	.59	.61	.60	.44	.62	.60	.51	.75	.60	.80	.61	.56	.68	.72	.55	.64	1				
23	.57	.53	.56	.68	.57	.60	.58	.58	.51	.59	.57	.53	.67	.57	.62	.58	.54	.70	.68	.60	.60	.75	1			
24	.54	.45	.70	.68	.77	.52	.56	.50	.43	.78	.77	.45	.58	.77	.81	.78	.73	.56	.88	.58	.80	.69	.80	1		
25	.68	.64	.97	.70	.83	.49	.57	.47	.70	.74	.83	.64	.49	.83	.73	.79	.86	.67	.73	.63	.88	.60	.53	.73	1	
26	.52	.48	.73	.57	.70	.61	.54	.71	.51	.72	.70	.48	.62	.70	.86	.67	.64	.57	.80	.56	.73	.74	.59	.73	.70	1

*Name of the varieties are given in Table 1

ACKNOWLEDGEMENT

The study forms a part of the M Sc. thesis work of the senior author. Junior Research Fellowship provided to the senior author from an ICAR aided adhoc project is gratefully acknowledged.

REFERENCES

Bhat. K.V., Bhat, S.R. and Chandel, K.P.S. 1992a. Survey of isoenzyme polymorphism for clonal identification in *Musa.* I. Esterase, acid phosphatase and catalase. *J. hort. Sci.* 67:501-507

Bhat, K.V., Bhat, S.R. and Chandale, K. P. S. 1992b. Survey of isozyme polymorphism for clonal identification in *Musa.* II. Peroxidase, superoxide dismutase and malate dehydrogenase. *J. hort. Sci.* 67:737-745

De Wald, M.G., Moore, G.A. and Sherman, W. B. 1988. Identification of pineapple cultivars by isoenzyme genotypes. *J. Am. Soc. hort. Sci.* 113:935-938

Feldmann, P. 1985. Identification of varieties of sugarcane (*Saccarum* sp.) by isozyme electrophoresis. *Agronomic Tropicale* 40 (2): 124-128

Gottlieb, G. D. 1981. Electrophoretic evidence and plant populations. *Prog. Phytochem* 7: 1-46

Kanakamani, M. T., Namboodiri, N. and Babu. L.C. 1985. Key for identification of the different cultivars of pepper. *Indian Cocoa, Arecanut, Spices J.* 114:486-491

Parfitt, D.E and Arulseker, S. 1989. Inheritance and isoenzyme diversity for GPI and PGM among grape cultivars. *J. Am. Soc. hort. Sci.* 114: 486-491

Ravindran, P. N. and Babu, N. 1994. Genetic resources of black pepper. *Advances in Horticulture: Vol. 9. Plantation and Spice Crops Part I* (eds. Chadha K. L. and Rethinam, P.), Malhothra Publishing House, New Delhi

Sebastian, A., Sujatha V. S., Nybe, E. V., Nair, G. S. and Augustine, A. 1996. Isoenzyme variation and species relationship in the genus *Piper. J. trop. Agric.* 34:85-92

Shaw, C. R. and Koen, P. L. 1968. Starch gel zone electrophoresis of enzymes. *Chromatographic and Electrophoretic Techniques* (ed. Smith, I). Vol. 2, 2nd edn., John Wiley, New York

Sokel, R. R. and Sneath, P. H. A. 1963. *Principles of Numerical Taxonomy.* W. H. Freeman and Company, San Francisco

Sujatha, V. S., Seshadri, V. S., Srivastava, K. N. and More, T. A. 1991. Isoenzyme variation in muskmelon (*Cucumis melo* L.). *Indian J. Genet.* 51: 438-444

Valizadeh, M. 1977. Esterase and acid phosphatase polymorphism in the fig trees (*Ficus carica* L.). *Biochem Genet.* 15: 1037-1048

Weeden, N. F., Reisch, B.I. and Martens, M. H. E. 1988. Genetic analysis of isoenzyme polymorphism in grapes. *J. Am. Soc. hort. Sci.* 113:765-769