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Efficiency of isozyme polymorphism and its relation to expression of heterosis in rice

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ABSTRACT

The present investigation was carried out with the objective to assess the genetic relationships among the fourteen genotypes through biometrical and biochemical approaches. The 14 genotypes were grouped into five clusters by Mahala- nobis D^2 analysis. Cluster I consisted of ten genotypes, cluster II, III, IV and V consisted of single genotype each viz., ADT 40, CR 1009, HA 891037 and Improved White Ponni, respectively. Number of filled grains per panicle followed by days to 50 per cent flowering contributed the maximum to the genetic divergence. The genotype ACK 198 exhibited greater divergence from CR 1009, CO 43 and ADT 40 with regard to isozyme pattern while with morphological analysis it showed divergence only from CR 1009 and ADT 40 and not from CO 43 with which it grouped in a single cluster. Thus the isozyme markers classified the genotypes with more resolution than the morphological markers. Genetic distances based on isozyme analysis were also correlated with mean performance and heterosis showed that there was no significant correlation between genetic distance and heterosis for any of the biometrical characters

Key words : *rice, isozyme, morphological markers, genetic diversity*

Rice germplasm collection in India is considered as a rich source of diversity due to the existence of high degree of phenotypic variability (Rao *et al*., 1979 and De *et al*., 1988). Hybrids showing strong heterosis were usually developed from parental lines diverse in relatedness, ecotype, geographic origin etc., (Lin and Yuan, 1980; Yuan and Cheng, 1986 and Yuan, 1985). Genetic diversity in rice has been studied by various means such as biometrical analysis, biochemical analysis (isozyme analysis) and molecular markers (RAPD).

Sinha *et al*. (1991) studied the genetic divergence using Mahalanobis D^2 statistics in 30 traditional upland rice varieties from nine states of India including the North Eastern Region. Based on 10 agromorphological characters, these varieties were grouped into six clusters. Characters like number of secondary branches panicle^{-1}, yield plant^{-1} and number of fertile grains panicle^{-1} had sizeable contribution to total divergence.

Thousand grain weight was found to contribute maximum to- wards genetic divergence followed by anther length and stigma length in 66 restorers and maintainers of the CMS line V 20A, which were grouped into ten clusters by Pradhan *et al.* (1995). These characters could therefore, form the basis for selection of parents from distantly placed clusters to obtain high heterotic combinations.

Isozymes or multiple molecular forms of enzymes are enzymes that share a common substrate but differ in electrophoretic mobility (Market and Moller, 1959). Isozymes have been utilised for the classification of varie- ties within *Oryza sativa*. Based on three esterase loci, Nakagah- ra*et al*. (1975) and Nakagahra (1977) could build a simple clas- sification which fits well with classification based on other factors. Second (1982) surveyed 40 loci, 25 of which were polymorphic within *Oryza sativa*. The varieties clearly tended to cluster into the *indica* and *japonica* types with 14 polymorphic loci. Glaszmann *et al*. (1984) similarly found a strong differentiation of the varieties towards the *indica* and *japonica* types. They pointed out that the *javanica* varieties (Bulu ecotype of Java), the typical upland rices from Africa and America and most upland rices from South East Asia belonged to the *japonica* group. Eighty three Aus cultivars, 168 Chinese land races, 26 Javanica

cultivars and 60 cultivars from other sources were analysed for 20 isozyme loci. Aus varieties as a whole showed a higher level of diversity than other varietal groups and contained a larger proportion of intermediate types which were not classifed into i*ndica* or *japonica*. The esterase loci were more polymorphic in Yunnan varieties than in other groups. However, Yunnan land races did not display high allelic richness and may be a minor group distributed through Burma from the whole diversity in the Indian sub continent (Wan and Ikehashi, 1997).

MATERIALS AND METHODS

All the fourteen parents were raised in a Randomised Block Design with three replications in the field during dry season at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. A spacing of 20 x 20 cm and a fertilizer dose of 120:60:60 NPK kg ha^{-1} were given. Each parent was raised in five rows; each row consisted of 10 plants. Seven biometrical traits were recorded. The data were subjected to genetic diversity analysis using D^2 statistics as described by Mahalanobis 1936.

The isozyme variation among the 14 genotypes was studied using native polyacrylamide gel electrophoresis for the Esterase, Peroxidase, Polyphenol oxidase and Superoxide dismutase were studied. Seedlings were raised in the Glass house, fresh young tender leaves were collected under ice-cold conditions and stored at -20°C. Leaf sample of 500 mg was homogenized in 500 µl (0.5 ml) of 0.5M ice cold Tris HCl buffer (pH 8.0) in a prechilled pestle and mort- er. Sample homogenate was centrifuged at 10,000 rpm for 20 minutes in centrifuge at -8°C. The supernatant was stored frozen at -20°C and used for isozyme analysis. Sample buffer was prepared by dissolving 5 ml of Tris HCl buffer with pH 6.8, 5 g of sucrose and 1 ml of Bromophenol blue in 10 ml of water. Electrophoresis was performed on polyacrylamide gel using Biorad protein II electrophoresis unit following the method described by Walker (1986). After the completion of electrophoresis, the gel was stained with respective staining solutions and visualised through the transilluminator. All the isozyme phenotypes (electromorphs) observed from zymogram were scored for their presence or absence of the band as '1' or '0 ' respectively. The isozyme scores were subjected to estimate the similarity coefficient and clustering the genotypes based on SAHN [Sequential Agglomerative Hierarchial Nonover lapping (Nested) Clustering] using UPGMA (Unweighted Pair Group Method Using Arithmetic averages) method (Sneath and Sokal, 1973). The statistical package used was NTSYSpc. version 2.0 developed by Rohlf (1992).

The crossing block was sown during dry season. The parents were raised thrice at an intervel of 15 days to ensure synchronization in flowering for the purpose of hybridization. Clipping method was followed for crossing. Forty hybrids were produced by crossing four lines (CR 1009, ADT 40, CO 43 and Improved White Ponni) with ten testers (ADT 43, ASD 20, HA 891037, IS 14, AS 95035, CB 97033, CB (DH) 95298, ACK 198, IET 15341 and TNAU 841434). Seeds from 40 cross combinations along with fourteen parents were raised in randomised block design with 3 replications during wet season and dry season. A spacing of 20 x 20 cm and a fertilizer dose of 120:60:60 NPK kg ha^{-1} was given and uniform practices were adopted. Each hybrid was raised in 1.8 m x 0.6 m plot size. At flowering, the true hybrids were identified based on the parental characters and tagged. In the parents also five plants were selected at random and tagged. At the time of maturity, data were recorded on all the tagged plants in each replication. The mean values recorded for the seven characters in parents and F_1 generation were used for statistical analysis. The analysis was done using the INDOSTAT statistical package.Estimation of heterosis was followed by procedure given by Fonesca and Peterson, 1968 and its significance was worked out by the formula given by Snedecor and Cochran, 1967.

RESULTS AND DISCUSSION

The mean values of seven morphological characters (Table 1) of the fourteen parents were transformed into standardised uncorrelated values and D^2 values were computed for all pair wise combinations *i.e.,* (14 x (14-1)/2 = 91 pairs). The genotypes were grouped into five clusters when the Tocher value was fixed at 6,500.00 (Fig 1). Cluster I consisted of ten genotypes *viz.,* ACK 198, TNAU 841434, IET 15341, AS 95035, IS 14, ASD 20, CB 97033, CO 43, CB (DH) 95298 and ADT 43. Four genotypes *viz.,* ADT 40, CR 1009, HA 891037 and Improved White Ponni remained individually in four

Table 1. Mean values of the quantitative traits of parents

Parents	Days to 50% flowering	Plant height	Numbers of productive tillers plant^{-1}	Panicle length	Number of filled grains panicle^{-1}	Hundred grain weight	Grain yield plant^{-1}
CR 1009	130.74	98.69	13.83	23.13	128.52	2.17	31.03
ADT 40	100.60	117.16	13.08	22.46	101.29	2.71	21.00
CO 43	114.43	80.70	16.47	18.40	140.36	2.03	30.79
I. W. Ponni	111.60	117.46	14.56	23.60	209.76	1.53	25.03
ADT 43	74.33	76.96	15.46	22.06	142.78	1.86	26.71
ASD 20	84.33	79.26	13.50	21.65	100.13	2.14	25.28
HA 891037	70.00	71.26	10.46	19.31	70.85	2.02	16.73
IS 14	94.16	73.33	13.69	21.36	107.46	2.45	19.90
AS 95035	99.66	77.32	16.45	20.36	120.53	1.92	20.23
CB 97033	98.16	81.70	14.05	23.25	150.16	1.89	24.49
CB (DH) 95298	102.16	88.86	14.66	21.46	122.50	2.50	16.63
ACK 198	99.50	93.93	14.06	21.26	102.13	2.20	28.56
IET 15341	101.33	80.78	17.27	23.95	100.56	2.18	27.69
TNAU 841434	98.16	90.14	12.85	22.18	115.73	2.68	29.42

clusters. The average intra and inter cluster D^2 values are presented in Table 2. At the intra cluster level, the maximum D^2 value (56.28) was noticed in cluster I. Whereas in clus- ters II, III, IV and V the intra cluster distance was zero, because all these clusters had only one genotype. At the inter cluster level, the maximum D^2 distance (249.17) was observed between clusters IV and V followed by that between clusters I and V (168.10). The least inter cluster distance (77.04) was recorded between cluster II and III. The characters, number of filled grains panicle^{-1} followed by days to 50 per cent flowering contributed the maximum towards divergence. Ramesh and Bateshwar Kumar (1998) reported that grain yield plant^{-1}; total spikelets and seeds panicle^{-1} and grain weight panicle^{-1} were mainly responsible for genetic divergence. Classification of rice varieties based on duration groups was reported by Vairavan *et al*. (1973). The four genotypes that deviated from cluster I had distinct characters. The genotype CR 1009 formed a separate cluster due to its long duration and more number of filled grains per panicle (128.52) and ADT 40 recorded medium duration with moderate number of filled grains per panicle. Likewise, the genotype HA 891037 recorded very low number of filled grains (70.85) and also had very early duration in flower- ing and formed a separate cluster. Similar results were reported by Sardana *et al*. (1997). Highest number of filled grains panicle^{-1} (209.77) was recorded by Improved White Ponni, which also remained single in a cluster. The divergence of these four genotypes from the genotypes in cluster I may also be due to the involvement of differ- ent ancestral pedigrees or uncommon parentage. In cluster I, all the 10 genotypes except two (IS 84 and TNAU 841434) have IRRI cultivars in their parental ancestry. The four genotypes *viz.*, ACK 198, TNAU 841434, IET 15341 and AS 95035 in cluster I also had a common parent Peta in their ancestry which may be a reason for close relationship among them.

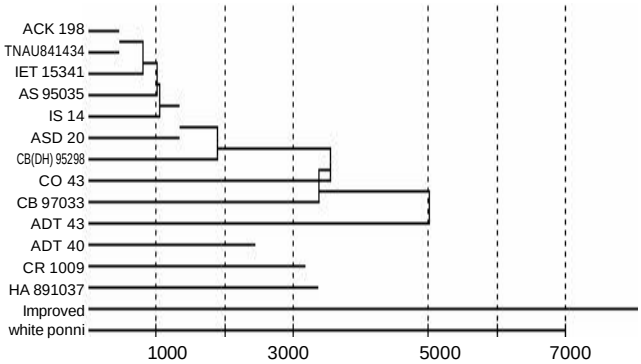

Fig 1. Cluster Diagram based on quantitative traits

The isozyme polymorphism can be a useful tool for the estimation of genetic divergence among the genotypes of a species. A total of four enzyme systems *viz.*, esterase, peroxidase, superoxide dismutase and

Table 2. Estimates of average intra and inter cluster D^2 for the clusters constructed from 14 genotypes

Clusters	I	II	III	IV	V
I	56.28	82.84	87.15	101.13	168.10
II	82.84	0.00	77.04	128.99	161.49
III	87.15	77.04	0.00	161.69	140.83
IV	101.13	128.99	161.69	0.00	249.17
V	168.10	161.49	140.83	249.17	0.00

polyphenol oxidase were studied involving all the 14 genotypes. The banding pattern of different marker alleles for all the enzymes are given in plates 1 to 4.

Eight different markers were generated for esterase activity. Among them, Est 1 was absent in ADT 43 and IET 15341. Est 4 was present in CR 1009, Improved White Ponni, HA 891037, IS 14, AS 95035 and ACK 198, Est 6 was present only in ACK 198. Est 8 was absent in ADT 43, ASD 20, HA 891037 and TNAU 841434. A total of four markers were generated. Prx 1 was present in ADT 40, CO 43, Improved White Ponni, ADT 43, ASD 20, IS 14, CB(DH) 95298 and TNAU 841434; Prx 2, Prx 3 and Prx 4 were absent in ADT 40 and ACK 198. A total of four alleles were observed among the fourteen genotypes. However, there was no polymorphism observed among the genotypes. Polyphenol oxidase was observed in five forms. Ppo 1 was present in six genotypes. In TNAU 841434 the locus Ppo 2 was absent but Ppo 5 was present. Ppo 4 was absent in 1ET 15341. The similarity co-efficient matrix obtained for each pairwise comparison of fourteen genotypes is given in Table 3.The similarity coefficient was found to be the highest (0.8667) between Improved White Ponni and CB 97033; between AS 95035 and CB(DH) 95298; and between CB(DH) 95298 and ACK 198. The lowest similarity coefficient value (0.20) was observed between 1ET 15341 and TNAU 841434.

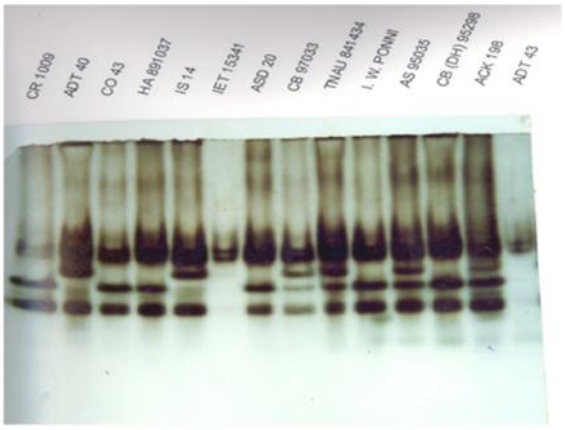

Plate 1. Esterase pattern of parental genotypes

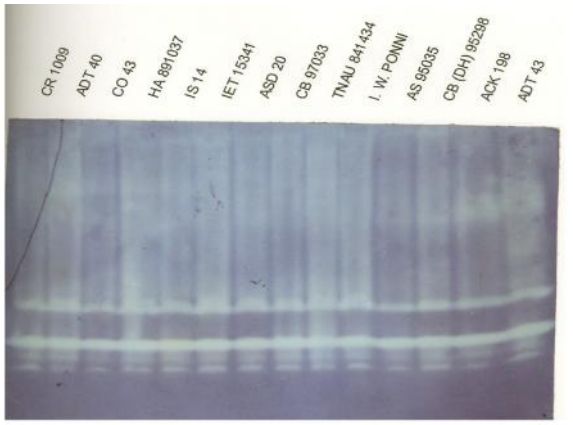

Plate 3. Superoxide dismutase pattern of parental genotypes

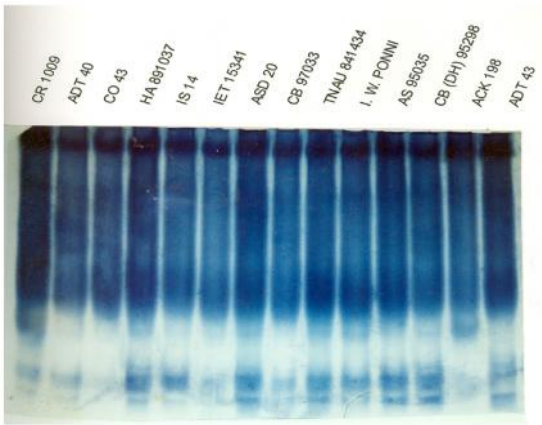

Plate 2. Peroxidase pattern of parental genotypes

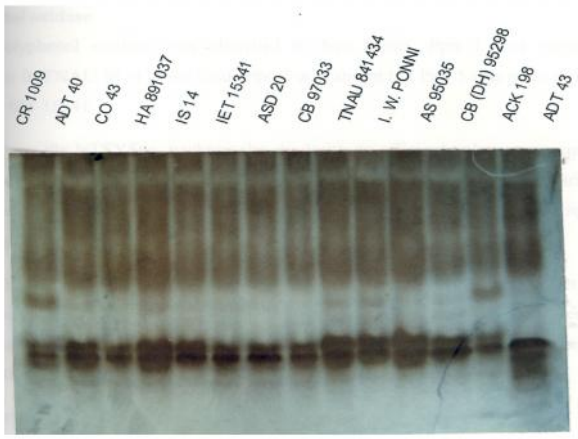

Plate 4. Polyphenol oxidase pattern of parental genotypes

Table 3. Similarity coefficient index for fourteen genotypes based on isozyme polymorphism

Genotypes	CR 1009	ADT 40	CO 43	I. W. Ponni	ADT 43	ASD 20	HA 891037	IS 14	AS 95035	CB 97033	CB(DH) 95298	ACK 198	IET 15341	TNAU 841434
CR 1009	1.0000													
ADT 40	0.4667	1.0000												
CO 43	0.4667	0.3333	1.0000											
I. W.Ponni	0.7333	0.6000	0.4667	1.0000										
ADT 43	0.6667	0.8000	0.4000	0.6667	1.0000									
ASD 20	0.4000	0.4000	0.6667	0.4000	0.4667	1.0000								
HA 891037	0.8000	0.5333	0.5333	0.8000	0.7333	0.6000	1.0000							
IS 14	0.5333	0.5333	0.6667	0.5333	0.7333	0.7333	0.7333	1.0000						
AS 95035	0.5333	0.8000	0.4000	0.8000	0.7333	0.3333	0.7333	0.6000	1.0000					
CB 97033	0.7333	0.6000	0.3333	0.8667	0.6667	0.4000	0.8000	0.5333	0.8000	1.0000				
CB (DH) 95298	0.6667	0.6667	0.5333	0.6667	0.6000	0.3333	0.7333	0.6000	0.8667	0.6667	1.0000			
ACK 198	0.6667	0.6667	0.6667	0.6667	0.6000	0.4667	0.7333	0.6000	0.7333	0.6667	0.8667	1.0000		
IET 15341	0.5333	0.4000	0.5333	0.4000	0.4667	0.3333	0.3333	0.4667	0.4667	0.5333	0.4667	0.3333	1.0000	
TNAU 841434	0.5333	0.5333	0.4000	0.4000	0.4667	0.6000	0.6000	0.4667	0.4667	0.5333	0.6000	0.7333	0.2000	1.0000

The clustering of fourteen genotypes was carried out by SHAN clustering programme using UPGMA method and the dendrogram was drawn (Fig. 2). At 0.42 similarity co-efficient two major clusters were formed. The first major cluster was subgrouped into seven sub clusters at 0.76 similarity coefficient level and the second major cluster contained only one genotype ACK 198. Hence in total eight clusters were observed. The genotypes HA 891037 and Improved White Ponni in sub-cluster II, were found to be very closely related to each other probably because their parents have genotypes from Chinese source. Sub-cluster III had TNAU 841434 and AS 95035. Closeness between these two genotypes was probably due to involvement of Peta as a common parent in their pedigree. The genotypes ADT 40 and IS 14 have a common Chinese source in their pedigree which might have resulted in maximum similarity. Similarly the genotypes IET 15341 and CB 97033 have IRRI source such as IR 8 and IR 72 respectively in their pedigree. The isozyme analysis clearly indicated that the genotype ACK 198 is highly divergent from other genotypes especially with the genotypes CR 1009 and Improved White Ponni. Genetic distances based on isozyme analysis were also correlated with mean performance and heterosis (Table 3). The results showed that there was no significant correlation between genetic distance and mean performance as well as heterosis for any of the biometrical characters.

Comparing the above two approaches, the genotypes CO 43 and ADT 43 showed more divergence in isozyme analysis, whereas in case with morphological markers, both these genotypes were in the same cluster.

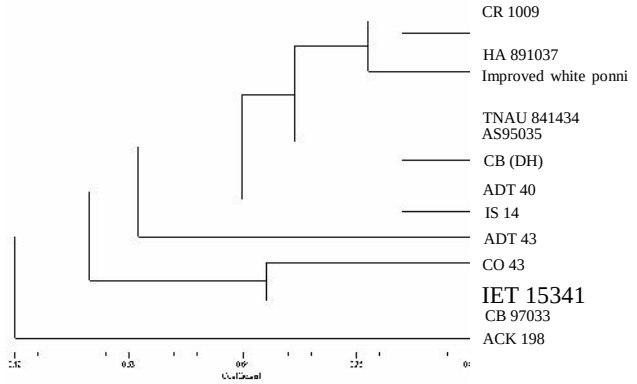

Fig.2. Clustering of genotypes based on isozyme polymorphism

Similarly, the genotype ACK 198 exhibited greater divergence from CR 1009, CO 43 and ADT 40 with regard to isozyme pattern, while with morphological analysis it showed divergence only from CR 1009 and ADT 40 and not from CO 43 with which it was grouped in a single cluster. Thus, the isozyme markers classified the genotypes with more resolution than the morphological marker characters.

REFERENCES

De RN, R Seetharaman, M.Sinha and SP Banerjee 1988. Genetic divergence in rice. Indian J. Genet., 48:189 - 194.

Fonesca S and FL Patterson 1968. Hybrid vigour in a seven parent diallel cross in common winter wheat. Crop Sci., 8: 85-88.

Glaszmann JC, H Benoit and M Arnaud 1984. Clessification desriz cultures (*Oryza sativa* L.) . Utilization deta variabilite isoenzymatique. Agron. Trop., 39: 51-66.

Glaszmann JC 1985. A.varietal classification of Asian cultivated rice (*Oryza sativa* L.) based on isozyme polymorphism. In: Rice genetics. International Rice Research Institute, Manila, Phillipines. pp. 83-90.

Glaszmann JC 1987. Isozymes and classification of Asian rice varieties. Theor. Appl. Genet., 74: 21-30.

Lin SC and LP Yuan 1980. Hybrid rice breeding in China. International Rice Research Institute. Shanghai Academic and Technical Press, Shenghai, China.

Mahalanobis PC 1936. On the generalized distance in statistics. Proc. Natt. Inst. Sci, India. 132: 49-55.

Market CL and F Moller 1959. Multiple forms of enzymes, tissue, ontogenetic and species specific pattern. Proc. Natl. Acad. Sci., 45: 753-763.

Nakagahra M 1977. Genetic analysis for esterase isozymes in rice cultivars. Jpn. J. Breed., 27: 141-148.

Nakagahra M, T Akihama and KI Hayashi 1975. Genetic variation and geographic origin of esterase isozymes in native rice varieties. Jpn. J. Genet., 50: 373-380.

Pradhan SB, SN Ratho and PJ Jachuck 1995. Analysis of genetic divergence among maintainers and restorers for use in hybrid rice breeding. Oryza, 32: 12 - 17.

Ramesh B and Bateshwar kumar 1998. Genetic divergence in rice Indian J. Pl. Genet. Resources, 11: 13-17.

Rao UP, AV Rao and TE Srinivasan 1979. Combining ability of short - statured rice cultivars from north-east India. Indian J. Genet., 39: 511 - 515.

Rohlf FJ 1992. NTSYS-pc, Numerical texonomy and multivariate Analysis system. Exeter software, New York.

Sardana S, BN Borthakur and TN Lakhanpal 1997. Genetic divergence in rice germplasm of Tripura. Oryza, 34: 201 - 208.

Second G 1982. Origin of the genetic diversity of cultivated rice (*Oryza* spp.): Study of the polymorphism scored at 10 isozyme loci. Jpn. J. Genet., 57: 25-57.

Sinha PK, VS Chauhan, K Prasad and JS Chauhan 1991. Genetic divergence in indigenous upland rice varieties. Indian J. Genet., 51: 47 - 50.

Sneath PHA and Sokal RR 1973. Numerical taxonomy. W. H. Freeman, Sanfrancisco.

Snedecor GW and WG Cochran 1967. Statistical methods. Oxford and IBH publishing Co.,New Delhi, p.593.

Vairavan S, EA Siddiq, V Arunachalam and MS Swaminathan 1973. A study on the nature of divergence in rice from Assam and N.E. Himalayas. Theor. Appl. Genet.,43: 213-221.

Walker JN 1986. Manual on techniques in molecular biology (proteins). Deparment of Biochemistry, Tamil Nadu Agricultural University, Coimbatore.

Wan J and H Ikehashi 1997. Identification of two types of differentiation, in cultivated rice (*Oryza sativa* L.) detected by polymorphism of isozymes and hybrid sterility. Euphytica, 94: 151-161.

Yuan LP 1985. Exploration of super high yielding hybrid rice. Hybrid Rice, 3:1 - 3.

Yuan LP and HX Cheng 1986. Hybrid rice breeding and cultivation. Hunan Science and Technology Press, Hunan, China.