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Inter- and Intraspecific Variations in Wild Rice Species Detected Through Isozyme Markers

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Abstract: Isozyme analyses were used to detect inter- and intraspecific variation in different accessions of wild rice species, *O. officinallis* and *O. punctata*. Some F_1 hybrids between cultivated and wild rice species were also utilized. Fresh leaf extracts were electrophoresed on polyacrylamide gels. Different isozymes viz., Esterases, Glutamate oxaloacetate transaminases and peroxidases were used as biochemical markers. On the basis of isozyme banding profiles, distinct variations were observed between and within the two wild rice species and their different accessions. Based on polymorphic isozyme profiles, hybrids of rice cultivars, Basmati 198 x *O. officinalis*, Nonabokra x *O. malampuzhaensis* ace: 100957 and EF-6 x *O. malampuzhaensis* ace: 100957 were also identified. Our study demonstrated that isozyme markers can successfully be used for detection of genetic diversity as well as for the identification of hybrids.

Key words: Isozymes, Esterase, Glutamate Oxaloacetate Transaminase, Peroxidase, Inter- and Intraspecific variations, Wild rice

Introduction

Rice is a staple food of nearly 40% of the world's population. It is grown in different regions of the world with diverse climatic and agronomic conditions. The genus *Oryza* is recognized in 20 different species (Roschevicz, 1931). Most of these species are important reservoir of genes for high biomass, grain yield and quality, resistance against diseases and abiotic stresses (Jena & Khush, 1986) and can be used for creation of genetic diversity in cultivated rice varieties. However, before such utilization, it is imperative to detect inter- and intraspecific variability in the wild germplasm so that the right species and (or) accessions can be utilized in crop improvement programe.

To detect such genetic variations among species and varieties, morphological markers have been used which have some limitations (Riesberg and Ellstrand, 1993), as morphology could greatly be affected by environmental factors and number of available markers is very low. Isozyme markers on the other hand have certain advantages as isozyme loci are usually co-dominant and there is rarely any epistatic interaction (Kimura, 1983). The technique is very cheap and at a time, a number of samples can be analyzed. In Plant Molecular Breeding group of NIAB, isozyme markers are being routinely used to detect inter- and intraspecific variability among species and accessions of wild and cultivated rice varieties. In the present study variations detected through the use of isozyme markers in different accessions *of O. officinalis* and *O. punctata* and the use of such variation for the identification of F1 interspecific hybrids are being presented.

Materials and Methods

Material used in this study comprised 10 different accessions of two wild rice species: 5 each of *O. punctata* and *O. Officinalis*, 3 F_1 hybrids of rice cultivars EF-6 and Nonabokra with *O. malampuzhaensis* accession 100957 and Basmati-198 with *O. officinalis* accession: 105322 and their parents. Polyacrylamide Gel Electrophoresis (PAGE) was used for the separation of different isozymes of Esterases (Est), Glutamate Oxaloacetate Transaminases (Got) and Peroxidases (Pox). Fresh leaves from the seedlings at three leaves stage were collected from the test material, crushed with sample buffer (Tris-HC1 with glycerol, pH 6.8) and centrifuged before loading on to the gel. The extracts were loaded (25μl) onto the stacking gel (2.5% Polyacrylamide, pH 6.8) where the isozymes form stacks when electrphoresed in an electric field. These stacks than enter the resolving gel (8-10 % Polyacrylamide, pH 8.8) where they are separated into different bands according to the size and charge. A non-dissociating discontinuous buffer system (Laemmli 1970) was used with Tris-glycine (pH 8.3) as electrode buffer. After electrophoresis, the gels were removed and treated with histochemical stains specific for each enzyme. The bands of different isozymes appearing on gels were photographed under white light. The 10 different accessions of *O. punctata* and *O. officinalis* were analyzed for 12 isozyme loci Est-1, Est-2, Est-3, Est-4, Est-5, Got-1, Got-2, Pox-1, Pox-2, Pox-3, Pox-4 and Pox-5. The numbering of the isozyme loci was done as described by Weeden & Marx (1984) in which the fastest migrating zone was designated as Est-I.

Results

Each accession of the two wild rice species showed a unique allozymic banding pattern (Table 1) and on the basis of that pattern they were identified. The percentages of allozymic polymorphism in *O. punctata* and *O. officinalis* are given in Table 2.

Esterases: Localized areas of Esterase isozyme activity were obtained as colored bands on the polyacrylamide gels. Weeden & Marx (1987) detected three isozyme loci from pea leaves but in this study 5 isozymes were found in the leaves of two wild rice species. Allozymes of Est-1 and Est-2 were stained as dense bands while rest of the allozymes appeared as light bands on the gels. It was concluded that allozymes of Est-1 and Est-2 having more catalytic activities in the leaves of wild rice species than Est-3, Est-4 and Est-5. Within the *O. punctata*, Est-1 was polymorphic while Est-2, Est-3, and Est-4 were monomorphic. All detected loci of Esterases were polymorphic within the *O. officinalis* and between *O. punctata* and *O. officinalis*.

When the Esterases isozyme profiles of the 3 Fl hybrids (EF-6 and *O. malampuzhaensis accession:* 100957, Nonabokra and *O. malampuzhaensis accession:100957*, Basmati 198 and *O. officinalis accession:* 105322) were compared with the profiles of their parents, only hybrid of EF-6 x *O. malampuzhaensis* was confirmed. The hybrid contained two bands of slow migrating isozymes of Est-2, (one from each parent) but fast migrating isozymes of Est-1 showed the same pattern as one of its parents *O. malampuzhaensis*. Hybrids between Nonabokra x *O. Malampuzhaensis* and Bas-198 x *O. officinalis* 105322 showed the same pattern of Est-1 and Est-2 as those of their wild parents. The intensity of fast moving isozyme bands of Est-1, however, was intermediate as compared to the parents (Fig. 2a).

Table 1: Isozyme polymorphism for 12 isozyme loci in *Oryza. punctata* and *O. officinalis*

Isozyme	Isozyme Locus	Number of Allozymes	
		O.punctata	*O. officinalis*
Esterases	Est-1		1 p(w,b)
	Est-2	1 p(w,b)	2 p(w,b)
	Est-3	1 p(w,b) 1 p(w)m (b)	2p(w,b)
	Est-4	2 p(b) m(w)	2 p(w,b)
	Est-5	2 p(b) m(w)	2 p(w,b)
Glutamate Oxaloacetate	Got-1	1 m(w,b)	1 m(w,b) 3 p(w,b)
Transaminase	Got-2	3 p(b) m(w)	1 m(w,b) 1 m(b)p(w)
Peroxidases	Pox-1	1 p(w,b) 1 m(w)	p(b)1 p(w,b)
	Pox-2	3 p(b) m(w)	1 p(w) m(b)
	Pox-3	1 p(w,b) 1 m(w)	p(b)4 p(w,b)
	Pox-4	--------	1 p(b) m(b)
	Pox-5	--------	1 p(b) m(w)

P = Polymorphic w = within the species b = between the species m = Monomorphic bands

Table 2: Percentage of allozymic polymorphism in *Oryza. punctata* and *O. officinalis*.

Isozyme system	Total number of bands	Polymorphic bands between species (%)	Polymorphic bands within species	
			O. punctata (%)	*O. officinalis* (%)
Est	16	14 (87.50)	10 (62.50)	09 (56.25)
Got	10	06 (60.00)	00 (00.00)	04 (40.00)
Pox	15	12 (80.00)	02 (13.33)	06 (40.00)

Est = Esterases Got = Glutamate Oxaloacetate Transaminases Pox = Peroxidases

Glutamate Oxaloacetate Transaminases: Got-1 and Got-2 appeared as clear sharp bands on the gels when stained with specific histochemical stains. Both loci were found monomorphic within the *O. punctata* however between the two species Got-1 was found polymorphic. Three allozymes of Got-1 and one of Got-2 were polymorphic within the *O. officinalis* and between *O. punctata* and *O. Officinalis* (Fig. 1) In the profiles of Glutamate oxaloacetate transaminases, 2 of the 3 FI hybrids (Nonabokra x *O. malampuzhaensis* and EF-6 x *O. malampuzhaensis)* showed the three banded pattern of fast moving allozymes of Got-1 which is a characteristic pattern of a dimeric enzyme in a heterozygote and this confirmed the hybridity of the test material (Fig. 2b). The hybrid between Basmati 198 and *O. officinalis* 105322 showed same profile as that of *O. officinalis*.

Peroxidases: Within the 2 species, isozyme variations were detected at Pox-1 and Pox-2 in *O. punctata* and at Pox-1, Pox-2 and Pox-3 in *O. officinalis* while between the species, all the loci of Peroxidases were polymorphic. Allozymes of Pox-1 and Pox-3 in *O. punctata* and Pox-1 and Pox-4 in *O. officinalis* were stained as sharp clear bands but Pox-2 locus in *O. officinalis* showed broad area of ghost band (Fig. 1b).

Discussion

Isozymes are direct products of the genes and for this reason they are reliable biochemical markers for species and hybrid identification. Different regions of enzyme activity appearing on the gels included, isozymes coded by different genes, isozymes coded by different alleles of the same gene and post translationaly modified enzymes. In our study, allozymes of a single gene, which are mostly inherited codominantly, were visualized as colored bands in a particular region of the gel. Allozymes of Esterases were found polymorphic for the detection of genetic diversity between and within the wild rice species. Glaszman (1988) also used Esterase polymorphism for discrimination of different groups of rice classified according to their geographical distribution while Seconds (1982) reported genetic diversity in cultivated and wild rice species on the basis of different isozymes including Esterases. In the present study, allozyme profiles of Got-1 and Got-2 were found completely monomorphic in *O. punctata* and showed little variations in *O. officinalis*. Similar results have earlier been reported by Hart & Longston (1977) that Got can not differentiate different accessions of the *O, punctata*. Since Got is a dimer (Weeden & Marx, 1987) and the dimeric isozymes, which are composed of similar subunits, showed less difference in their expression. This could therefore be one of the reason(s) that Got can not differentiate different accessions. However between the species, allozymes of Got showed 60% polymorphism that was due to difference in the number of alleles for isozyme loci present in different species, therefore showing different number of bands.

Peroxidases scored a large number of allozymic bands but showed relatively low level of polymorphism within the species *O. punctata* (13.33%) and *O. officinalis* (40%) however, Peroxidases were found highly polymorphic (80.00%) between the species. Such observations have also been reported for cotton, where peroxidases showed considerable allelic polymorphism between different varieties of *Gossypium hirsutum* (Farooq *et al.*, 1999) but within the varieties only quantitative changes were observed (Farooq & Sayyed, 1999 a & b). In the second part of this study, isozymes Got and Est were used for the identification of hybrids. Based on Esterases profile which are both monomeric (Tanksley & Rick, 1980) and dimeric (Wehling & Schmidt-Stohn, 1984) in nature, 1 of the 3 hybrids EF-6 x *O. malampuzhaensis* was confirmed at Est-2 locus as the phenotypes of the isozyme was characteristic of a heterozygote (one band coming from each parent). The quaternary structure of the isozymes observed in the present study can also be predicted as monomer because heterozygote of a monomer isozyme displayed two bands one each from the contributing parental alleles (Kephart, 1990). Carlson, *et al*, (1972) also reported that the isozyme bands of a hybrid are the summation of those found in the parental species. The Est-1 locus of hybrids EF-6 x *O. Malampuzhaensis* and Nonabokra x *O. malampuzhaensis* acc: 100957 produced bands of intermediate intensity as compared to their parents where *O. malampuzhaensis* gave a ghost band occupying a broad region while EF-6 and Nonabokra each gave a sharp dense band at this locus. It can therefore, be predicted that the isozymes, of same mobilities but different activity levels, produced by contributing parental alleles, form a band of intermediate intensity in the resulting hybrids. While studying Got which is

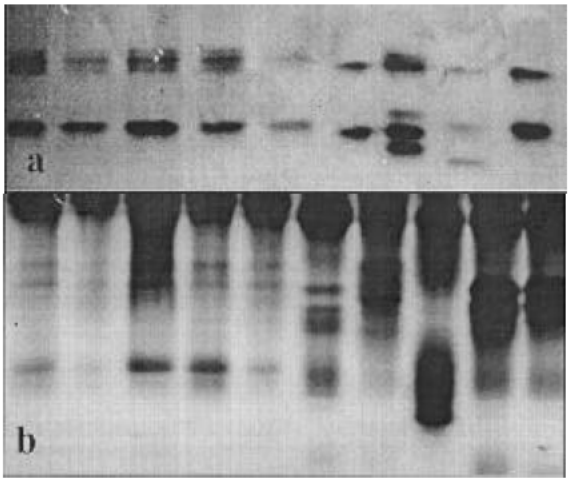

Fig. 1: Zymograms of isozymes Glutamate Oxaloacetate Transaminase (a) and Peroxidase (b) showing banding pattern polymorphism for five different accessions each of *O. punctata* and *O. officinalis*.

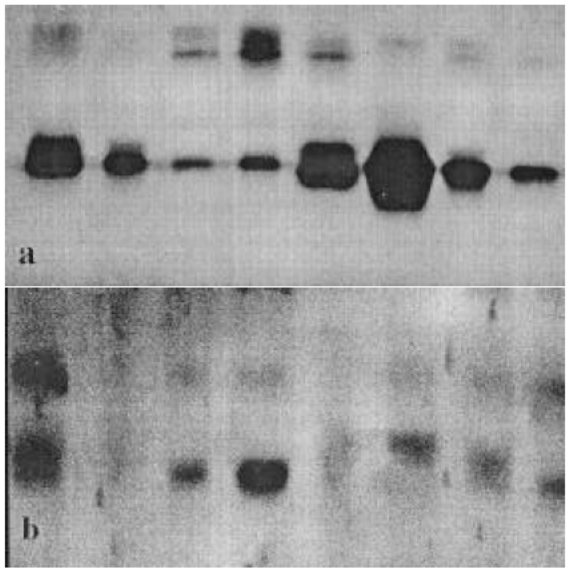

Fig. 2: Esterase (a) and Glutamate Oxaloacetate Transaminase (b) Isozyme patterns of F1 hybrids between rice cultivars and wild rice species along with the parents.

a dimer, two hybrids (EF-6 x *O. malampuzhaensis* and Nonabokra x *O. malampuzhaensis* acc: 100957) were confirmed at Got-1 locus. When the hybrid is produced by crossing two parents with homodimeric enzymes of different mobilities the hybrid is confirmed by the presence of a third activity region of intermediate mobility. The reason being that the products of two contributing alleles are associated at random in the cells of heterozygotes to form three types of two subunit proteins, two homodimer and one heterodimer and these intragenic dimeric allozymes migrate to different positions on the gel resulting in three banded pattern (Kephart, 1990). Based on these results it was concluded that hybrids can not necessarily be identified by all isozyme systems as somatic hybrid *Daucus carota x D. cappillifolius* showed the identical profiles to one of their parental types (Dudits *et al*, 1977). It can also be explained as when an isozyme locus is homozygous at two loci in a dimer, hybrid produces the same profile as that of their parents. Present study demonstrated that isozymes are of great value in the detection of genetic diversity present in plant populations as well as in the hybrid identification.

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