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IDENTIFICATION & CHARACTERIZATION OF INDIAN RICE CULTIVARS – MDH ELECTROPHORETIC STUDY

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Abstract
Rice (Oryza sativa) is one of the most important crop of the world and also it is principle food of the half of the world's human population. No considerable work on Indian rice cultivars have been done as yet. Therefore in this research attempts have been made to study the PAGE pattern of MDH enzyme and try to collect the data which is helpful in the identification of cultivars of Indian rice. MDH is an enzyme known to have multiple molecular forms (Kitto & Wilson 1966; Honold et.al 1967 plays an important role in the catabolic activities of the living organism. MDH catalyses the conversion of malate to oxaloacetate in Citric Acid Cycle.

Introduction

Malate dehydrogenase (MDH), an enzyme known to have multiple molecular forms (Kitto & Wilson, 1966; Honold et.al., 1967) plays an important role in the catabolic activities of the living organisms. It is one of the most important enzymes of carbohydrate metabolism. MDH catalyses the conversion of malate to oxaloacetate in Citric Acid Cycle.

It has been proposed that different isozymes of MDH are localize in different cellular compartments, such as mitochondria, peroxisome and even in chloroplasts (Rocha & Ting, 1971; Yang and Scandalios, 1974). This enzyme is concerned with the respiratory process and carboxylation phenomena in plants. The oxaloacetate produced by the carboxylase enzyme can be reduced to malate by MDH.

Mitra et.al. (1979) carried out electrophoretic work on this enzyme in the genus Hordeum. Dogra et.al., (1985) used this enzyme as a penamete in cultivar identification of fenugreek. MDH was studied alongwith many other enzymes in the identification of barley varieties by Nielson and Johansen (1986). Tiwari et.al. (1985) used it to differentiate the species of cucurbits. Jha (1989) successfully utilised this enzyme in identifying the cultivars of sesame. Wilkinson (1985) used this enzyme alongwith peroxidase, esterase and catalase for identifying different cultivars of *Nicotiana tabaccum*. Dogra (1992) used this enzyme in the identification of 60 Indian cultivar of chickpea along with other enzyme systems & seed general protein. Dogra & Dogra (1998) also attempted to distinguish the cultivars of pigeonpea with the help of this enzyme along with peroxidase, esterase & GDH.

Material and Method MDH Extract

Approximately 250mg seeds soaked overnight in 0.1M tris-HCI buffer (pH-8.0), crushed in 5cc of the same buffer, centrifuged for 20 minutes at 5,000g and supernatant kept in the test tube.

Electrophoretic Procedure

All the reagents were kept at room temperature for one hour before use. Clean and dried tubes were inserted vertically into the rubber holders of the gel polymerization tray. The reagents for separating gels were mixed in the proportion mentioned below and poured into the glass tubes upto $3/4^{th}$ the length with a pipette. The reagents were mixed in the proportion- Buffer A: Distilled water: Monomer C: Catalyset 'E' (1:1:2:4), pH8.9. After one and a half minute the tubes were over layered with distilled water droplets to obtain a smooth flat gel surface. Gels in the tubes were allowed to polymerise. After ploymerisation which takes approximately 40-45 minutes, water droplets were removed from the tubes and the tubes were again fixed into the polymerization tray. Stacking

gel solutions, prepared in the following proportion from the stock solution- Buffer B: Momoner D:Catalyst F: Distilled water (1:2:1:4), pH 6.7 was poured into the tubes, leaving the top 2-3mm of the tubes empty. After 5 minutes, the gels were again overlayered with distilled water droplets. After polymerization (nearly 20-25 minutes), the tubes were removed from the polymerization tray carefully and overlying water was drained out. The tubes were inserted vertically into the rubber grommets of the upper reservoir of the electrophoretic apparatus. Prior to insertion of the tubes, the lower reservoir was filled $3/4^{th}$ with Tris-glycine buffer (pH-8.3). This buffer was diluted 10 times before use. The same buffer was then poured into the upper reservoir leaving the upper ends of the gel tubes emerging out. Seed extracts were then applied in the gel tubes with the help of a capillary tube. Prior to this 20% sucrose solution and one drop of 1% aqueous bromophenol blue (tracking dye) was added in the sample. Lid was placed over the upper reservoir and the electric leads were connected with the power pack. A contrast current (5mA per tube for protein and 1.5-2mA per tube for enzymes) was supplied through the power pack. After supplying current the upper reservoir was filled gently with the diluted Tris-glycine buffer until all the tubes got immersed in it. The current was allowed to pass till the bromophenol blue marker reached near the bottom of the gel tubes.

After electrophoresis, the gel tubes were removed from the apparatus. The gels were removed from the tubes by injecting the same buffer (Tris-glycine) in the glass tube, along the wall of the tube, without damaging the gel, by a 5cc glass syringe attached to a 5cm. long 21 guage needle. The gels were then treated separately for protein and enzyme studies.

The staining technique of Brewer & Singh (1960) was followed for their enzyme.
The gels were incubated in 0.2 M Tris-glycine buffer (pH-7.5) for about 20-30 minutes and then transferred to the stain solution and kept under light at 37^{O}c.

Stain solution:

L-malic acid	350 mg
Nicotinamide adenine Dinucletotide(NAD)	13.2 mg
M.T.T. tetrezolium	15 mg
Phenazine methosulphate	5 mg
Magnesium chloride	81.2 mg
Sodium cyanide	5 mg
0.2 M Tris-HCI buffer (pH-7.5) upto 100 ml	

After 30 minutes of staining, dark violet bands of the isozymes developed. Their Rp values were calculated.

The control gel was incubated without the substrate L-malic acid.

Observation

Seven bands with MDH activity were observed in 62 cultivars of Indian rice studied. Zymogram for each cultivar was drawn (fig- 1-4) .The characteristics of each cultivar in terms of MDH banding is as follows:-

Cultivar

1. Four MDH bands having Rp values – 0.30, 0.45, 0.52, 0.6 were observed in this cultiar.
2. Three MDH bands having Rp values – 0.45, 0.52, 0.6 were observed in this cultivar.
3. This cultivar exhibited only one band of MDH activity having Rp values - 0.6.
4. In this cultivar four MDH bands having Rp values – 0.30, 0.45, 0.52, 0.6 were observed.
5. This cultivar is represented by four MDH bands having Rp values- 0.25, 0.3, 0.52, 0.6.
6. Two MDH bands with Rp values- 0.45, 0.6 were observed in this cultivar.
7. This cultivar exhibited three MDH bands having Rp values – 0.25, 0.6, 0.7.

8. Three different types of MDH bands having Rp values – 0.25, 0.6 , 0.7 were observed in this cultivar.
9. Only two MDH bands having Rp values 0.6, 0.7 were present in this cultivar.
10. This cultivar also exhibited two MDH bands having Rp values 0.45, 0.6.
11. This cultivar is represented by three MDH bands having Rp values – 0.45, 0.6, 0.7.
12. Three MDH bands having Rp values- 0.18, 0.6, 0.7 were observed in this cultivar.
13. Only one MDH band having Rp vaules – 0.6 was observed in this cultivar.
14. Three MDH bands having Rp values – 0.3 0.45, 0.6 were present in this cultivar.
15. Altogether five different bands of MDH activity were observed in this cultivar. Their Rp values are – 0.18, 0.25, 0.45, 0.52, 0.6.
16. This cultivar is represented by three MDH bands having Rp vaules – 0.45,0.52, 0.6.
17. Four different types of MDH band having Rp values – 0.3, 0.45, 0.52, 0.6 were observed in this cultivar.
18. Three MDH bands with Rp values – 0.3, 0.45, 0.6 were found in this cultivar.
19. This cultivar is represented by three MDH bands having Rp values – 0.3, 0.45, 0.52, 0.6.\
20. This cultivar exhibited three MDH bands having Rp values – 0.3, 0.45, 0.52, 0.6.
21. Like the previous cultivar, this cultivar also exhibited three MDH bands with Rp values- 0.3, 0.45, 0.6.
22. This cultivar exhibited four MDH bands having Rp values – 0.25, 0.6, 0.7 were recorded in this cultivar.
23. Four different types of MDH bands having Rp values – 0.25, 0.45, 0.6 0.7 were recorded in this cultivar.
24. This cultivar also exhibited four MDH bands having Rp values- 0.25, 0.45, 0.6, 0.7.
25. In this cultivar also four MDH bands having Rp values- 0.25,0.45, 0.6, 0.7 were observed.
26. Five different type of MDH bands having Rp values – 0.18, 0.45, 0.6, 0.7 were recorded in this cultivar.
27. Only three MDH bands having Rp values- 0.25, 0.45, 0.6 were observed in this cultivar.
28. Three MDH bands with Rp values- 0.25, 0.45, 0.6 were present in this cultivar.
29. Five different MDH bands having Rp values – 0.18, 0.25, 0.3 ,0.45 and 0.6 were present in this cultivar.
30. This cultivar exhibited four types of MDH isozymes having Rp values- 0.18, 0.3, 0.45, 0.6.
31. This cultivar also exhibited similar four MDH bands as found in cultivar 30 with Rp values – 0.18, 0.3, 0.45, 0.6.
32. Cultivar 32 represented by five MDH isozymes having Rp values – 0.18, 0.25, 0.3, 0.45 and 0.6.
33. Three MDH bands having Rp values – 0.3, 0.45, 0.6 were present in this cultivar.
34. This cultivar also exhibited three MDH bands having Rp values- 0.3, 0.52, 0.6.
35. four MDH bands with Rp values- 0.18, 0.3, 0.45, 0.6 were present in this cultivar.
36. Three MDH bands with Rp values – 0.3, 0.52, 0.6 were present in this cultivar.
37. Single MDH band with Rp values – 0.6 was observed in this cultivar.
38. Only two MDH bands having Rp values – 0.3, 0.6 were present in this cultivar.
39. This cultivar also exhibited two MDH isozymes having Rp values- 0.25, 0.3, 0.52, 0.6.
40. This cultivar exhibited four MDH bands with Rp values – 0.25, 0.3, 0.52, 0.6.
41. Three MDH bands having Rp values - 0.3, 0.52, 0.6 were present in this cultivar.
42. Similar three bands in cultivar 41, having Rp values – 0.3, 0.52, 0.6 were recorded in this cultivar.
43. Only two MDH bands having Rp values – 0.25, 0.6 were observed in this cultivar.
44. Three MDH bands with Rp values- 0.18, 0.3, 0.6 were present in this cultivar.
45. This cultivar also exhibited three MDH bands having Rp values- 0.3, 0.52, 0.6.
46. Two MDH isozymes with Rp values – 0.45, 0.6 were present in this cultivar.
47. This cultivar also exhibited only two MDH bands with Rp values- 0.45, 0.6.
48. Three MDH bands with Rp values- 0.45, 0.52, 0.6 were observed in this cultivar.
49. Like the previous cultivar, this cultivar also exhibited three MDH bands having Rp values – 0.45, 0.52, 0.6.

50. Only two MDH bands with Rp values – 0.45, 0.6 were present in this cultivar.
51. Four MDH bands having Rp values – 0.3, 0.45, 0.52, 0.6 were observed in this cultivar.
52. Four different types of MDH bands having Rp valuels – 0.18, 0.3, 0.6, 0.7 were observed in this cultivar.
53. This cultivar exhibited three MDH bands having Rp values- 0.3, 0.52, 0.6.
54. Single v band with Rp values – 0.6 was observed in this cultivar.
55. Three MDH bands observed in this cultivar have following Rp values- 0.3, 0.45, 0.6.
56. This cultivar also exhibited three MDH bands with Rp values- 0.25, 0.45, 0.6.
57. This cultivar is represented by four MDH bands having Rp values- 0.18, 0.3, 0.52, 0.6.
58. Like the previous cultivar this cultivar also exhibited four MDH bands with Rp values- 0.18, 0.3 ,0.45, 0.6.
59. This cultivar exhibited five MDH bands having Rp values- 0.18, 0.25, 0.3, 0.52, 0.6.
60. Four MDH bands having Rp values- 0.18, 0.25, 0.3, 0.6 were present in this cultivar.
61. This cultivar also exhibited four MDH bands with Rp values – 0.25, 0.45, 0.6, 0.7.

This cultivar is represented by three MDH bands having Rp values – 0.3, 0.45, 0.6.

Discussion

A total of seven MDH bands were observed for MDH isozyme system. These MDH bands are represented by following Rp values – 0.18, 0.25, 0.3, 0.45, 0.52, 0.6 and 0.7. Only one of these seven bands, having Rp value 0.6 was monomorphic, i.e. present in all the 62 cultivars studied. The other six bands were polymorphic, i.e. present in some and absent in other cultivars. The second most prevalent MDH band having Rp value 0.45 was noted to be present in 40 cultivars Band with Rp value 0.30 was present in 32 cultivars.
MDH band with Rp values – 0.18, 0.25, 0.52 and 0.7 were present in 14, 19, 19 and 12 cultivars respectively.

On the basis of MDH banding pattern, all the 62 cultivars of rice studied could be arranged into 24 groups. The first 12 groups consisted of one cultivar each. These groups alongwith their representative cultivar and their MDH banding pattern are :

Group	MDH Banding Pattern	Cultivar
1	0.6, 0.7	9
2	0.3, 0.6	38
3	0.25, 0.6	43
4	0.45, 0.6, 0.7	11
5	0.18, 0.6, 0.7	12
6	0.18, 0.3, 0.6	44
7	0.18, 0.3, 0.6, 0.7	52
8	0.16, 0.3, 0.52. 0.6	57
9	0.18, 0.25, 0.3, 0.6	60
10	0.18, 0.25, 0.45, 0.52, 0.6	15
11	0.18, 0.25, 0.45, 0.6, 0.726	
12	0.18, 0.25, 0.3, 0.52, 0.659	

The next three groups consisted of two cultivars each. The cultivars belonging to these subgroups alongwith their MDH banding pattern are –

Group	MDH Banding Pattern	Cultivar
13	0.25, 0.6, 0.7	7 & 8
14	0.25, 0.3, 0.52, 0.6	5 & 40

15	0.18, 0.25, 0.3, 0.45, 0.6	29 & 32

Group 16 included three cultivars. These cultivars with their MDH banding pattern are :

Group	MDH Banding Pattern	Cultivar
16	0.25, 0.45, 0.6	27, 28 & 56

Further three groups consisted of four cultivars each. These are :

Group	MDH Banding Pattern	Cultivar
17	0.6,	3, 13, 37 & 54
18	0.18, 0.3, 0.45, 0.6	30, 31, 35 & 58
19	0.45, 0.52, 0.6	2, 16, 48 & 49

Two groups (group 20 & 21) included five cultivars each. These cultivars belonging to the two groups alongwith their MDH patterns are :

Group	MDH Banding Pattern	Cultivar
20	0.3, 0.45, 0.52, 0.6	1, 4, 17, 19 & 51
21	0.25, 0.45, 0.6, 0.7	22, 23, 24, 25 & 61

The next two groups included six cultivars each. The groups alongwith their representative cultivars and MDH pattern are:

Group	MDH Banding Pattern	Cultivar
22	0.45, 0.6	6, 10, 39, 46, 47 & 50
23	0.3, 0.52, 0.6	34, 36, 41, 42, 45 & 53

The last group all the remaining seven cultivars.

Group	MDH Banding Pattern	Cultivar
24	0.3, 0.45, 0.6	14, 18, 20, 21, 33, 55 & 62

Thus it is evident that MDH activity of seeds could distinguish the 62 cultivars of rice studied into 24 groups. Obviously this isozyme system alone cannot be used for rice cultivar identification, but can be used in conjunction with other isozyme systems.

Discussion and Conclusion

Reliable identification of cultivars using classical method based on morphological and psychological character has become increasingly difficult because of large number of lines being released and convergence of these lines on a few of the most desirable character. Even the use of highest possible number of morphological character is often insufficient for distinguishing some cultivars Identification of Crop cultivars and genotype in breeding population at all stages of the life cycle is important in day-to-day handling of the breeding stocks. Two other aspects of genotype identification requires additional attention-maintenance of varietal purity and discriminatory markers for identification of protected varieties. Electrophoresis of seed extracts followed by appropriate protein or activity stains offer considerable promise in these areas. These techniques are all based on the concept that each cultivar is distinct and relatively homogeneous at the genetic level.

Seed material is relativily easy to handle with respect to protein extraction and more important, the seed may be regarded as a physiological state. In taxonomic studies, it is critical to compare organs at the same stage of development and this applies to chemistry as well as morphology. In this sense, the seeds and its proteins may be regarded as " conservative " unit, little affected by the environment, geographic origin, seasonal fluctuation and chromosomal rearrangements (Ladizinsky & Hymowitz, 1979; Vaughan, 1983). The biological characters compliment the morphological ones ensuring more reliability and stability. In addition, morphological characters are not simultaneous in time, hence their comparision requires a prolonged period of time.

MDH with 7 different bands distinguished the cultivars into 24 groups, out of which the first 12 groups consisted of one cultivar each and the largest group included 7 cultivars.

All these findings lead to the conclusion that seed proteins are a good reflection of the genotype of the individual cultivars and that they are a useful tool in the study of taxonomy of rice cultivars.

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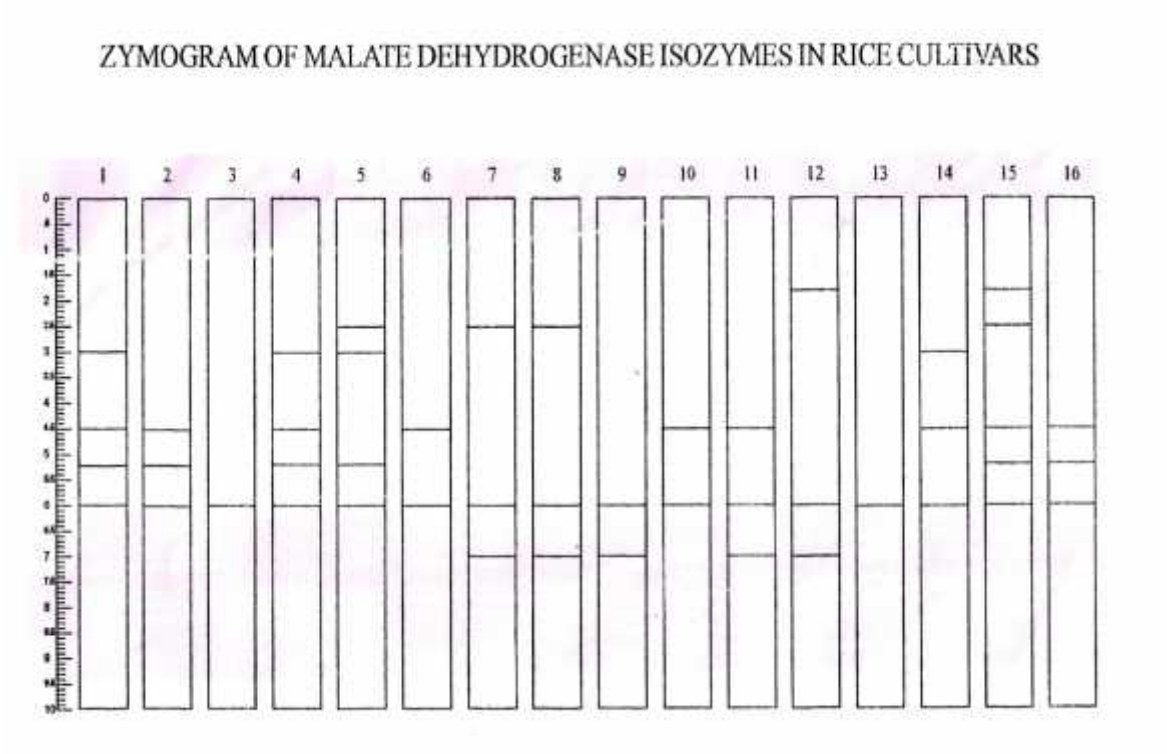

Figure 1

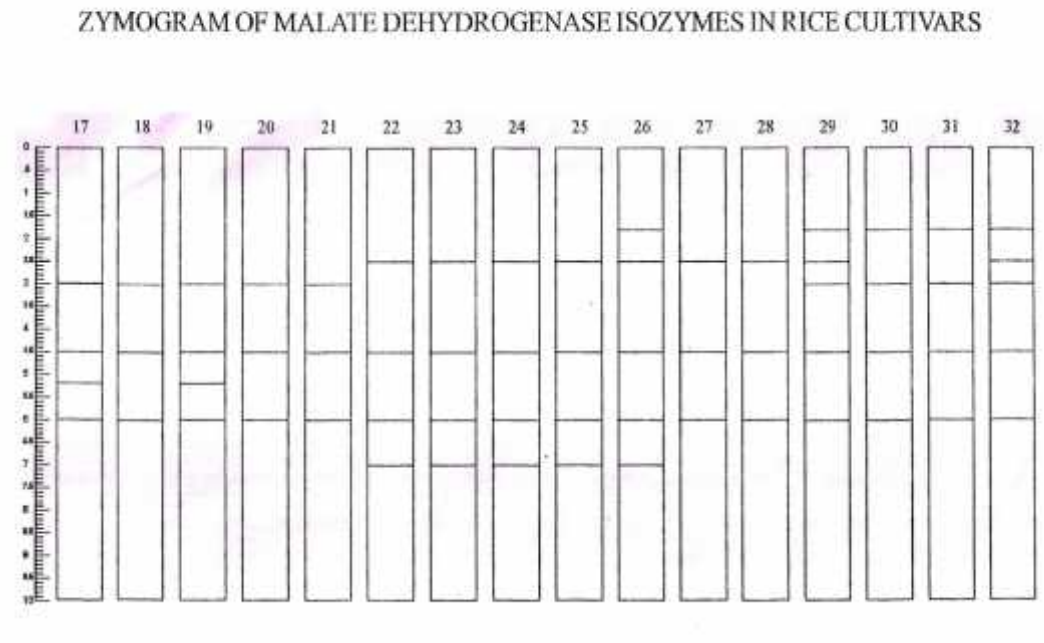

Figure 2

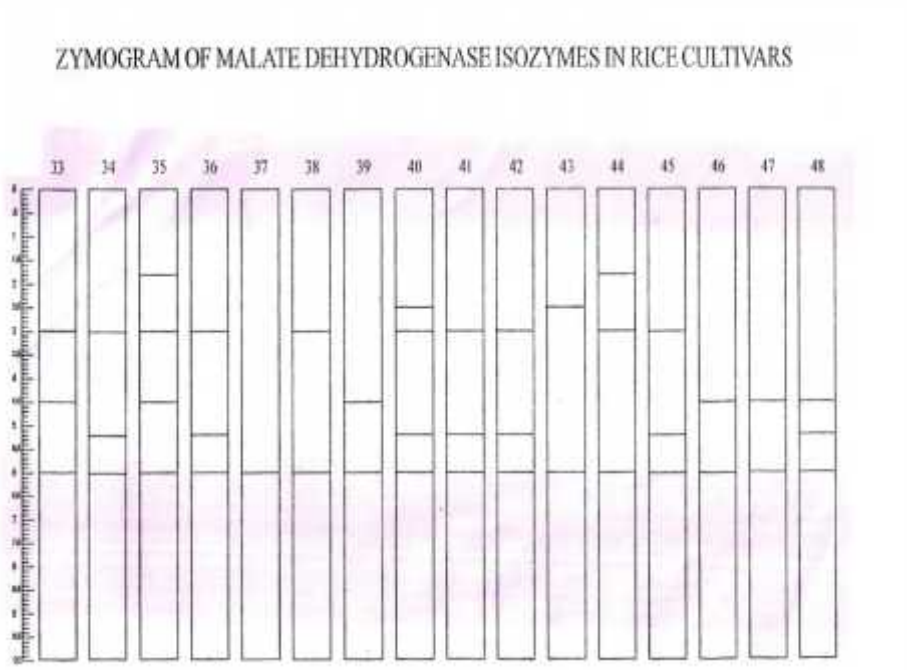

Figure 3

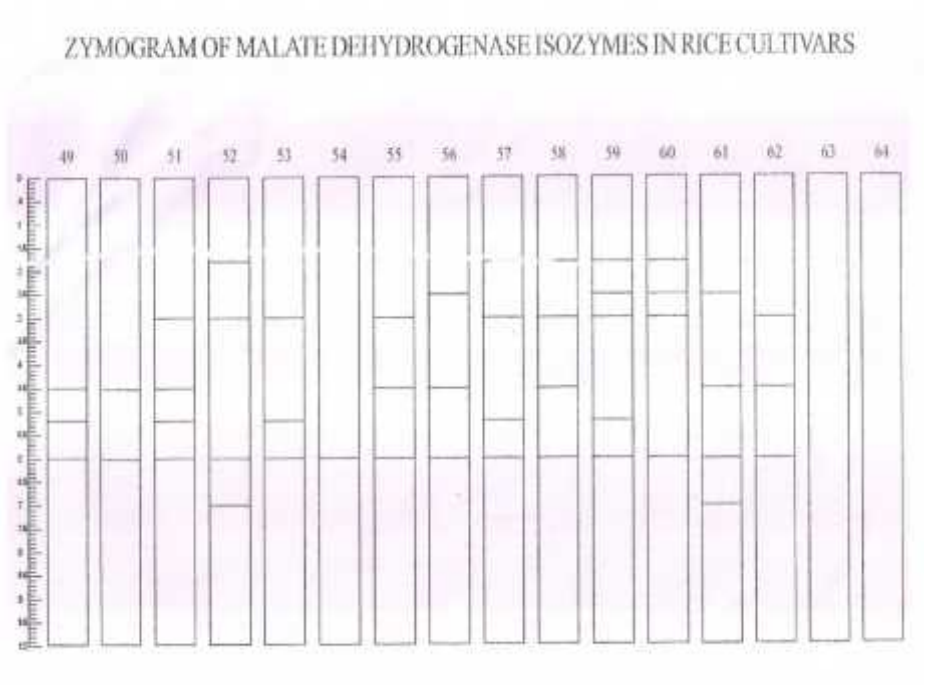

Figure 4