SHORT COMMUNICATION

ISOZYME ANALYSIS ON SOME TRADITIONAL RICE VARIETIES FROM SOUTH VIETNAM

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In a plant breeding context, many morphological markers have undesirable effects on plant phenotype and their use in rice improvement has been limited. Developments in the electrophoretic separation of proteins and in the exploitation of recombinant DNA technology have dramatically increased the number of genetic markers available for use in plant breeding. The most widely used protein markers in plant breeding are isozymes.

The application of isozymes in rice genetics has been reviewed (Glaszman 1988).

The intraspecific variation in *Oryza sativa* L. is remarkably extensive and subspecific classification has been important research for rice breeders and genetics.

Biochemical methods of investigation especially isozyme studies, have provided valuable tools for rice geneticists. Electrophoretically, identifiable isozymes have often been utilized the classification of varieties within *O. sativa*. Oka (1958), Chu (1967), Shahi et al (1969), Pai et al (1973), Pu and Pai (1979) showed the existence of the peroxidase alleles specific to indica and japonica groups.

Based on isozyme polymorphism, rice germplasm has been classified into six varietal groups (Glazsmann, 1987).

Location of isozyme loci on the rice chromosomes has simultaneously progressed by linkage analysis (Glaxsmann1985b, Nakagahra and Hayashi 1976, Pai et al 1975, Sano and Babier 1985, Sano and Morishima 1984, Pham et al. 1990) and by trisomic analysis (Ishikawa et al 1986, Ranjhan et al 1986, Wu 1987). Isozyme loci have alleles that are no longer transcribed or that code for defective polypeptides lacking enzymatic activity are generally referred to as 'null alleles' (Khush et al, 1995). Second (1982) surveyed 40 presumed loci, 25 of which were polymorphic within *O. sativa* both indica and japonica types. With 14 polymorphic loci, Glazsmann et al (1984) similarly found a strong differentiation of the varieties towards the indica and japonica types.

The cultivars in the indica group which were awnless, sensitive to potassium chlorate, sensitive to low temperature, resistant to drought and positive color reaction with phenol, were mostly distributed in continental Asia. e.g. India, South China and Indo-China.

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Fifty-three Vietnamese rice varieties in the Mekong Delta, and High Land were provided by Cuu Long Delta Rice Research Institute (CLRRI) (Table1). Two markers accessions (Acc23754 and IR36) were provided by the Genetic Resources Center (GRC) at the International Rice Research Institute (IRRI).

The Vietnamese rice varieties were analyzed through starch gel electrophoresis to 10 enzymes representing 19 loci: **Cat-1, Est-1, Est-2, Est-5, Est-9, Amp-1, Amp-2, Amp-3, Amp-4, Enp-1, Sdh-1, Icd-1, Adh-1, Got-1, Got-3, Pgi-1, Pgi-2, Pdg-1, Pgd-2.**

Ten seeds of accession were germinated in petri dishes lined with moist filter paper and were placed in a growth cabinet at 30^0C for 5 days. These seedlings will be used for the crude extract.

Four seedlings per accession were analyzed. The one seedling was ground in a spot plate by labeling each depression. The seedlings were cut into small pieces and adding 2 drops of 0.01% mercaptoethanol. The leaves sample were macerated with a glass rod to produce a crude extract. Four small strips of Whatman No.3 filter paper (5x15 and 5x12mm per strip) were put in each depression of the spot plate and allowed the crude extract to be absorbed.

Gel preparation

Each one gel mold was labeled buffer system I, and buffer system II. Then a thin coating of 50% glycerol was applied in each mold. 77g of starch was put into 1000ml erlenmeyer flasks and 27.5ml of system I buffer were added and 522.5ml distilled water were poured into the flasks. System II, 58g of starch and 45ml of system II buffer and 405ml distilled water were used. The above solution was stirred and heated using a hot plate stirrer until the gels thickened. Then the flasks was transferred into a microwave oven and was heated until they boil. After denaturating the solution was poured into the gel mold prepared earlier. Solid particles and air bubbles can be quickly removed with forceps. Each gel was covered with plastic wrapping film after cooling and can be left overnight inside refrigerator.

Loading the crude extracts

The gel insertion in each gel mold was prepared by first pulling back the plastic wrapping film from the cathodal end. Then a slit across the gel's width 5.0cm from the cathodal end was made by using a sharp scalpel and a straight edge. This slit is called the origin. The origin was lined with 1% bromphenol blue to serve as tracking dye by a Pasteur pipette. A pair forceps was used to take two strips of filter paper from the depression in the spot plate and the strips were blotted on a paper towel to remove excess crude extract. The filter paper strips were inserted into the origin side by side, starting at the left side working to the right. This procedure was repeated until all strips were aligned along the origin.

Electrophoresis

The plastic wrapping film was replaced on the gel's surface and the masking tape was removed from both ends of the gel mold tray.

Inside a refrigerator at 4-5^{0}C, the gel mold tray was mounted on to the electrode trays containing electrode buffer solution. This set up was connected to a direct current source, preferably a DC power supply for 4 hours at 50mA current.

Staining

After turning off the electric current the gel mold tray was removed from the refrigerator. All the plastic wrapping film and filter paper strips were removed and a second cut across the gel slab's width 9cm from the origin was made. The area between this cut and the origin is the gel slice, where the isozyme bands can be visualized after staining. The upper right of the gel slice was trimmed off to orienting it. The rest of the slab may be discarded. The gel slice was placed on a slicing bed and thin steel wire was down through the gel thickness to come up with 1mm slices for scoring. These 'scoring' slices were transferred to their appropriate plastic staining tray. The scoring slices were incubated in an oven. The incubating temperature will depend on the isozyme.

Scoring

The bands were scored by Glazsmann's methods (Glazsmann et al. 1988). After scoring the data were trasferred to the scoring sheets (Table 2).

Gel drying

In the staining tray, the scoring slices were trimmed some more with a scalpel, cutting approximately 1cm above and below their isozyme band lines. These trimmed gel strips will now be dried. The gel strips were aligned on top a filter paper at about 7 per sheet. The gel strips were covered with cellophane and the thick plastic sheet was replaced. The dryer was close and turn it on by setting its controls to 65^{0}C for 45 minutes. The gel dryer automatically shuts off at the end of the drying period.

Data analysis

Using the Algorithm of five diagnostic loci **(Pgi-1, Pgi-2, Amp-3, Amp-2, Amp-1**) to classify rice varieties (Glazsmann et al 1988).

The genetic polymorphism was analyzed by POPGENE software. The diversity at each locus and the frequencies of the alleles were calculated by the formula:

1. Frequencies of the alleles i = Number of alleles i / Total number of alleles. (where Number for alleles i = 2x (number of homozygote) for the allele i + number of heterozygote with alleles i + ...). Total number of alleles = 2x (total number of individuals)
2. Genetic diversity at locus "L" : $H = 1 - S P_i^2$ (where: P_i: frequency of the allele i; n: number of alleles observed at locus L).
3. Average number of alleles per locus = Total number of alleles observed at N loci / N

The clustering analysis was done on genotypes without missing data using the NTSYSpc.

The color reaction of grains was examined by using 2% phenol solution. Six seeds of accession was soaked in 2% phenol solution for 24 hours. Then the change in coloration was noticed.

Ten out of 19 investigated loci were polymorphic, they were **Est-1, Est-2, Est-9, Amp-3, Amp-4, Sdh-1, Icd-1, Pgi-1, Pgi-2, Pgd-1 (Figure 1)** and the percentage of polymorphic loci was 52.63% (table 3).

35 alleles were detected in the population and the average number of alleles per locus was 1.84 (Table 3).

The frequency of allele 3 of **Amp-3** was 1.91%, while allele 1 of **Amp-3** was 78.47%. Similarly, the frequency of allele 2 of **Pgi-1** was 15.09%, while allele 1 of **Pgi-1** was 84.91% (table 4).

The genetic diversity (H) was high for loci **Pgi-2** (0.3398), **Est-1** (0.4178), **Pgd-1** (0.4942), **Est-2** (0.5038), **Sdh-1** (0.5765); beside a very frequent one at loci was **Cat-1, Est-5, Amp-1, Amp-2, Enp-1, Adh-1, Got-1, Got-3, Pgd-2** (table 3**).** The average genetic diversity was 0.169.

The 53 investigated accessions were classified into group I (indica) (Table 2).

Five out of 53 accessions showed a negative color reaction with phenol. One accession was 5 points negative and 1 point positive; two accessions were 2 points negative and 4 points positive, and two accession was 1 point negative and 5 points positive. Forty eight accessions showed positive reaction (table 1). This confirms the positive phenol reaction is a good diagnostic criterion to discriminate indica and japonica varieties.

Within accession polymorphism was observed in 21 cases.

There were 17 cases showing two genotypes, 2 cases with three genotypes, and 2 cases with four genotypes (table 5 and 6).

The clustering analysis shows the existence of three main groups of varieties, at the level of similarity of 0.64 (Figure 2).

Although the clustering analysis was not performed for all genotypes, it shows that cases of within accession polymorphism have two kinds: either accessions includes very similar genotypes (e.g. Tau huong A and B), or very different genotypes (e.g. Nep than A and B *versus* Nep than C and D).

More accessions need to be analyzed to get a better picture of the isozyme polymorphism of the rice varieties from the Southern areas. This work shows that most of them are likely belonging to the isozyme group I and that about 40% of the accessions showed a within-accession variation.

Table 1. Isozyme analysis and phenol test of some traditional rice varieties originated from Southern areas

No.	Designation	Acc.No	Ecosystem of origin	Province	Isozyme analysis	Phenol test
32-129	Ba chuc	1545	DWR	An giang	I	++++++
25-111	Ba danh	1546	RF	Ca mau	I	++++++
35-58	Ba khieu	1547	DWR	Bac lieu	I	++++++
43-169	Bien tay		Upland	Bac lieu	I	++++++
28-219	Bong tay	1208	RF	Dong thap	I	++++++
34-38	Ca dung phen	1566	RF	Long an	I	++++++
47-105	Cai nuoc	1385	DWR	Ca mau	I	++++++
30-218	Di cu	1292	RF	Dong nai	I	++++++
49-63	Dich ran		Upland	Lam dong	I	+++++-
52-82	K135	1525	RF	Kien giang	I	++++++
15-133	K181	1510	RF	Kien giang	I	++++++
21-140	K194	1517	RF	Kien giang	I	++++++
14-139	K229	1515	RF	Kien giang	I	++++++
7-178	K234	1506	RF	An giang	I	++++++
37-56	K414	1520	RF	An giang	I	++++++
8-191	K429	1528	RF	An giang	I	++++++
19-128	K702	1519	RF	An giang	I	++++++
11-196	K732	1518	RF	An giang	I	++++++
48-73	Kieu lua		DWR	Vinh long	I	++++++
45-46	Koi mia	2360	Upland	Lam dong	I	++++++
53-185	Koitap	2362	Upland	Lam dong	I	++++++
46-202	Liep den trang	2370	Upland	Lam dong	I	++++--
2-216	Lua thom KG		RF	Kien giang	I	++++++

Table 1. (continued)

No.	Designation	Acc.No	Ecosystem of origin	Province	Isozyme analysis	Phenol test
4-212	Lun can		DWR	Kien giang	I	++++++
23-26	Lun duoi	1893	DWR	Ca mau	I	++++++
29-142	Men nhen	1647	RF	An giang	I	+++++-
6-211	Mot bui		DWR	Kien giang	I	++++++
13-153	Mot bui	1460	DWR	Ca mau	I	++++++
10-167	Mua so2		RF	Can tho	I	++++++
20-168	Mua94		RF	Can tho	I	+-----
26-119	Nang huong	2385	RF	Long an	I	++++++
33-130	Nang loan	1499	DWR	Vinh long	I	++++++
27-217	Nang sau do	0758	DWR	Binh thuan	I	++++++
3-214	Nang thom		RF	Long an	I	++++++
51-98	Nanh chon	1475	RF	Tra vinh	I	++++++
41-30	Nen con	1526	RF	An giang	I	++++++
42-10	Nep than	1704	RF	Soc trang	I	++++++
24-122	Nho vang	0887	RF	Long an	I	++++++
50-97	Soc nau		RF	An giang	I	++++++
5-215	Tai nguyen		DWR	Long an	I	+++++-
36-49	Tai nguyen		DWR	Can tho	I	++++++
22-126	Tau huong	1665	DWR	Long an	I	++++++
39-41	TD2	1182	RF	Can tho	I	++++++
38-50	TD6	1986	RF	Can tho	I	++++++
1-213	Than nong mua		RF	Kien giang	I	++++++
12-204	Thang cho dai	1449	DWR	Can tho	I	++++++
40-51	Thom vang	1763	RF	Long an	I	++++++
16-136	Tieu chet	1537	DWR	Soc trang	I	++++++
18-181	Tieu chet	1764	DWR	Can tho	I	++++++
44-88	Tieu chet	1766	RF	Soc trang	I	++++++
31-44	Trang chum		DWR	Can tho	I	++++++
9-172	Trang hoa binh	1482	DWR	Soc trang	I	++++++
17-156	Ve phich	1483	RF	Tra vinh	I	++++++

** RL: Rainfed lowland rice, DWR: deep water rice*

Table 3. Allelic richness and genetic diversity

No.	Loci	Sample size	No.of alleles	Genetic diversity (H)	Alleles
1	Cat-1	416	1	0.0000	$Cat1^1$
2	Est-1	296	2	**0.4178**	$Est1^1$, $Est1^0$
3	Est-2	344	3	**0.5038**	$Est-2^0$, $Est-2^1$, $Est-2^2$
4	Est-5	408	1	0.0000	$Est-5^1$
5	Est-9	368	3	0.2130	$Est-9^0$, $Est-9^1$, $Est-9^2$
6	Amp-1	424	1	0,0000	$Amp-1^1$
7	Amp-2	416	1	0.0000	$Amp-2^2$
8	Amp-3	418	3	0.3246	$Amp-3^1$, $Amp-3^2$, $Amp-3^3$
9	Amp-4	424	2	0.0372	$Amp-4^1$, $Amp--4^2$
10	Enp-1	424	1	0.0000	$Enp-1^1$
11	Sdh-1	424	4	**0.5765**	$Sdh-1^1$, $Sdh-1^2$, $Sdh-1^3$, $Sdh-1^4$,
12	Icd-1	424	2	0.0186	$Icd-1^1$,$Icd-1^3$
13	Adh-1	408	1	0.0000	$Adh-1^1$
14	Got-1	424	1	0.0000	$Got-1^1$,
15	Got-3	424	1	0.0000	$Got-3^{1'}$
16	Pgi-1	424	2	0.2562	$Pgi-1^1$, $Pgi-1^2$
17	Pgi-2	424	2	**0.3398**	$Pgi-2^1$,$Pgi-2^2$
18	Pgd-1	420	3	**0.4942**	$Pgd-1^1$, $Pgd-1^2$, $Pgd-1^3$,
19	Pgd-2	416	1	0.0000	$Pgd-2^1$
	Sum		35	3.2133	
	Average	**407**	**1.8421**	**0.1691**	

Table 4. Allele frequency of population

No	Loci	ALLELES								
		Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9
1	Cat-1	1.0000								
2	Est-1	0.7027								0.2973
3	Est-2	0.1686	0.6570							0.1744
4	Est-5	1.0000								
5	Est-9	0.8804	0.0054							0.1141
6	Amp-1	1.0000								
7	Amp-2		1.0000							
8	Amp-3	**0.7847**	0.1962	**0.0191**						
9	Amp-4	0.9811	0.0189							
10	Enp-1	1.0000								
11	Sdh-1	0.5236	0.3821	0.0566	0.0377					
12	Icd-1	0.9906		0.0094						
13	Adh-1	1.0000								

14	Got-1	1.0000		
15	Got-3	1.0000		
16	Pgi-1	**0.8491**	**0.1509**	
17	Pgi-2	0.7830	0.2170	
18	Pgd-1	0.3000	0.0571	0.6429
19	Pgd-2	1.0000		

Table 5. Isozyme loci involved in the within-accession polymorphism

No.	Loci	Number of cases
1	Cat-1	0
2	Est-1	2
3	Est-2	4
4	Est-5	0
5	Est-9	1
6	Amp-1	0
7	Amp-2	0
8	Amp-3	5
9	Amp-4	1
10	Enp-1	0
11	Sdh-1	6
12	Icd-1	1
13	Adh-1	0
14	Got-1	0
15	Got-3	0
16	Pgi-1	2
17	Pgi-2	5
18	Pgd-1	6
19	Pgd-2	0

Table 6. Within- accession polymorphism: number of genotypes observed from the 4 seeds of each accessions

	Number of Genotype			
	1	2	3	4
Number of cases	32	17	2	2

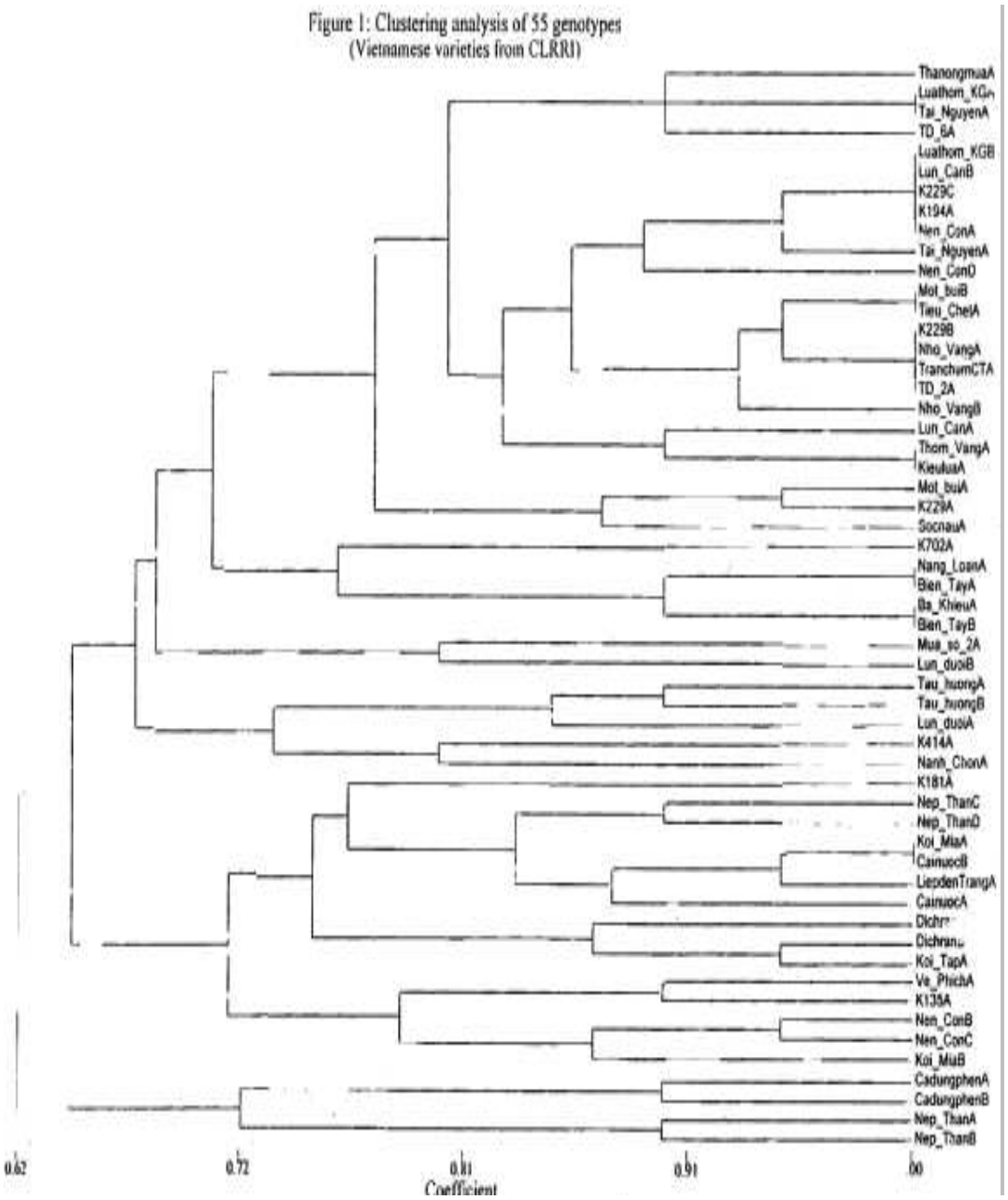

Figure 1. Polymorphism of Amp-1, Amp-2, Amp-3, Amp-4, Pgi-1, Pgi-2, Est-1, Est-2, Est-5, Est-9.

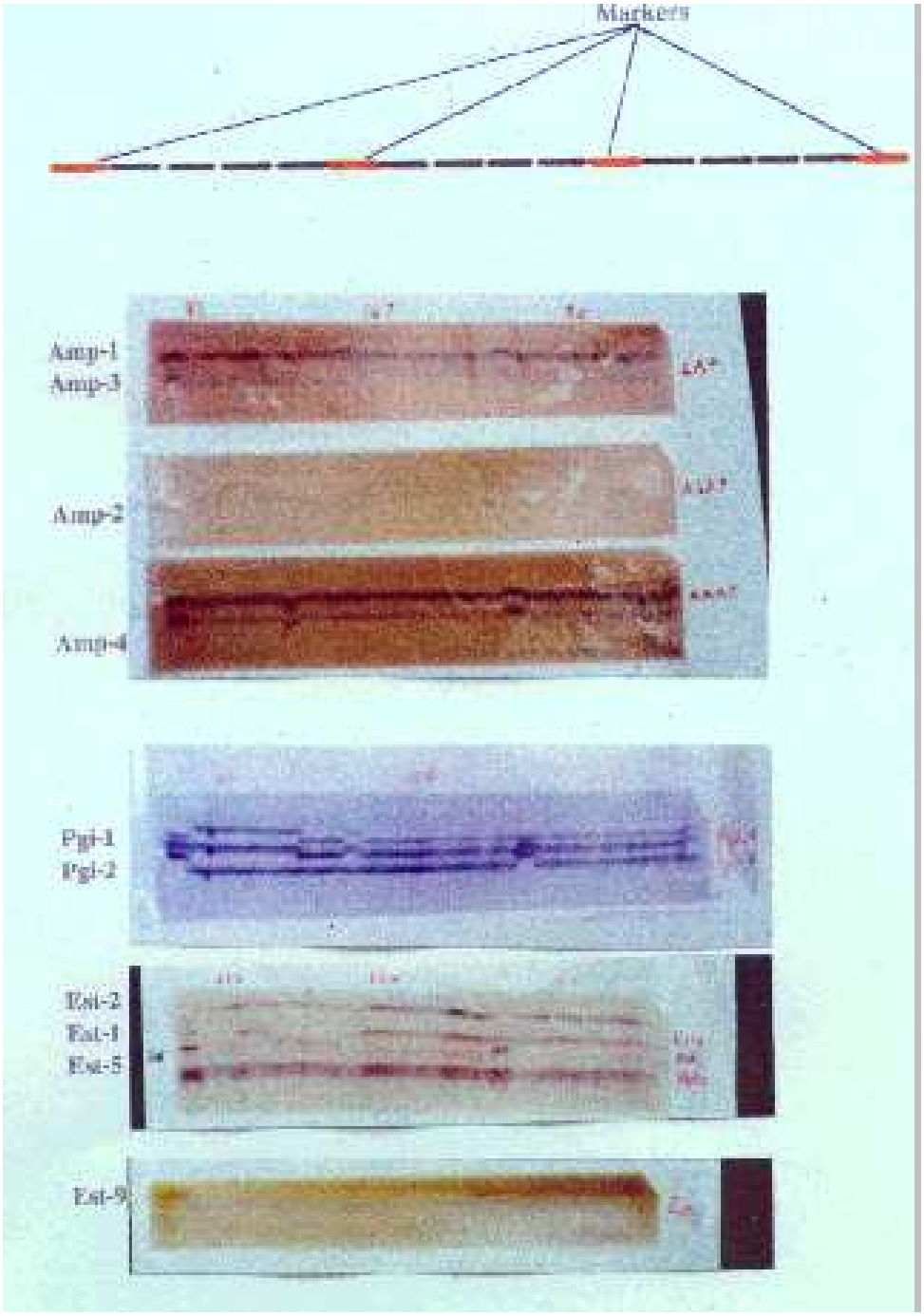

Figure 2.

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TÓM TẮT

Phân tích sự đa dạng di truyền bằng isozyme đối với một số giống lúa địa phương ở miền Nam

Mức độ đa hình về mặt di truyền của 53 mẫu giống lúa địa phương ở miền Nam đã được phân tích trên 19 loci của 10 isozyme. Các loci sau đây có mức độ đa hình cao là: ***Cat-1, Est-1, Est-2, Est-5, Est-9, Amp-1, Amp-2, Amp-3, Amp-4, Enp-1, Sdh-1, Icd-1, Adh-1, Got-1, Got-3, Pgi-1, Pgi-2, Pdg-1, Pgd-2.*** Theo phân loại chuẩn của Glazsmann, tất cả 53 mẫu giống đang nghiên cứu thuộc nhóm I (indica). Số giống lúa có phản ứng dương tính đối với phenol ở hạt thóc là 48 mẫu, và 5 mẫu có phản ứng âm. Mức độ đa hình trong cùng một mẫu giống cũng được ghi nhận trong 21 trường hợp của 53 mẫu giống, cho thấy sự đa dạng ngay trong một giống lúa địa phương. Phân tích nhóm di truyền chưa thể hiện đầy đủ đối với những kiểu gen này. Trong trường hợp đa hình trong cùng một mẫu giống, có hai dạng được ghi nhận: mẫu giống có kiểu gen hoàn toàn như nhau (thí dụ Tàu Hương A và B), mẫu giống có nhiều kiểu gen khác nhau (thí dụ Nếp Than A và B khác với Nếp Than C và D).