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STUDY OF PEROXIDASE ISOZYME BANDING PATTERNS IN MULBERRY VARIETIES

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The present investigation deals with peroxidase isozymes banding patterns in eight mulberry varieties.Of these Berhampur local is having five bands, whereas Sukasakwa, Jakkur local, Mutants, M.R. Midew, Coonoor-11, Selection –41 and Mysore local have revealed less bands. The significant changes were observed in banding patterns and in their Rt values. The possible relation between plant morphology and peroxidase is discussed, on the basis of similarity index.

Keywords: Isozyme; Mulberry varieties; Peroxidase.

Introduction

Peroxidase is a metallic group of enzyme catalyses the removal of oxygen from hydrogen peroxidase. Its greater variability among higher plants, tissues and immense physiological interest because of its association with numerous catalytic functions, peroxidase are usually characterized by a monogenic control ; monomeric behaviour and the presence of null alleles[1], and are described as glycoproteins and haemoproteins[2], dimeric enzymes have been made studied in oryza perennis[3], whereas repeated observations have been made in several plant species/varieties of epistatic genes which suppress the expression of peroxidase isoenzymes by post transcriptional modifications and or inhibiting the appearance of some isozymes, thus affecting segregation ratios[4]. Electrophoretic studies of peroxidase in the varieties were done by many workers[5-14]. It has been postulated that these peroxidase isozymes may have role in plant development because of their ability to oxidize the plant hormones IAA[15]. The present investigation was undertaken to observe the distribution of peroxidase isozyme in some mulberry varieties.

Material and Method

The material for the present investigation was collected from the germplasm bank established at Department of Sericulture, Jnanabharathi, Bangalore University, Bangalore. Varieties viz. Berhampur Sukasakwa, Jakkur local, Mutants, M.R.Mildew, Coonoor-11, Selection-41(S41), Mysore local. 10 plants were maintained for each variety and they were planted in 60X60cm spacing. The peroxidase isozyme, separation was carried out with 7.5% polyacrylamide tube gel electrophoresis according to the method of Clarke[16]. One gram of fresh leaf material (3rd – 6th leaf from top of the shoot) was weighed and homogenized in a mortar using 8ml of tris-buffer (0.1 m tris,0.04M NaCl and 0.02M EDTA, pH 7.5), the extract was centrifuged at 18,000 rpm for about 30 minutes at 4°C. To each ml of the extract thus obtained, 0.25ml of 20% Glycerol containing Bromophenol blue (0.1%) was added and 0.2ml of it was loaded for each tube. The gels were stained after the electrophoretic run for peroxidase enzyme activity, according to the Scandalios[17], using 50 ml of ammonium benzidine solution mixed with 10 ml of 30% ammonium chloride and 2 ml of 0.2% hydrogen peroxide.Finally the gels were preserved in 7% acetic acid.The degree of electrophoretic similarity among the varieties was determined by calculating the similarity index values (S) for each of the possible pairs of varieties[18].The formula used to calculate similarity index (SI) is as follows:

$$SL = \frac{\text{Number of similar bands}}{\text{Total number of bands}} \times 100$$

Results and Discussion

For population studies, isozymes (enzyme from alleles of the same gene loci enzymes and isozyme are those enzymes coded from different gene loci) make possible comparisons between individuals and populations on the basis of several gene loci, rather than just one or two. Moreover, if the analysis is accompanied by investigations of progenies of the organisms analyzed. Mendelian segregation ratios can be obtained without the trouble of isolating parents and making crosses. Two parameters have been extensively used. Proportion of enzyme loci for which the population is polymorphic and

the mean numbers of loci for which individuals are heterozygous. A review by Salander[19] comparing these parameters in various populations has been followed by many other studies. The present study was used to evaluate the importance of heterozygocity in natural populations. The strength of isozyme analysis for testing hypotheses is well illustrated by the contribution of the Soltis[20].

The data generated from enzyme electrophoresis differ fundamentally from other information routinely employed by plant systematists because the banding patterns in gels is produced by staining for specific enzymes[21-25]. Different banding patterns may be equated to different alleles at a gene locus or to alleles at different loci. Allozymes are inherited as codominants in a simple Mendelian fashion, which allows one to ascertain allelic frequencies for a population of plants, species, etc. From these data, one can quantify the similarity and differences between populations, groups of populations, species, etc. Electrophoresis also allows one to ascertain the number of isozymes (and therefore the number of gene loci) of particular enzymes included in a study. For a variety the enzymes normally examined electrophoretically, there is a highly conserved minimal number of isozymes in diploid plants[26]. The means that only increase in isozyme number is indicative of gene duplication at the diploid level or duplication due to polyploidy. Gottieb[27] discussed the utility of isozyme number for studying electrophoretic relationships in plants.

Among the eight varieties studied; Mysore local, Jakkur local and Selection – 41 (S41) showed more height whereas, Berhampur, Sukasaskwa revealed less height ,on the other hand mutants, M.R.Mildew and Coonoor-11 have showed more number of primary and secondary branches (Table 1).

Table 1. Morphological characters in Mulberry varieties.

Sl. No.	Variety	Shoot Length	No. of primary branches	No. of secondary branches
1.	Berhampur	104.21	11.41	1.17
2.	Sukasakwa	86.22	10.21	4.00
3.	Jakkur local	109.24	12.00	10.41
4.	Mutants	88.75	25.21	35.21
5.	M.R. Mildew	86.41	25.24	36.21
6.	Coonoor-II	85.52	23.31	41.44
7.	Selection-41	120.40	15.14	20.44
8.	Mysore local	134.20	10.00	25.00

The peroxidase enzyme of these varieties showed much differetiation in isoenzyme banding patterns. Among these varieties studied selection-41 showed 2 bands,

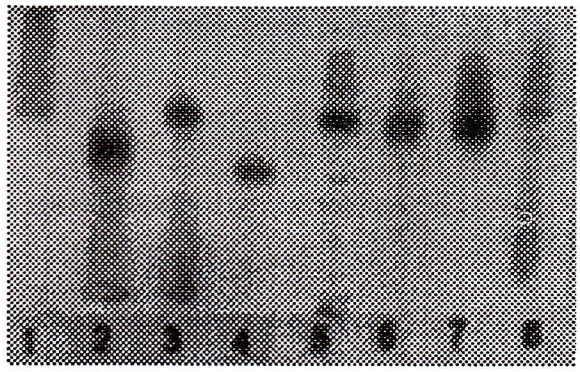

Fig.1.

Sukasakwa, Mutants showed three bands, while Jakkur local, Coonoor-11 and Mysore local revealed four bands each.Whereas,Berhampur local is having five bands (Fig.1). The details of RF values and band intensities of these eight varieties are given in Table 2.

Table 2. Showing details of number of bands and Rf values.

Sl. No.	Varieties	No. of Bands	RF Values
1.	Berhampur	5	0.12, 0.15, 0.20, 0.37 and 0.40
2.	Sukasakwa	3	0.12, 0.51, 0.57
3.	Jakkur local	4	0.12, 0.16, 0.21, 0.41
4.	Mutants	3	0.12, 0.16, 0.21, 0.41
5.	M.R. Mildew	5	0.12, 0.16, 0.20, 0.31 and 0.40
6.	Coonoor-II	4	0.12, 0.16, 0.35, 0.40
7.	Selection-41	2	0.12, 0.40
8.	Mysore local	4	0.12, 0.16, 0.20, 0.35

Analyses of the results have indicated that all the varieties of the Mulberry have revealed differentiation in the electrophoretic mobility, and this peroxidase may play an important role in regulation of cell growth and differentiation. The Link between peroxidase and hormones is supported by the finding that mutations affecting morphological characters, growth and development of differentiation may reveal abnormal peroxidase activity[28].The exact physiological metabolism of peroxidase in plant kingdom is still obscure due to multiplicity of its functions, the peroxidase activity was co-related with growth, development and hormonal activity[29]. The occurrence of each isozyme band is controlled by a gene[30], though it may be due to polygenic in function[31]. These studies revealed the utility of leaf peroxidase banding pattern in resolving the genetic differenes the seemingly types of Morus, particularly at intraspecific level.

Further, the similarly index value (Table 3) between Berhampur local and M.R.Mildew is 80.00. And

Table 3. Matrix of similarity index (%) of peroxidase isozymes of different mulberry varieties.

Sl. No.	Mulberry varieties	Berhampur	Sukasakwa	Jakkur local	Mutants	M.R.Mildew	Coonoor -11	Selection -11	Mysore local
1	Berhampur	—	14.28	80.00	25.00	80.00	50.00	40.00	50.00
2	Sukasakwa	14.28	—	16.66	50.00	14.28	16.66	25.00	16.66
3	Jakkur Local	25.00	16.00	—	28.57	14.44	37.50	33.33	37.50
4	Mutants	25.00	33.33	28.57	—	25.00	28.57	20.00	28.57
5	M.R.Mildew	80.00	14.28	44.44	25.00	—	33.33	28.57	33.33
6	Coonoor – 11	50.00	16.66	37.50	28.57	33.33	—	33.33	25.00
7	Selection-11	40.00	25.00	33.33	20.00	28.57	33.33	—	16.66
8	Mysore local	50.00	16.66	37.50	28.57	33.33	25.00	16.66	—

50 % seems to be the highest of all the varieties compared, and leaves are thick larger and without lobe and well developing roots. Whereas the leaves M.R. Mildew showing less and medium in leaf variation. The similarity index value between Coonoor- 11, Berhampur, Mutants, Selection- 41 and Mysore local is the next highest of 50 and 40 % respectively which shows that these also more closely related than the other varieties studied. The similarity index values between Coonoor- 11, M.R.Mildew, and Jakkur local are 50,44and 37.50 %. The values between selection, Jakkur local is 33.33 % because the morphologically, Jakkur local revealed small leaves with lobe, medium colour and fast growing root. The similar results is also noticed between Selection – 41 , Coonoor-11 , M.R. Mildew, Jakkur local , Mysore local and Mutants revealed more or less 33.33 percentage, whereas other varieties also express similar values. Therefore the closeness between these different varieties showing morphological similarity but electrophoretically heterozygous character.

The low degree of similarity index is noticed between M.R. Mildew and Sukasakwa is 14.28, Coonoor-11 and Sukasakwa is 16.66, Mysore local and Selection - 41 is 16.66 and Selection-41 and Mutans have 20.00 percentages. These results clearly indicate that these varieties sufficiently differ from each other although they belong to the same genus (Table 3). Therefore, the classification based on the morphology agrees well with the results obtained from electrophoretic data. However, variety 7 shows low number of genes being represented (n2) and could be homozygous for Pox -Group II and heterozygous for group I while variety 2 is homozygous for Pox group- 1. While varieties 1, 5, 8 show heterozygous for Pox group-1.

1. Since Pox in this case shows a maximum of 7 iso from 8 varieties and are distinct from one another. The RF valuses for each band have been calculated from mid point, wherever thick zones of activity are seen. The RF values in conjunction with visual scoring of bands will not lead to misinterpretation.
2. Each variety tested in a pooled sample of ten individuals randomly collected and hence would be representative population.
3. It has supported POX being a monomer the interpretation is straight forward and simple as is followed in statistical analysis.

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