Biochemical Marker (Protein) Based Characterization of Rice Accessions Bio-Diversity in International Rice Molecular Breeding Programme

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ABSTRACT :

Rice (*Oryza sativa L.*) is the world's single most important food crop. Rice is one of the few crop plants with rich genetic diversity, having a great demand of increased productivity. Genetic diversity is the key to overcome to biotic and abiotic stresses, so exploitation of germplasm diversity is the only solution. Thus, present study was planned to characterize the rice biodiversity of a set of thirty rice accessions collected from IRMBP. Protein was extracted from these rice seed using easy and user friendly protocol and SDS-PAGE.

There were 7 major protein bands present across all accessions, medium mobility of them present in all accessions while, and remaining low mobility bands we found polymorphic. The similarity coefficient was analyzed using NTSYS-pc program, which ranged from 0.42 to 1.00. Biochemical based marker (Protein) was efficient for the assessment of genetic diversity. All the accessions were different and genetically distant from each other at the biochemical level.

The results of the present study as the representative accessions of IRMBP led to a conclusion that the rich biodiversity still exists and should be explored for allele mining, and rice improvement programs.

Keywords: SDS-PAGE, Biodiversity, IRMBP, diversity, Biochemical (Protein) markers.

INTRODUCTION:

Rice is the world's most important crop. It is the stable food for nearly one- half of the global population. Rice is the abundant in carbohydrates and is a major source of protein accounting for 35 to 60 percent calories. World's rice production must increase by 60 percent by 2025 to feed the alarming rate of increasing population of rice consumers [1].

Rice has been closely linked with the evaluation of human society, both as a commercial product and as a creator of communities. In addition, rice has been traded in all directions from its places of origin, and today it is cultivated on every continent. The diversity of rice (*Oryza sativa* L.) evolved during several years leading to multiplicity of rice varieties in different agro-ecological conditions with resistant to insects, pests and diseases [2-3]. Tens of thousand of varieties of rice have evolved in different growing areas of the world. India contains a very rich genetic diversity of cultivated rice and its progenitor species. Exploitation of biodiversity permits humanity to adapt to local and global changes. With the continuing growth of population, the needs or efficient sustainable and highly productive agro-ecosystems are vital [4-5].

Bio-diversity is sometimes called genetic resources, because they can be used as the starting point for new products or processes. Bio-diversity refers to variety and variability among ecological complexes in which they occur. Bio-diversity is affected by both natural and human selection pressure of the agro-ecosystem and the structure crop population itself [6-7]. Protein markers, seed storage protein and isozymes, which are commonly used for the estimation of genetic purity, were used in this work to estimate genetic purity in sunflower hybrids [8].

Seed proteins are not influenced by external conditions rendering them invariable. Therefore, their electrophoretic protein profile obtained from SDS-PAGE can serve as reliable genetic markers [9]. Indica and

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japonica cultivars differ in many characters when typical varieties are composed but now show overlapping variations. The indica and japonica types are each characterized by an association of certain diagnostic characters, such as resistance, cold tolerance, apiculus hair length and phenol reaction [10].

Three key problems responsible for showed breeding progresses are lack of novel genetic variation, genetic drag associated with use of exotic germplasm, and the inability to trace and identify novel variation foe target traits in breeding progenies.

A range of biochemical and molecular approaches were used to study diversity of conserved rice germplasm. Variation at the biochemical level, such as isozyme and seed proteins has also been investigated in an attempt to classify taxonomic group of cultivars [11].

In the present study thirty rice accessions collected from International Rice Molecular Breeding programme (IRMBP) were selected and used as a basic material.

Characterization of these thirty unique rice lines with biochemical (protein) will provide information on the genetic diversity of thirty lines which mill make efficient use of these genotypes in rice breeding programme. The result of cluster analysis of biochemical marker and some related phenotypic traits indicated that the biochemical based marker system has distant advantages. The results of the present study showed the biochemical markers are limited in numbers and they do not reliably reflect genetic relationship.

MATERIALS AND METHODS:

The present investigation was carried out at the Department of Biotechnology, Indira Gandhi Agricultural University, Raipur (CG).

The experimental material, thirty accessions of rice (*Oryza sativa* L.) was collected from the Donor Gene Pool of the International Rice Molecular Breeding Program (IRMBP), which were randomly selected from the core collections (Table- 1).

Sr.No.	IC No.	Code	Variety name	Origin
1	IRMBP-7	C8	Feng-Ai-Zan	China
2	IRMBP-9	C11	Hua-Gen-Xian 74	China
3	IRMBP-11	C15	Shen-Nong 89366	China
4	IRMBP-13	C17	Yunhai-290	China
5	IRMBP-15	C19	Yu-Xiang-Zan	China
6	IRMBP-18	C22	Zhong-You-Zao 81	GRC
7	IRMBP-19	C24	Bg 90-2	India
8	IRMBP-20	C25	Basmati-370	India
9	IRMBP-22	C27	IR-72	India
10	IRMBP-23	C28	PR-106	India
11	IRMBP-27	C32	Cisedane	Indonesia
12	IRMBP-28	C33	Amol 3 (Sona)	Iran
13	IRMBP-30	C35	IR-64	IRRI
14	IRMBP-32	C37	Gayabyeo	Korea
15	IRMBP-34	C39	Hmibyeo	Korea
16	IRMBP-37	C42	MR 84	Malaysia
17	IRMBP-39	C44	Manawthukha	Malaysia
18	IRMBP-40	C45	Shwe-Thwe-Yin- Hyv	Malaysia
19	IRMBP-41	C46	Bg-300	Srilanka
20	IRMBP-43	C48	Cr-203	Vietnam
21	IRMBP-44	C49	Om-997	Vietnam
22	IRMBP-50	C55	IR-66897 B	IRRI
23	IRMBP-51	C56	IR-58025 B	IRRI
24	IRMBP-52	C57	B-4122	India
25	IRMBP-58	D2	Ai-Zi-Dao	China
26	IRMBP-61	D8	Jiangxi-Si-Miao	China
27	IRMBP-62	D10	Lao-Hu-Dao	China
28	IRMBP-63	D13	Peng-Shan-Tie-Gan-Zan	China
29	IRMBP-70	D25	Milagrosa,Zawa banday	GRC
30	IRMBP-71	D28	Basmati	India

Table 1: List of rice accessions from the collection of International Rice Molecular Breeding Programme (IRMBP)

Seed protein extraction and profile studies:

Seed protein profile patterns of all thirty accessions were studied to find out the variation among the accessions. Twenty grains of each rice accessions under investigation were dehusked and grounded to fine power using mortar and pestle. 15 mg powdered sample was taken for extraction of protein, mixed with 500µ1 of extraction buffer (0.05M Tris HCL buffer, pH 8.0, containing 0.2% SDS, 5M Urea and 1% β- Mercaptoethanol). The contents were mixed thoroughly using vortex mixer and the samples were centrifuged for 5 minutes at 5000 rpm, the supernatant of the sample was stored in deep freezer until further use.

20µ1 of the supernatant of the sample was mixed with 5 µ1 of Bromophenol Blue dye (0.05% w/v) and placed on PAGE at 100 volts for 30min. and 150 volts for further 2hrs. and were than stained and de- stained for observation.

Poly Acryl amide Gel Electrophoresis:

Gel was prepared following the method of with minor modifications [12]. Gel contained 33.35% of separating and 12.2% of stacking gel.

Polyacrylamide (for 50ml): Acrylamide (30%w/v) 14.6gm and Bis- acrylamide 0.4gm dissolved in 30ml Double Distilled Water and adjust the volume 50ml. Filter over Whatmman NO.1 Filter paper and store in dark brown bottle at 4˚C.

SDS 10%: 2gm SDS dissolved in 15ml DDW and adjust the volume 20 ml

Upper Tris (pH 6.8): Tris base 3.03gm, 10% SDS 2 ml, dissolve in 30ml Double Distilled water and adjust the pH 6.8 with concentrated HCL. Adjust the final volume to 50ml store at three month at 4 ˚C.

Lower Tris (pH 6.8): Tris base 18.15gm, 10% SDS 2 ml, dissolved in 80 ml distilled water and adjust the pH 8.8 with concentrated HCL and make up the volume to 100ml.

APS (Ammonium per sulphate): 0.1gm APS dissolved in 0.9ml double distilled water. Every time make the fresh APS.

Gel composition for 14 x 16cm Vertical Slab Gel plates.

Distilled water	10.4ml
Lower Tris	6.25ml
Polyacrylamide	8.40ml
Ammonium Per Sulphate	100µl
TEMED	10µl
Total	5.165ml

(Lastly Isopropanol in added for leveling)

Distilled water	4.86ml
Lower Tris	2ml
Polyacrylamide	1ml
Ammonium Per Sulphate	300µl
TEMED	10µl
Total	8.17ml

(The gel was overlaid with water to avoid desiccation)

Electrophoresis was carried out with a constant voltage of 150 volts till the Bromophenol blue dye crossed the staking gel. The voltage was increased to 200 volts in the separating gel till the dye reached the last of the gel.

Staining and distaining of Gel:

Coomossive Brilliant Blue R-250 (for staining): 1.2gm of Coomossive Brilliant Blue R- 250 dissolved in 220ml of methanol. Add 4.6ml of Glycerol Acetic acid make up the volume to 500ml with distilled water. Filter the solution through Whatmman NO. 1 paper and store in a brown bottle.

Staining of proteins: The gel was stained in Coomossive brilliant blue R-250 dye for 2½ hours, and then destained with 2-3 washings in the solution containing 5% Methanol and 7% Glacial acetic acid for 24 hours till sharp bands appeared.

Resolution Factor: The distance traveled by the dye front and the protein bands from the top of the running gel was recorded. The resolution factor for each band was calculated as: Distance travelled by protein band divided by Distance travelled by the dye front

Positions of the bands were expressed in Resolution factor (RF) values. The Bromophenol blue dye front at the bottom of the gel was arbitrarily give the value 1, while the top of the gel was given a value of O. The RF value of a particular variant band was proportional to the distance between two reference standards i.e.0 to 1. Thus RF value for each band was computed.

Data analysis:

The sharp bands for each rice accession were scored as present (1) or absent (O). The similarity matrix was calculated from the data using the program similarity for qualitative data (SIMQUAL) dice coefficient. Cluster analysis was carried out by the UPGMA method and dendrogram was generated using SAHN subroutine of NTSYS – PC.

RESULTS AND DISCUSSION:

Biochemical Studies:

To characterize the set of thirty rice accessions at biochemical level, seed protein was extracted from all the 30 rice accessions use in the present investigation and was subjected to SDS-PAGE to generate protein profiles. The observations were scored for the presence (1) or absent (0) of bands on the protein profiles and the data was then subjected to generate similarity ma-

trix using the program similarity for qualitative data (SIMQUAL) dice coefficient [13]. Cluster analysis was carried out by the UPGMA method and dendrogram thus generated using SAHN subroutine of NTSYS-pc is summarized in (Figure-1, Table-2).

The distance traveled by the dye and the protein bands from the top of the running gel was noted and thus electrophoretogrames were prepared. The computed resolution factor was used to prepare Zymogram (Figure- 2) Across the 30 lines 7 bands were observed for which the RF value ranged from 0.44 to 0.97. Three major protein bands of RFs 1) 0.97, 0.95, 0.90 2) 0.67, 0.63, 3) 0.46, 0.43 were observed and were designated as fast, medium and slow mobility bands respectively. Two slow mobility bands with RFs 0.46 and 0.43 were observed in the slow mobility group and were present in all the protein profiles of rice accessions but were absent in accession ≠ 20,30,52 (RF 0.43) and ≠ 18, 20, 30 52 (RFs 0.46) and thus indicating polymorphism in the slow mobility group for the rice accession ≠ 18. Two medium mobility protein bands were present with RFs 0.67, 0.63. The medium mobility protein with RFs 0.63 was present in # 7, 9, 11, 18, 19, 20, 23, 27, 28, 30, 32, 34, 37, 39, 40, 41, 43, 44, 50, 51, 58, 61, 62, 63, and were absent in ≠ 13, 15, 22, 52, 70, 71 where as the proteins bands with 0.67 RFs were present in all accessions except accession # 52. The medium mobility proteins thus indicated to be polymorphic for the accessions #13 and #15. Three fast mobility bands with RFs 0.97, 0.95, 0.90 were resolved. The fast mobility group showed polymorphism and the protein bands with RF 0.90 were absent in rice accessions# 7, 19, 20, 22, 71 with RFs 0.95 were absent in rice accessions # 70, 71 and with RFs 0.97 were absent in the rice accessions # 62, 63, 70, 71.

Similarity coefficient:

A perusal of dendrogram indicated that the similarity coefficient ranged from 0.42 to 1.00 among 30 rice accessions based on biochemical analysis. The diversity in biochemical traits was only 0.58 indicating that rice accessions did not show much variation at the translational level. The rice accessions were grouped into two major clusters A and B. The cluster A contains 29 accessions while the cluster B has only one accession and shared 42% similarity. The cluster A was sub grouped as A1 and A2, and consisted of 27 and 2 rice accessions and shared 68% similarity. The two rice accessions clustered in the sub group A2 shared 86% similarity. The sub group A1 was further sub divided into, A1(a) and consisted of 24 and 3 rice accessions and shared 68% similarity. A1 (a) was further divided into two A1 (a1) and A1(a2) and consisted of 21 and 3 rice accessions respectively and shared 79% similarity. The cluster A1(b) and consisted of 2 and 1 rice accession and shared 78% similarity.

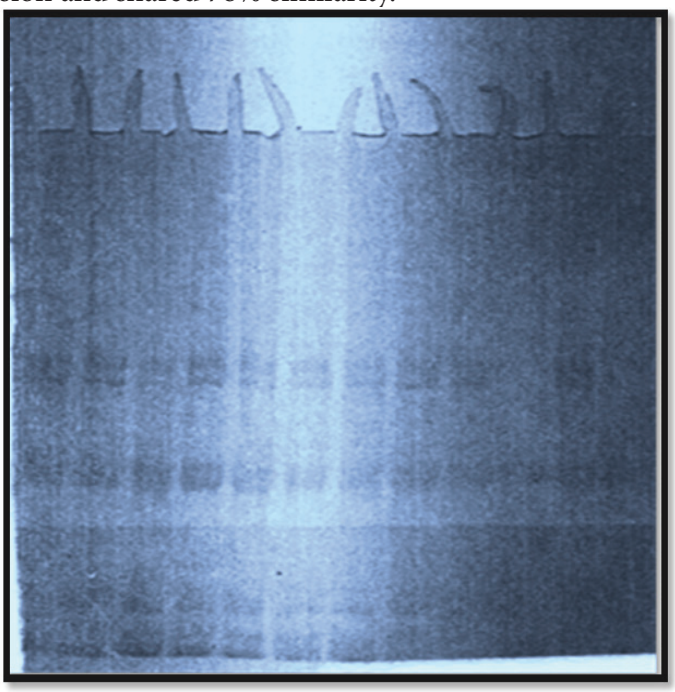

Figure 1: Seed protein profile using SDS-PAGE

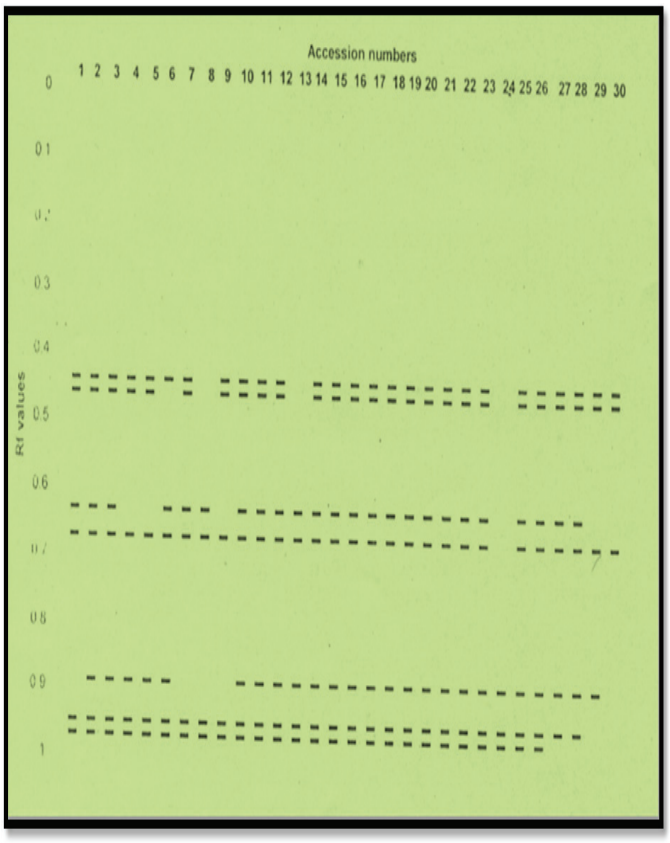

Figure 2: Zymogram representing seed protein profile of 30 rice accessions

Four groups were identified within which all the accessions share 100% similarity for biochemical characters. These groups are shown in Table 2.

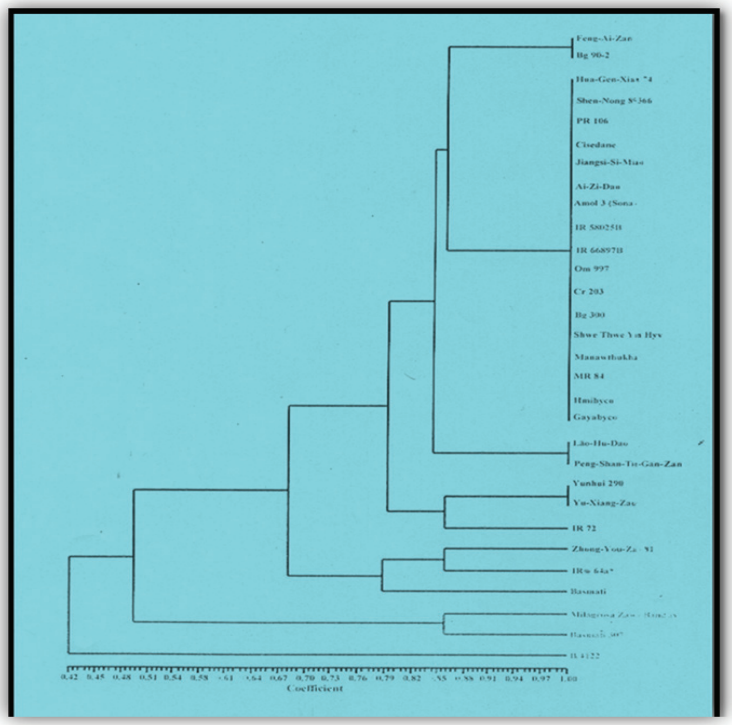

Figure 3: Dendrogram of 30 rice accessions developed from RAPD using NTSYS- Pc analysis. The scale is based on Nei and Li's coefficient similarity

S.N	Group	Accession No.	Cluster
1	Group 1	7,19	Cluster No A1(a1-1-1)
2	Group 2	9,11,23,27,61,58,28,51,50,44,43,41,40,39 37,34,32	Cluster No A1(a1-1-2)
3	Group 3	62,63	Cluster No A1 (a1-2)
4	Group 4	13,15	Cluster No A1 (a1-2)

Table 2: Seed protein profiles differences in the few rice accessions were revealed.

Accession No.	Code	Variety name	Origin
22	C 27	IR 72	India
18	C 22	Zhong-You- Zao 81	GRC
30	C 35	IR-64a*	IRRI
71	D 28	Basmati	India
70	D 25	Milagrosa, Zawa Banday	GRC
20	C 25	Basmati- 370	India

Table 3: The accessions collected from International Rice Molecular Breeding Programme (IRMBP) were not similar.

Protein profile revealed that the rice accession B 4122 showed more protein bands which are different from rest of all 29 rice accessions.

Molecular markers representing locus-specific DNA variation can be detected at the gene protein level or directly at the DNA as with RFLP's Isozymes markers have been use widely in rice to classify cultivated rice varieties and their relatives [14]. Isozymes are tissue specific and are affected by both environment and the stage of development [15].

It has been suggested that it is because of the fact that of translational level these is not difference in the synthesis of protein in plants of different varieties or genotypes. Some studies reported that the seed proteins are mainly storage proteins and are not likely to be changed in dry mature seeds of different age [16]. This phenomenon is confirmed by studying rye grass [17]. However, the composition of seed proteins is affected slightly by environmental conditions and seasonal fluctuations [18-19].

The classification based on phenotypic traits though showed high amount of diversity but was not able to discriminate the accessions and may involve lot of hu-

man and environmental influences questioning the correctness of the similarities and dissimilarities of the accessions. Fingerprinting is an absolute measure of genetic makeup of an individual or a line, and must be unique to that individual or line in order to distinguish it from all others.

Sr. No.	Variety name	Code	Origin
Cluster A			
Cluster A1(a)			
Cluster A1(a1)			
Cluster A1(a1-1)			
Cluster A1(a1-1-1)			
1	Feng-Ai-Zan	C8	China
2	Bg 90-2	C24	China
3	Hua-Gen-Xian 74	C11	China
4	Shen-Nong 89366	C15	China
5	PR-106	C28	India
6	Cisedane	C32	Indonesia
7	Jiangsi-Si-Miao	D8	China
8	Al-Zi-Dao	D2	China
9	Amol 3 (Sona)	C33	Iran
10	IR-58025 B	C56	IRRI
11	IR-66897 B	C55	IRRI
12	Om-997	C49	Vietnam
13	Cr 203	C48	Vietnam
14	Bg-300	C46	Sri Lanka
15	Shwe-Thwe-Yin- Hyv	C45	Malaysia
16	Manawthukha	C44	Malaysia
17	MR 84	C42	Malaysia
18	Hmibyeo	C39	Korea
19	Gayabyeo	C37	Korea
Cluster A1(a1-2)			
20	Lao-Hu-Dao	D 10	China
21	Peng-Shan-Te-Gan-Zan	D 13	China
Cluster A1(a2)			
22	Yunhai-290	C17	China
23	Yu-Xiang-Zan	C19	China
24	IR-72	C27	India
Cluster A1(b)			
Cluster A1(b1)			
25	Zhong-You-Zao 81		
26	IR-64a*	C35	IRRI
Cluster A1(b2)			
27	Basmati	D28	India
Cluster A2			
28	Milagrosa Zawa Banday	D 25	GRC
29	Basmati 370	C25	India
Cluster B			
30	B 4122	C57	India

Table 4: The list of genotypes of cluster analysis based on Seed protein profile analysis with the Variety name, Code and Origin

CONCLUSIONS:

In present study we characterized the total bio-diversity at total seed crude protein of the rice present in IRMBP. Protein profile revealed that the rice accession 'B 4122' showed more protein bands which are polymorphic from rest of all 29 rice accessions. It confirms the presence of different alleles in possible combinations in the sub set of rice accessions of IRMBP thus suggesting for the presence of natural variation in the core collections. These biochemical markers are useful to fingerprint varieties, establish phylogenies, determine similarities among inbreed, and mapping entire genomes.

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