Global Journal of Biotechnology & Biochemistry 5 (1): 19-26, 2010
ISSN 2078-466X

Variations of Genotypes of Radish at Molecular Level Using Isozyme Analysis for the Identification of Self-Incompatible Lines

[1]N.A. Ivy, [2]M.S. Biswas, [3]G. Rasul, [4]T. Hossain and [3]M.A.K. Mian

[1]Genetics and Plant Breeding, BSMRAU, Gazipur-1706, Bangladesh
[2]Department of Horticulture, BSMRAU, Gazipur-1706, Bangladesh
[3]Genetics and Plant Breeding, BSMRAU, Gazipur-1706, Bangladesh
[4]Crop Botany, BSMRAU, Gazipur-1706, Bangladesh

Abstract: The experiment was conducted to characterize 78 radish genotypes using esterase and peroxidase isoenzyme. The results of the experiment revealed a wide range of diversity among the genotypes based on their esterase and peroxidase isoenzyme banding patterns. Five bands at different Rf values varying from 0.06 to 0.56 were observed in peroxidase enzyme system and formed 10 zymotypes. Zymotype P_1 was the most frequent which includes 26.92% of the total genotypes. Lowest frequency of 2.56% was observed in the zymotype P_{10}. Band 3 and 4 consist of same frequency (23.31%) of the genotypes at Rf values 0.20-0.30 to 0.30-0.50. It was also found that there was no common band for all the genotypes. Thus the genotypes have wide range of genetic variation. On the other hand thirteen electrophoretic zymotypes (P_1-P_{13}) were observed in Esterase enzyme system formed by 13 bands at different Rf values varying from 0.04 to 0.93. It appeared that electrophoretic zymotype P_6 was the most frequent (25.64%) and zymotype P_{13} was least frequent (2.56%). The zymotype P_{11} had maximum number of bands. From the distribution of esterase bands among the radish genotypes, it was observed that band 3 had the most frequent of 18.75% followed by band 2 and 4 had the same frequency 16.67% among all the genotypes. A dendogram based on the two polymorphic enzyme activities all the genotypes were classified into eight major clusters designated as I, II, III, IV, V, VI, VII and VIII. The highest number of 21 genotypes was found under the cluster number I that represented 27% of the total genotypes followed by cluster VI contained 17 genotypes. The lowest number (4) of genotypes was found under cluster VII followed by cluster III contained 5 genotypes. The result indicated that zymotype of higher frequency are the representative of less variation. The lower frequency of the germplasm in different zymotypes indicated higher variation among the genotypes.

Key words: Radish · Esterase · Peroxidase isoenzyme · Characterization

INTRODUCTION

Radish is the second major vegetable crop in terms of area and first major winter vegetable crop in Bangladesh in terms of production. It is widely cultivated in the cool season of Bangladesh. Its production can be boosted up by utilizing hybrid technology. Thus, development and use of new hybrid varieties can increase the yield potential of radish compared to the production of traditional varieties [1]. Molecular marker is an important tool for crop improvement and marker assisted selection for traits of interest. The different molecular forms of an enzyme which catalyze the same reaction are called isozymes if they or their polypeptide constituents are coded by more than one gene locus and allozymes when coded by different alleles of the same locus [2]. In other words, isozymes have been defined as different variants of the same enzyme, having identical or similar function and present in the same individual [3]. In plants, most enzymes routinely assayed have several isozyme forms often with specific sub-cellular locations and the majority of isozymes have different allozymic variants. Isozyme played an important role in numerous aspects of biological studies in plant [4,5]. Isozymes, the molecular form of a protein can be differentiated by electrophoresis. The primary evidence observed in studies of protein electrophoretic variation is a band of color in a acrylamide gel. Electrophoretic data in plants have often been used

Corresponding Author: N.A. Ivy, Associate Professor, Genetics and Plant Breeding, BSMRAU, Gazipur-1706

to accumulate information about genes *per se* for the purpose of describing the amount and pattern of genetic variability in populations and the extent of divergence among them and between species. Electrophoretic analysis of isozyme has been used extensively to provide rapid, quantitative estimates of the extent of genetic variation within the species [4,6]. The conventional methods used for identifying different crop plants are based on the phenotypic expressions of the plant, plant parts, or seeds. Such expressions are strongly influenced by the environment thus these conventional means are gradually being replaced by chemical methods. One of the main methods is isozyme electrophoresis by which the chances of correct cultivar identification are highly improved.

Considerable variability with respect of root shapes and quality of radish are available in this country. Such variability can be confirmed through isozyme analysis. To date there is no report on such study in radish. Therefore, the isozyme study was carried out for two-enzyme system to know the banding pattern among the parents and hybrids and also the inheritance pattern of parental bands by all the hybrids indicated the authenticity of the hybrids of radish.

MATERIALS AND METHODS

Young leaf material were taken from plant sample into a small eppendorf tube with a small amount of sea sand as a crushing agent. Exactly 0.70 µl extraction buffer solutions were added and crushed the sample using a glass rod. The sample was dissolved by vortex until become homogenization. Dissolved samples were centrifuged at 15000 rpm for 4°C for 3 minutes [7]. Samples were kept in a refrigerator until it used.

Gels of different concentrations were prepared using the stock solution. The stock solutions were stored in dark and cold condition. Freshly prepared ammonium per sulphate (APS) solution was used. The glass plates (one was notched and the other was plain) and the gaskets with the clips were assembled first to make plate sandwich. A comb was inserted into the sandwich. A level of marking was made on the glass plate, 4-5 mm lower than the teeth of the comb, by using marker pen. The comb was then removed. The solution of separation gel except APS and tetramethylene diamine (TEMED) were gently mixed in a 50 ml conical flask. After words APS and TEMED were added and mixed very carefully. Immediately after adding APS and TEMED, the separation gel solution was poured with its notched glass plate upside. Small amount of water or ethanol overlaid to a level of 2-3 mm over the gel solution carefully. Then the solution was allowed for polymerization about 30-60 minutes until a clear line between the gel and water is seen.

After polymerization of the separating gel the overlaid water was discarded by using tissue paper. The solution for stacking gel were mixed as separating gel and introduced to fill the upper portion of the glass plates. Immediately a comb was inserted through the stacking gel solution carefully, so that the comb did not trap any air bubbles. About 20-30 minutes was allowed for polymerization. The comb was removed by pushing it up and washed the wells with a small amount of electrode buffer.

Electrophoresis: After removing the clips and gaskets, the glass plate with gel was attached to the electrode assembly. About 430 ml of the electrode buffer was poured in the electrophoresis chamber slowly and gently to avoid trapping of air bubbles at the bottom of the gel. About 70 ml of electrode buffer was poured on to the upper portion of the electrode assembly and the level of the buffer would be 2-3 ml lower from the top of the glass plates to submerge the top of the upper gel.

Ten to twenty micro liter of the homogenized sample was loaded into each well with a micro syringe. Electrophoresis was performed by connecting the electrode assembly with the power supply for 3-4 hours depending on the concentration of gels at 20 mA constant current per gel. The electrophoresis was stopped at the time when tracking marker stain reaches 4-5 mm to the gel bottom.

Staining of Isozymes: After removing the gel sandwich from the electrode assembly, the gel was separated from the glass plate gently with a steel spatula keeping under slow running tap water. The gel was then put into the staining solution. The isozyme, which was stained to observe the variation in banding pattern are esterase and peroxidase.

Staining of Peroxidase: Solution A was prepared by mixing 40 ml methanol and 200 mg of 0-phynyl-diamine in 50ml of 1M Na-acetate buffer. To the mixture 50ml methanol was added. The gel was incubated in solution A in the dark at 30°C for 30 minutes. Then 2 mg of solution B (30% H_20_2) was added to the solution A and the tray was shaken for at least 20 minutes.

Staining of Esterase: The gel was incubated in buffer for 10 minutes under continuous shaking. Solution A was prepared by mixing 100mg fast violet B salt in 100ml buffer. Solution B (substrate buffer) was prepared by adding 100mg 1-naphthyl acetate and 100mg 2-naphthyl acetate in 10 ml acetone and then 10ml water was added to it. After incubation in buffer for 10 minutes under continuous shaking the gel was transferred to the staining solution for one minute. Solution B was added to solution A, the gel was incubated until bands appeared. The gel was then washed with water and fixed in fixing agent for at least 2 hours and dried by a rapid dryer.

Band Identification: Isozyme banding patterns were recorded on the basis of number and the relative front (Rf) values of the bands. The Rf value of each respective bands on schematic isozyme patterns was determined to allow precise comparisons among the various germplasm. The Rf value is the mobility of each isozyme band that traveled from the origin divided by the distance traveled by the front tracking dye [8]. An electrophoretic pattern comprised genotype with the same isozyme patterns [9].

Cluster Analysis: For each enzyme similarity coefficient (S_{xy}) values of electrophoretic pattern were subjected to cluster analysis employing the unweighted pair group method using arithmetic averages (UPGMA). For cluster analysis of overall isozyme electrophoretic patterns, the value 1 was put for the presence of the electrophoretic pattern and value 0 was used against the absence of the pattern for each genotype. Zymotypes were used for clustering and the Euclidean distance method [10] was used for the dissimilarity. The original data was transformed to Z-scores prior to cluster analysis [11,12].

RESULTS AND DISCUSSION

Peroxidase Enzyme Variability: Five bands at different Rf values varying from 0.06 to 0.56 were observed in peroxidase enzyme system (Fig.1). Genotypes under each peroxidase zymotypes were listed in Table 1. Zymotype P_1 was the most frequent which includes 26.92% of the total genotypes. Zymotype P_2 was found to be the next frequent patterns and included 14.10%. Lowest frequency of 2.56% was observed in the zymotype P_{10}. From the distribution of peroxidase bands among the zymotypes of 78 radish genotypes, it was observed that band one present in maximum 33.33% genotypes (Table 2). Lowest band frequency was observed in 4.76% genotypes at Rf value 0.50 to 0.60. Band 3 and 4 consist of same frequency (23.31%) of the genotypes at Rf values 0.20-0.30 to 0.30-0.50. It was also found that no common band was not present for all the genotypes. Thus the genotypes have wide range of genetic variation.

Esterase Enzyme Variability: Thirteen electrophoretic zymotypes (P_1-P_{13}) were observed in Esterase enzyme system formed by 13 bands at different Rf values varying from 0.04 to 0.93 (Figure 2). Genotypes under each esterase zymotype were listed in Table 3. It appeared from the table that electrophoretic zymotype P_6 was the most frequent (25.64%) and zymotype P_{13} was least frequent (2.56%). The zymotype P_{11} had maximum number of bands. On the other hand the zymotype P_6, P_9 and P_{12} had only single band. Among the zymotypes band number 3 was found among 9 zymotypes groups at Rf values 0.18. The result supports the isozyme tissue-observed in the plant species as described Evans and Alldridge [13] Hess [14] Scandalios [15]. Bands at 0.25 and 0.33 were found

Table 1: Zymogram patterns and observed number of electrophoretic zymotypes of peroxidase isoenzyme in 78 radish genotypes

Zymo types	Description (the bands are present)	Number of genotypes	Total number of genotypes	Percent (%)
P1	1, 3, 4,	RS 04, RS 05, RS 06, RS 07, RS 08, RS 09, RS 10, RS 11, RS 14, RS 15, RS 16, RS 17, RS 18, RS 19, RS 21, RS 22, RS 23, RS 24, RS 26, RS 35, RS 48	21	26.92
P2	1, 2, 3	RS 28, RS 32, RS 54, RS 56, RS 58, RS 62, RS 64, RS 65, RS 67, RS 69, RS 71	11	14.10
P3	1, 4	RS 01, RS 20, RS 37, RS 38, RS 39, RS 40, RS 44, RS 45, RS 75, RS 76	10	12.82
P4	1, 2, 3, 4	RS 12, RS 33, RS 41, RS 43, RS 46, RS 73	06	7.69
P5	1, 3,	RS 30, RS 34, RS 49, RS 50, RS 52, RS 57, RS 60, RS 68	08	10.26
P6	3	RS 61, RS 63, RS 66, RS 70, RS 72	05	6.41
P7	5	RS 27, RS 29, RS 34	03	3.85
P8	4	RS 03, RS 74, RS 77, RS 78	04	5.13
P9	1	RS 25, RS 33, RS 36, RS 42, RS 51, RS 53, RS 55, RS 59	08	10.26
P10	1, 2, 4	RS 02, RS 47	02	2.56

Table 2: Distribution of the peroxidase bands among zymotypes in radish

	Relative frequency of bands					
Zymotypes	0.02-.010 (1P)	0.10-0.20 (2P)	0.20-030 (3P)	0.30-0.50 (4P)	050-0.60 (5P)	Number of bands
P1	√		√	√		03
P2	√	√	√			03
P3	√			√		02
P4	√	√	√	√		04
P5	√		√			02
P6			√			01
P7					√	01
P8				√		01
P9	√					01
P10	√	√		√		03
Total	07	03	05	05	01	21
Band frequency	33.33	14.29	23.81	23.81	4.76	

Table 3: Zymogram patterns and observed number of electrophoretic zymotypes of esterase isoenzyme in 78 radish variety

Zymotypes	Description (the bands are present)	Number of genotypes	Total	Percent (%)
P1	2, 3, 4, 5, 6	RS 09, RS 10, RS 14, RS 15, RS 19, RS 21, RS 24, RS 25, RS 28, RS 30	10	12.82
P2	5, 8, 9	RS 31, RS 54, RS 55, RS 56, RS 60, RS 65, RS 71	07	8.97
P3	1, 2, 3, 8, 9	RS 32, RS 34, RS 36, RS 78	04	5.13
P4	1, 3, 6	RS 01, RS 03, RS 04, RS 73	04	5.13
P5	2, 3, 4	RS 37, RS 39, RS 63, RS 64	04	5.13
P6	5	RS 26, RS 29, RS 33, RS 40, RS 41, RS 42, RS 44, RS 45, RS 47, RS 48, RS 52, RS 53, RS 61, RS 62, RS 66, RS 67, RS 69, RS 70, RS 72, RS 74	20	25.64
P7	2, 3, 4, 7	RS 12, RS 22, RS 27, RS 49, RS 50, RS 51, RS 75, RS 76, RS 77	09	11.54
P8	2, 3, 4, 10, 11	RS 08, RS 11, RS 13, RS 23, RS 35, RS 38	06	7.69
P9	8	RS 57, RS 58, RS 68	03	3.85
P10	4, 9	RS 43, RS 46, RS 59	03	3.85
P11	2, 3, 4, 5, 6, 12,13	RS 16, RS 17, RS 18	03	3.85
P12	6	RS 06, RS 07, RS 20	03	3.85
P13	6, 8	RS 02, RS 05	02	2.56

Table 4: Distribution of the esterase bands among zymotypes in radish

	Relative frequency of bands													
Zymotypes	0.02-.010 (1P)	0.10-0.15 (2P)	0.15-0.20 (3P)	0.20-0.25 (4P)	0.25-0.35 (5P)	0.35-0.45 (6P)	0.45-0.55 (7P)	0.55-0.75 (8P)	0.75-0.80 (9P)	0.80-0.85 (10P)	0.85-0.87 (11P)	0.87-0.90 (12P)	0.90-0.95 (13P)	Number of bands
P1		√	√	√	√	√								05
P2					√			√	√					03
P3	√	√	√					√	√					05
P4	√		√			√								03
P5		√	√	√										03
P6					√									01
P7		√	√	√			√							04
P8		√	√	√					√	√				05
P9								√						01
P10				√					√					02
P11		√	√	√	√	√						√	√	07
P12		√	√	√			√							04
P13		√	√	√						√	√			05
Total	02	08	09	08	04	03	02	03	04	02	01	01	01	48
Band frequency	4.17	16.67	18.75	16.67	8.33	6.25	4.17	6.25	8.33	4.17	2.22	2.22	2.22	

Table 5: Distribution of 78 Radish genotypes under different clusters based on nonspecific peroxidase, esterase polymorphism

Cluster No.	Total no accessions in cluster	Genotypes included in different clusters
I	21	RS 31, RS 33, RS 52, RS 54, RS 55, RS 56, RS 57, RS 58, RS 59, RS 60, RS 61, RS 62, RS 64, RS 65, RS 66, RS 67, RS 68, RS 69, RS 70, RS 71, RS 72
II	6	RS 04, RS 05, RS 06, RS 07, RS 20, RS 74
III	5	RS 12, RS 75, RS 76, RS 77, RS 78
IV	6	RS 16, RS 49, RS 50, RS 51, RS 53, RS 63
V	8	RS 08, RS 09, RS 10, RS 22, RS 23, RS 24, RS 35, RS 73
VI	17	RS 11, RS 13, RS 14, RS 15, RS 17, RS 18, RS 19, RS 21, RS 25, RS 26, RS 27, RS 28, RS 29, RS 30, RS 32, RS 34, RS 36
VII	4	RS 01, RS 02, RS 03, RS 38
VIII	11	RS 37, RS 39, RS 40, RS 41, RS 42, RS 43, RS 44, RS 45, RS 46, RS 47, RS 48

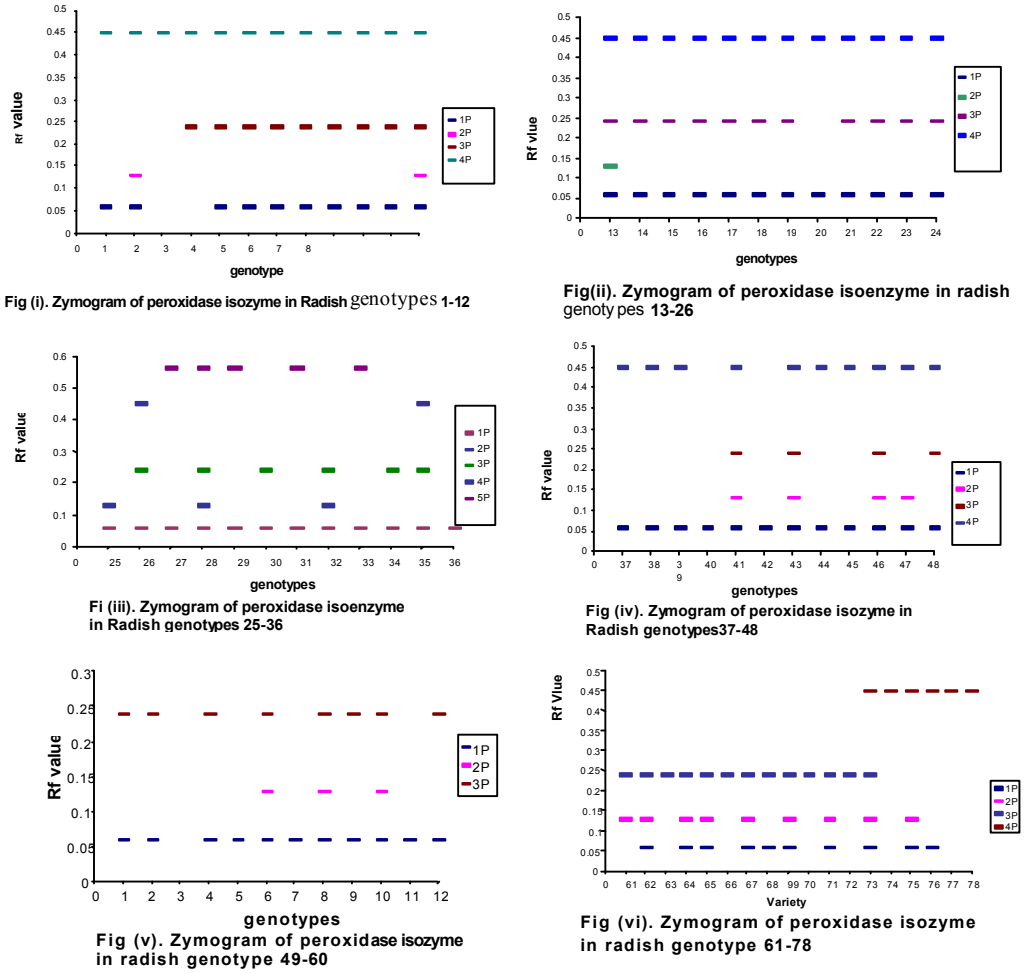
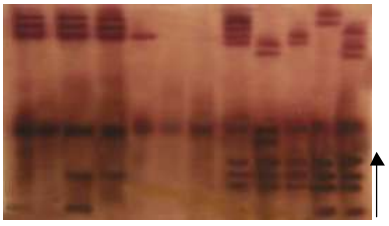


Fig. 1: Variation in banding patterns and zymogram of peroxidase enzyme in different radish genotypes

7.69% frequent of total population. Bands at Rf value 0.07 and 0.38 were found in the zymotypes E_1 and E_5 had the lowest frequency of 3.85%. From the distribution of esterase bands among the radish genotypes (Table 4), it was observed that band 3 had the most frequent of 18.75% followed by band 2 and 4 had the same frequency 16.67% among all the genotypes. Zymotypes P_{11}, P_{12} and P_{13} had the lowest band frequency.

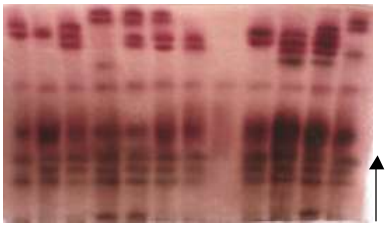
The result indicated that zymotype of higher frequency are the representative of less variation. The lower frequency of the germplasm in different zymotypes indicated higher variation among the genotypes.

A dendogram based on the two polymorphic enzyme activities all the genotypes were classified into eight major clusters designated as I, II, III, IV, V, VI, VII and VIII in the Table 5 and Fig. 3. The highest number of 21 genotypes



1 2 3 4 5 6 7 8 9 10 11 12

Plate (a). Esterase banding pattern in radish genotypes 01-12(arrow indicates the direction of migration of sample)



13 14 15 16 17 18 19 20 21 22 23 24

Plate (b). Esterase banding pattern in radish genotypes 13-24(arrow indicates the direction of migration of sample)

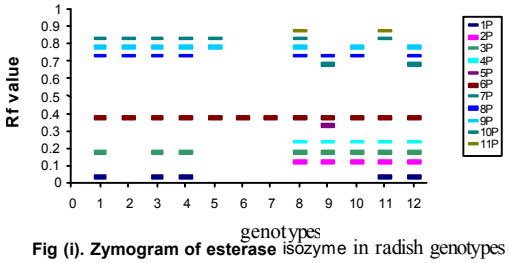


Fig (i). Zymogram of esterase isozyme in radish genotypes **01-12**

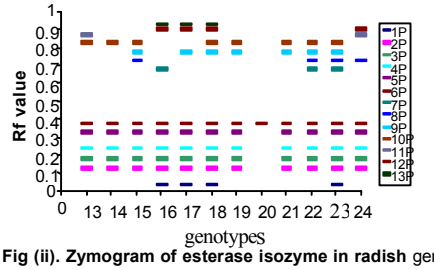
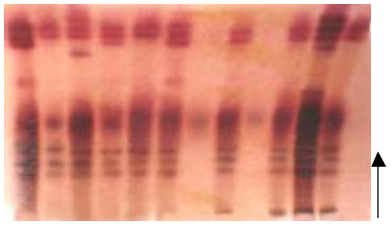
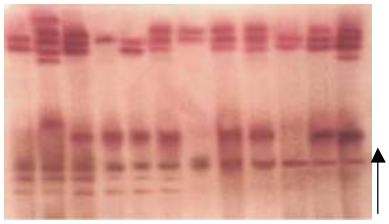


Fig (ii). Zymogram of esterase isozyme in radish genotypes **13-26**



25 26 27 28 29 30 31 32 33 34 35 36

Plate (c). Esterase banding pattern in radish genotypes 25-36 (arrow indicates the direction of migration of sample)



37 38 39 40 41 42 43 44 45 46 47 48

Plate (d). Esterase banding pattern in radish genotypes 37-48 (arrow indicates the direction of migration of sample)

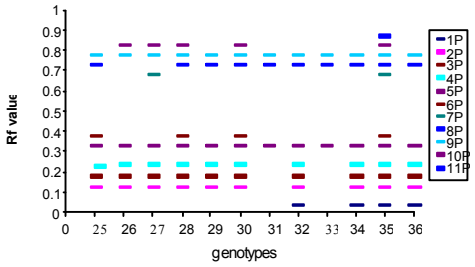


Fig (iii). Zymogram of esterase isozyme in Radish 25-36

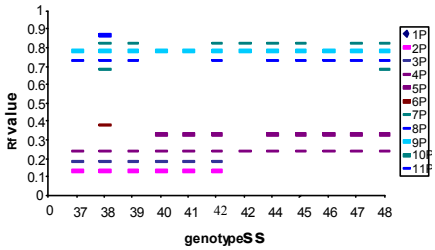
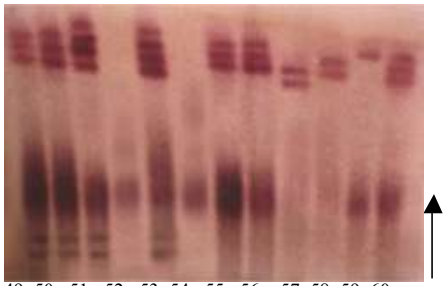
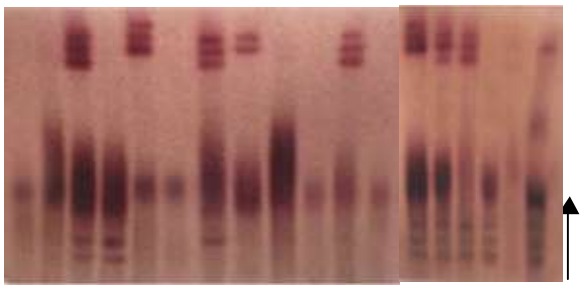


Fig (i). Zymogram of esterase isozyme in Radish genotypes 37-48



49 50 51 52 53 54 55 56 57 58 59 60

Plate (e). Esterase banding pattern in radish genotypes 49-60(arrow indicates the direction of migration of sample)



61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78

Plate (f). Esterase banding pattern in radish genotypes 60-72(arrow indicates the direction of migration of sample)

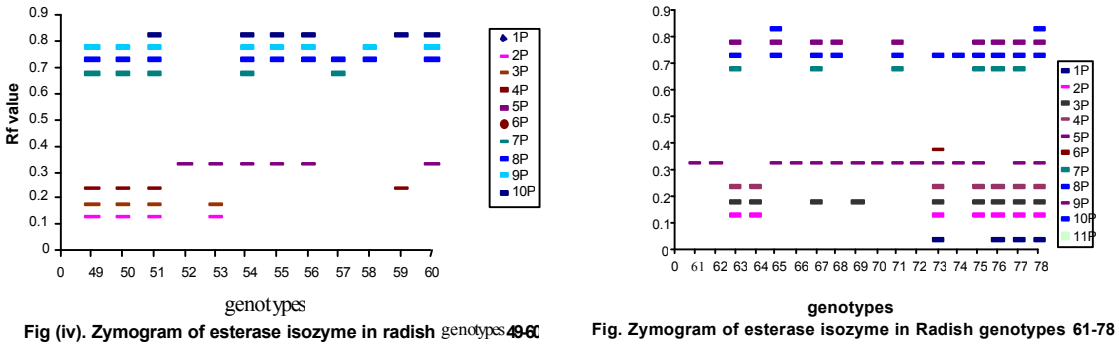


Fig (iv). Zymogram of esterase isozyme in radish genotypes 49-60

Fig. Zymogram of esterase isozyme in Radish genotypes 61-78

Fig. 2: Variation in banding patterns and zymogram of esterase enzyme in different radish genotypes

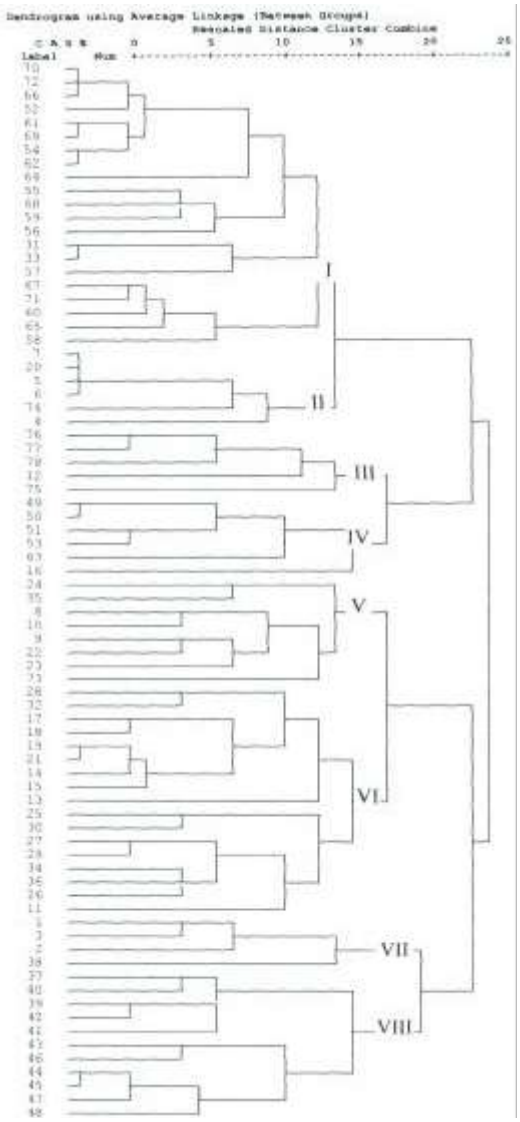


Fig. 3: Dendrogram showing hierarchical clustering of 78 radish genotypes based on zymotypes of peroxidase and Esterase

was found under the cluster number I that represented 27% of the total genotypes followed by cluster VI contained 17 genotypes. The lowest number (4) of genotypes was found under cluster VII followed by cluster III contained 5 genotypes.

REFERENCES

1. Tyagi, I.D., 1972. Variability and correlation studies in bottle gourd (*Lagenaria siceraria*). Indian J. Hort., 29(2): 219-222.
2. Gottlieb, L.D., 1981. Electrophoretic evidence and plant populations . Progr. Phytochem., 7: 1-46.
3. Markert , C.L. and F. Moller, 1959. Multiple forms of enzymes; tissue, ontogenic and species patterns. Proc. Natl. Acad. Sci. USA., 45: 753-763 .
4. Tankasley, S.D. and T.J. Orton, (eds) 1984. Isozymes in Plant Genetics and Breeding . North-Holland Publ. Co. Amsterdam.
5. Harris, H., 1966. Proc. Roy. Soc. London B, 164: 298.
6. Bailey, D.C., 1983. Isozymic variations and plant breeder rights. In: S.D. Tanksley T.J. and Orton (Eds). Isozymes in plant genetics and breeding, Part A, Elsevier, Amsterdam, pp: 425-440.
7. Bhowmik, A., 1995. Gel electrophoresis Techniques, IPSA-JICA project publication No. 25. Institute of Post Graduate Studies In Agriculture, pp: 23.
8. Powers, H.R. Jr. D. Lin and Hubbes, 1988. Interspecific and intraspecific differentiation within the genus *Cronartium* by isozyme and protein pattern analysis. Plant Disease, 73: 691-694.
9. Simox, K.D., W.L. Pedersen and D.L. Niukrent, 1993. Isozyme diversity in *Setosphaeria turcica.*, Can. J. Plant Pathol., 15: 91-96.
10. Nourish, M.J., 1993. SPSS for windows professional statistics-Release 6.0 SPSS Inc. 444N. Michigan Avenue Chicago.

11. Anderberg, M.R., 1973. Cluster analysis for application. Academic Press. New York., pp: 27.
12. Romesburg, H.C., 1984. Cluster analysis research. Belmont, Calif, Lifetime Learning Publication, pp: 24.
13. Evans, J.J. and N.A. Allridge, 1965. The distribution of peroxidases in extreme dwarf and normal tomato (*Lycopersicon esculentum*). Phytochemistry, 4: 499-503.
14. Hess, D., 1967. Multiple Formen der Phenolase und Peroxidase in reinen Linen von Petunia hybrida. Z. Pflanzenphysiol, 56: 295-298. (English summary).
15. Scandalios, J.G., 1968. Genetic control of multiple molecular forms of Catalase in Maize Ann. N.Y. Acad. Sci., 151: 272-293.