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Isozyme as Biomarker for Identification of Rice varieties

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ABSTRACT

Rice varieties of Assam, which are in seed production chain, lack in compilation of key diagnostic characters. Systematic studies to develop diagnostic markers of these varieties are essential which are morphologically very similar, to carryout scientific seed production, certification and also for plant variety protection. Eleven rice varieties of Assam, which are in high demand in seed chain were characterized based on SDS-PAGE of total soluble protein and two isozyme systems viz., esterase and aspartate amino transferase to use as biomarker for rapid identification of the varieties.. The electrophoretic banding profile of total protein exhibited no specific bands for identification of the varieties. However, variations were observed among the varieties with respect to Esterase banding pattern. Varieties could be grouped into five clusters in the dendrogram based on isozymes. Among the two isozyme systems, esterase isozyme was found to be more suitable for identification of the rice varieties.

KEYWORDS :

Introduction

Rice (*Oryza sativa* L.), which has two cultivated subspecies, *indica* and *japonica*, is one of the leading food crops in the world and staple for more than half the world's population[1]. The rice genotypes that have close genetic relationship are difficult to differentiate only with morphological characters. The use of protein and isozyme markers expands the possible use in genotype identification. These markers can be used as indicator of distance similarity calculation of different genotypes and their genetic constitution. Thus it can also speed up identification of closely related rice cultivars.

There are a number of rice varieties in Assam, many of which are in seed production chain. However, there is lack of compilation depicting the key diagnostic characters of these varieties, which are very essential to carry out scientific seed production and certification. This necessitates a characterization of the rice varieties for efficient management and proper utilization in seed production programme as well as in other rice improvement programmes. Information about the identity, or even genetic history, of a grain sample is possible to read from the grain-protein composition. As direct products of gene transcription and translation, proteins contain a wealth of genetic information, ready to be read off, given the appropriate techniques. Analysis of protein composition would thus be expected to provide a better basis for varietal identification than the study of morphology [2]. Electrophoretic identification can provide assurance that seed is true to label for sowing, but can also indicate the nature of off-type plants or ad-mixers during propagation [3]. Isozyme markers have been successfully used to classify rice cultivars into different taxonomic groups [4]. Scientist reported that changes in antioxidant isozymes in rice spikelet can be used as a biomarker for characterizing high temperature stress tolerance in rice spikelet [5].The present investigation attempts to identify popular 11 rice varieties of Assam using SDS-PAGE of total soluble protein and two isozyme systems viz., esterase (EST) and aspartate amino transferase (AAT).

Materials and Methods

The total soluble seed protein was extracted following the method of Laemmli (1970) [6]. The protein extract was estimated by the method given by Lowry's *et al* (1951) [7]. The protein samples after quantification were subjected to 12% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) following the method of Laemmli (1970) [7]. Gel staining was done in 0.05% coomassie brilliant blue R250.

Overnight soaked seeds of 11 rice varieties were germinated at 25°±2°C in a germinator and shoots of 3 days old etiolated seedlings were used for isozyme analysis. Isozyme analysis was carried out after separating soluble proteins on native-PAGE, following the procedure given by Davis (1964) [8]. Then appropriate staining was done for respective enzymes.

Data analysis

Jaccard's similarity coefficients for isozyme data were calculated and Unweighted Pair Group (UPGMA) methodology was used for dendrogram construction (Sneath and Sokal, 1973) [9].

Results and Discussion

The SDS-PAGE protein profile (Fig. 1) showed that molecular weight (MW) of the polypeptides ranged from 6.0 to 106.75 KD. All together 14 numbers of bands could be seen in all the varieties studied. Most of the bands were found to be monomorphic except few minor bands.

Altogether 11 numbers of bands were observed in EST isozyme (Fig 2a). Three zones of activity for EST isozyme (EST I, II and III) were observed in the present study (Fig-2b). Maximum variations were observed in EST III zone. Bands at Rm 0.051 and 0.711 were present in all the varieties studied. The frequency of occurrence of each polymorphic band ranged from 0.090 to 0.909.

Two to four numbers of bands were observed in AAT isozyme (Fig 3a). AAT isozyme banding pattern showed three zones of activity namely AATI, AATII and AAT III (Fig.3b). Two bands (Rm 0.473 and 0.852) were found to be monomorphic, while bands at Rm 0.105 and 0.189 were found to be polymorphic. The frequencies of occurrence of the two polymorphic bands were 0.454 and 0.275. The similarity coefficient ranged from 0.308 to 0.889 (Table 1). The dendrogram (Fig. 4) based on isozyme grouped the rice varieties into five main clusters, with two sub clusters (I, IIa, IIb, III, IV and V). A highest of four varieties was included in cluster II, whereas cluster IV and V each included the lone varieties Solpona and Mahsuri respectively.

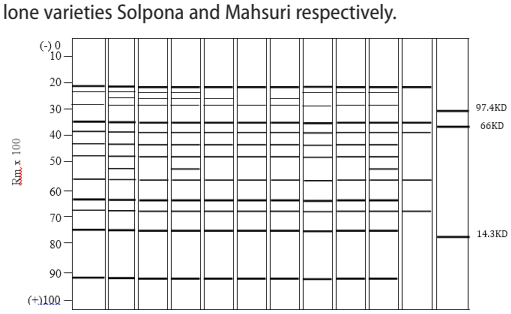

FIG. 1: Electrophoretic banding pattern of total soluble protein of eleven rice varieties

1 : Solpona
2 : Rongadoria
3 : Jyotiprasad
4 : Piyalee
5 : Bishnuprasad
6 : Rangilee
7 : Monoharsali
8 : Lakhimi
9 : Mo iram
10 : Satyaranjan
11 : Mahsuri

SDS-PAGE of soluble protein of 11 rice varieties showed 5 to 14 numbers of bands and an intense band with MW~ 20 KD was found. In basmati rice, three major protein bands of MW~ 14.5 KD, 20.4 KD and 33.1 KD were reported by Steenson and Sathe (1995) [10]. The electrophoretic banding profile clearly distinguished the varieties Bishnuprasad and Jyotiprasad, which may be otherwise difficult to distinguish through morphological markers, as these are the two sister lines having common parentage. However, no specific band was observed which could be used as the 'built in' marker for the varieties. Therefore, SDS-PAGE of total protein marker cannot be used for characterization of these 11 varieties. It is also possible that varieties showing similar patterns for proteins may have differences in certain enzyme(s). Therefore, further study was carried out using isozymes for characterization of the rice varieties. More than 15 enzyme systems have been detected in rice, more than 40 polymorphic genes encoding isozyme's have been reported in the *sativa* group of *Oryza* [11,12].

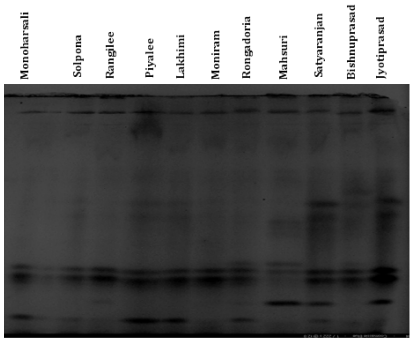

FIG. 2a ESTERASE (EST)

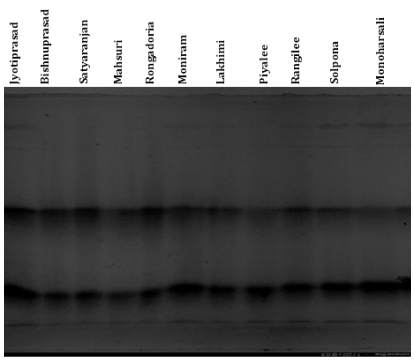

FIG. 3a ASPARTATE AMINO TRANSFERASE (AAT)

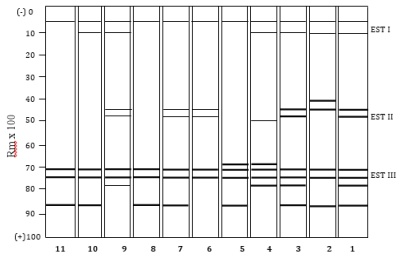

fig. 2b Zymogram of Esterase

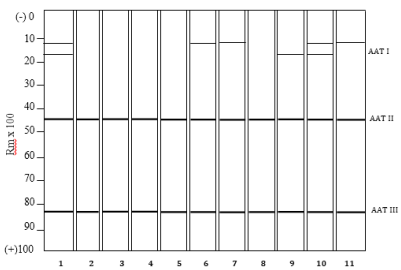

FIG. 3b Zymogram of aspartate amino transferase(AAT)

1 : Solpona
2 : Rongadoria
3 : Jyotiprasad
4 : Piyalee
5 : Bishnuprasad
6 : Rangilee
7 : Monoharsali
8 : Lakhimi
9 : Moniram
10 : Satyaranjan
11 : Mahsuri

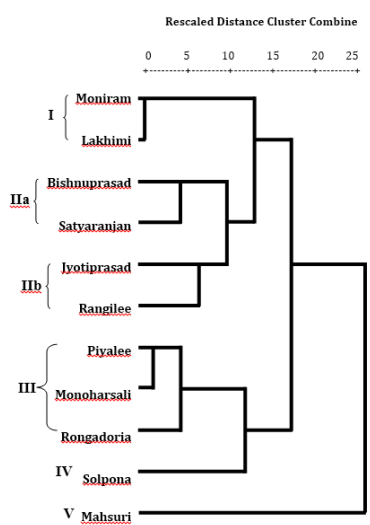

Fig. 4. Relationship analysis of 11 rice varieties based on isozyme data.

Table 1. Similarity Matrix between each two genotypes of 11 rice varieties (with respect to isozymes)

Variety	Jyotiprasad	Bishnuprasad	Satyaranjan	Mahsuri	Rongadoria	Moniram	Lakhimi	Piyalee	Rangilee	Solpona	Monohar sali
Jyotiprasad	1.000										
Bishnuprasad	0.692	1.000									
Satyaranjan	0.692	0.800	1.000								
Mahsuri	0.400	0.417	0.545	1.000							
Rongadoria	0.429	0.600	0.600	0.500	1.000						
Moniram	0.615	0.700	0.545	0.333	0.500	1.000					
Lakhimi	0.692	0.800	0.636	0.308	0.600	0.889	1.000				
Piyalee	0.462	0.667	0.667	0.400	0.857	0.556	0.667	1.000			
Rangilee	0.469	0.727	0.727	0.500	0.417	0.636	0.583	0.455	1.000		
Solpona	0.692	0.636	0.636	0.417	0.600	0.545	0.636	0.667	0.583	1.000	
Monoharsali	0.538	0.600	0.600	0.364	0.750	0.667	0.778	0.857	0.417	0.778	1.000

Out of the two isozyme systems AAT and EST studied, EST isozyme banding pattern was appeared to be highly polymorphic and informative. In EST isozyme, 9 bands were found to be polymorphic out of 11 bands obtained, whereas in AAT isozyme two bands were polymorphic out of the total four bands observed. Glaszmann (1985) [13] reported 10 loci controlling esterase isozyme in rice. Devi and Hazarika (2000) [14] observed five bands of esterase in isozymic study of 26 cultivated rice varieties of Assam. The contrasting result of the present study may be due to tissue, stage and species specificity of isozymes. Bon *et al* (2006) [15] reported reduced isozyme polymorphism is due to loss of allelic variation among the seed collections and could be described due to factors relating to collecting sample size, and procedural practices in handling germplasm for ex situ regeneration and conservation while studying the thirty-nine ex situ accessions of Philippine *Oryza officinalis* genotypes. In some studies in *Camellia japonica* reported two zones of activity for AAT isozyme [16]. The bands at Rm value 0.505 and 0.752 in Mahsuri and the band at Rm value 0.360 in Bishnuprasad were found to be distinguishing bands for the two varieties in case of EST isozyme. The lowest frequency (0.090) of occurrence of polymorphic bands indicates better discriminating power of this isozyme in the rice varieties under study. Thus the EST isozyme system was found to be suitable in characterizing the rice varieties under study. In recent study isozyme variability for esterase, alcohol dehydrogenase, glutamate dehydrogenase was success fully utilized in their study in genetic relationship among indigenous rice varieties [17].

Conclusion

From the present study it can be concluded that better phylogeny can only be achieved when both the data of isozyme and total soluble protein markers were used in combination than the individual data for appropriate characterization of the rice varieties. More number of isozyme systems should be employed to gain a better picture of the varieties under study.

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