ISOZYMES VARIABILITY IN RICE MUTANTS INDUCED BY FAST NEUTRONS AND GAMMA RAYS

J.L. FUENTES, A. ALVAREZ
Centro de Estudios Aplicados al Desarrollo Nuclear (CEADEN),
Miramar, Playa, Havana, Cuba

L. GUTIERREZ, J.E. DEUS
Instituto de Investigaciones del Arroz,
Bauta, Havana, Cuba

Abstract

The isozyme variability of a group of rice mutants induced through gamma and fast neutron (14 MeV) irradiation was studied. Polymorphisms were detected using esterase, peroxidase, polyphenol oxidase and alcohol dehydrogenase systems. The mean value of genetic similarity among the different cultivars, which arose from isozymes, was 0.75. The dendrogram was constructed based on genetic similarity matrices, designed with isozyme data using the unweighed pair group method arithmetic average (UPGMA) method. The efficiency of the UPGMA model for the estimation of genetic relationship among cultivars was supported by cophenetic correlation coefficients. Such values indicate that the distortion degree for the estimated similarities was minimal. It was found that both gamma rays and fast neutrons generated a wide range of variability which can be detected by means of isozyme patterns, even in closely related cultivars.

1. INTRODUCTION

Practical application of plant mutation breeding has made good progress. This breeding technique has yielded genetic variants in many plant species and a certain proportion was found to be useful [1]. Among them, rice has received special attention: already by 1991, 251 new varieties had been introduced into the production [2].

In Cuba, a rice breeding program using fast neutrons and gamma radiation was initiated in 1988. In this program nearly thirty new mutants have been obtained [3].

However, there is very little information about the genetic variability generated by mutagens on these materials, which is crucial for the effective use of genetic diversity resources in breeding programs.

Biochemical methods of investigation, especially isozyme studies, have provided valuable tools for rice geneticists. Electrophoretically identifiable isozymes have often been utilized for the classification of varieties within *O. sativa* [4] and they have proved useful in diversity surveys of rice induced mutants [5]. In the present work the enzymatic polymorphisms in a group of rice mutants was studied.

2. MATERIALS AND METHODS

2.1. Plant material

Three varieties and 8 mutants, 2 to 4 from each variety, were studied (Table I); 4 mutants were induced by fast neutrons and 4 by gamma rays.

TABLE I. GENOTYPES USED IN THE SURVEY AND THEIR ORIGIN.

Variety/line	Origin	Radiation used[a]
Jucarito-104	IR-480-5-9-2/IR-930-16-1	--------
3762	Mutant of Jucarito-104 (20 Gy)	FN
3763	Mutant of Jucarito-104 (20 Gy)	FN
3764	Mutant of Jucarito-104 (20 Gy)	FN
3263	Mutant of Jucarito-104 (20 Gy)	FN
Gloria	--------	--------
G-C_{10}-2-1-7	Mutant of Gloria (300 Gy)	GR
G-C_{10}-2-1-8	Mutant of Gloria (300 Gy)	GR
Basmati	--------	--------
B-14-2-2	Mutant of Basmati (200 Gy)	GR
B-14-2-3	Mutant of Basmati (200 Gy)	GR

[a] FN = Fast neutrons, 14 meV; GR = ^{60}Co, gamma rays.

2.2. Isozyme assays

Electrophoretic assays were performed in vertical polyacrylamide gels with discontinuous buffer system basically as described earlier [6]. The staining techniques used for the esterase and peroxidase systems were reported by Iglesias and Gonzalez [7].

2.3. Data analysis

For bands' scoring, polyacrylamide gels were replicated at least three times. Only consistent and reproducible bands were considered.

Polymorphisms were scored for the presence or absence of bands and the data was analyzed using the NTSYS-PC version 1.8 [8]. The average proportion of alleles that are shared between any two of the accessions screened, was used as the measure of similarity. For inbreeding species, such as the present Cuban rice varieties, this corresponds to using the Nei and Li coefficient [9] or their algebraic equivalent, Dice's coefficient [10] for co-dominant marker data (isozymes). Cluster analyses were based on similarity or distance matrices using the UPGMA and relationships between accessions were visualized as dendrograms. The Mantel matrix correspondence test [11] was used to compare cophenetic and similarity matrices in order to define the congruence degree in the estimation of genetic relationships.

3. RESULTS AND DISCUSSION

The esterases and peroxidases isozyme systems were chosen because they revealed a good level of polymorphism in a previous study on fast neutron and γ-induced mutants [5]. In the present survey higher levels of polymorphism were detected with peroxidases rather than with esterases. Different authors [12, 13] have also reported this isozyme system as the most polymorphic one in plants.

In the peroxidase system, there were eleven polymorphic bands out of twelve, whereas esterase showed six polymorphic bands out of eight. Likewise, whereas the peroxidase system showed eight isozyme patterns among the analyzed genotypes, in the esterase system only six different patterns appeared (data not shown). In this survey, both disappearance and appearance of new bands were observed.

The number of isozyme patterns found in the peroxidase system, related to the Jucarito-104 genotypes, was higher than that obtained in previous studies [5], in which eleven cultivars were used. However, in the esterase system relatively less polymorphism was detected.

The wide distribution of genotypic variants across the different isozyme patterns is good evidence that a high degree of genetic variability was generated (Table II).

TABLE II. FREQUENCY (%) OF THE ISOZYME PATTERN VARIANTS OBTAINED.

Variant[a] No.	Isozyme system	
	Peroxidase	Esterase
1[b]	18.2	18.2
2	9.1	27.3
3	18.2	9.1
4	9.1	18.2
5	9.1	9.1
6	9.1	18.2
7	9.1	-
8	18.2	-

[a]Data not shown
[b]Variant in which the parental profiles are included.

Table III shows the similarity matrix calculated using the Dice index [10]. The genetic similarity mean value from isozymes data was S = 0.75. In general, the isozyme systems used could detect the existing variability, differences between the surveyed lines. These results agree with those obtained in other studies, where the genetic core of the Cuban rice germplasm bank was analyzed [14].

TABLE III. SIMILARITY MATRIX CALCULATED WITH DICE INDEX, USING BINARY DATA OBTAINED FROM PAGE ISOZYMES PROFILES.

	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.81	1.00									
3	0.88	0.94	1.00								
4	0.81	1.00	0.94	1.00							
5	0.88	0.81	0.88	0.81	1.00						
6	0.79	0.64	0.73	0.64	0.73	1.00					
7	0.86	0.73	0.80	0.73	0.80	0.94	1.00				
8	0.85	0.71	0.73	0.71	0.73	0.81	0.88	1.00			
9	0.66	0.57	0.60	0.57	0.73	0.69	0.77	0.69	1.00		
10	0.66	0.71	0.66	0.71	0.80	0.62	0.71	0.69	0.84	1.00	
11	0.66	0.71	0.66	0.71	0.80	0.62	0.71	0.69	0.85	1.00	1.00

The dendrogram that was constructed based on genetic similarity matrix using isozymes data and the NTSYS-PC computer pack [8] is shown in Fig. 1. All the studied varieties conformed to three well defined clusters. The first one included the J-104 cultivar and mutants, the second included Gloria and related mutant lines. Both mutant lines can be considered as Group I following Glaszmann's classification [4], which includes Indica rice type. The third one, however, is the more distant cluster (68% of genetic similarity with the rest of analyzed lines). The Basmati varieties are included in Group V of Glaszmann's classification [4]. This group holds intermediate type varieties, between the Indica and

Japonica morphological types, which are clearly differentiated from Group I [4]. This explains the large differences found in our survey between Basmati type varieties and the other cultivars.

Likewise, the efficiency of the UPGMA in the estimation of genetic relationships between varieties was corroborated by means of the cophenetic correlation coefficient (0.86, $P < 0.01$) calculated using Mantel test [11]. Such a value indicates that the distortion degree in the estimated similarity relationship was minimal. Furthermore, the results support the conclusion that the dendrogram in Fig. 1 shows a proper representation of the associated similarity matrix.

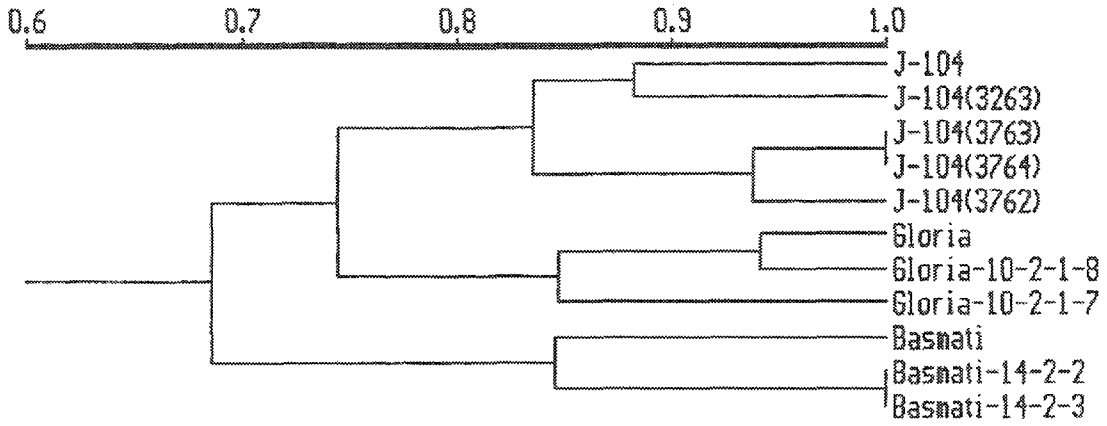

FIG. 1. Dendrogram corresponding to the similarity matrix (Table III) constructed using UPGMA analysis.

The dendrogram generated by the isozyme data agreed well with the genealogy of the rice materials studied (Table 1), showing the usefulness of isozyme markers in diversity surveys of induced mutants in rice.

The results in the present paper suggest that isozyme polymorphisms should be used in varietal certification. However, based on our experience with induced mutants in rice, good polymorphisms are not always obtained. The differential response of plant genotypes to ionizing radiations and the low loci number that can be surveyed by isozymes, should be the determining factors in the degree of the obtained polymorphism (data not published).

Analyses of data bases obtained from different markers types such as AFLP, RAPD and isozymes, offer great potentials in varietal validation, particularly in fast neutron - and gamma-induced mutants.

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