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Electrophoretic Analysis of Seed Proteins from Different Varieties of Rice Cultivated in Sindh

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Abstract: SDS-PAGE electrophoresis is the most effective as well as one of the simplest methods for separating proteins. The extraction and fractionation technique was applied to characterize rice seed proteins. Identification and screening of rice proteins carried out from 14 varieties of rice collected from Nuclear Institute of Agriculture (NIA) Tandojam. Rice seed proteins were extracted with phosphate buffer for total proteins. After quantification, proteins were separated by SDS-PAGE Electrophoresis. Analysis of rice seed proteins was performed on the results of SDS-PAGE using gel documentation system (Biorad) to find out the molecular weight of different varieties of rice followed by cluster analysis using SPSS to find out the diversity among different rice varieties.

Key words: SDS-PAGE, seed storage proteins, genetic diversity

INTRODUCTION

The food and feed value of most cereals could be improved by increasing their levels of protein content. The molecular properties of proteins contribute to the functionality of food ingredients, i.e specific proteins contribute to the selected functional behavior of food. Proteins functionality in food is mainly determined by the molecular composition and structure of the individual protein (Kinsella and Shetty, 1979). Thus characterization of the individual seed proteins provides useful information for the isolation and utilization of rice seed protein.

Rice is highly consumable food and source of different variety of proteins in Asian countries. Proteins contents varies greatly in mature rice grain (Julialo *et al.*, 1968). In brown rice it ranges from 5-7% (Julialo *et al.*, 1964; 1968). The protein content of milled rice average 7% (Athwal, 1975).

Water soluble and salt soluble proteins (Albumin and Globulin) amount for a minor part of the total proteins of milled rice. In general, globulins are 7-11% of the total protein and albumins 0.5-6% (Houston and Mohammad, 1970). Electrophoretic analysis of grain protein composition has been extensively used to provide useful analysis of identity (Naomi Pollard *et al.*, 1996).

The purpose of this work is to characterize rice seeds proteins cultivated in Sindh on the basis of their genetic similarities and dissimilarities.

MATERIALS AND METHODS

Fourteen varieties of rice cultivated in Sindh were obtained from Nuclear Institute of Agriculture (NIA) Tandojam. The grain was dehulled and milled to get a fine powder the total soluble protein was extracted for

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) by suspending overnight 0.5 gram in 1 ml extraction buffer (phosphate buffer pH 7.5). The suspension was sonicated for 30 min to optimize solubilization. Then centrifuged for 20 min at 14000 rpm. (Protein samples were solubilized in the sample buffer (0.5 M Tris-HCl pH 6.8, containing 5% SDS, 5% 2-mercaptoethanol, 30% glycerol, 0.06% BPB) by heating for 5 min in a boiling water bath. 20 µl of each of the sample and 5 µl of the standard (ferment as pristine protein marker) were loaded in individual well (1 mm thick 12 well comb) on the gel for discontinuous SDS-PAGE by using AE-6530 mini slab gel electrophoresis (Naomi Pollard *et al.*, 1996).

SDS-PAGE electrophoresis was performed, following the procedure of Leammli (1970) using 10% acrylamide gel slabs overlaid with a 4% stacking gel (Leammli, 1970) at a constant range of 30 mA for approximately 3 h at 20°C.

After completion of electrophoresis, the gel slabs were stained with 0.25% Coomassie Brilliant Blue R250/2-propanol: Acetic acid: H₂O 5:1:5 and destained in 10% acetic acid and 30% methanol until the background became transparent and protein fractions became visible in the form of blue colored, light and dark bands. Gel was photographed by BioRad Gel Documentation System to provide the data of molecular weight for each of the protein fractions. All the visible bands of electrogram were scored and were used in the analysis. Each band was given score of 1 for presence and 0 for absence. Cluster analysis on the basis of obtained data were conducted on similarity estimates using the Unweighed Pair-Group Method for Arithmetic Averages (UPGMA) and the resulting clusters were expressed as dendrograms.

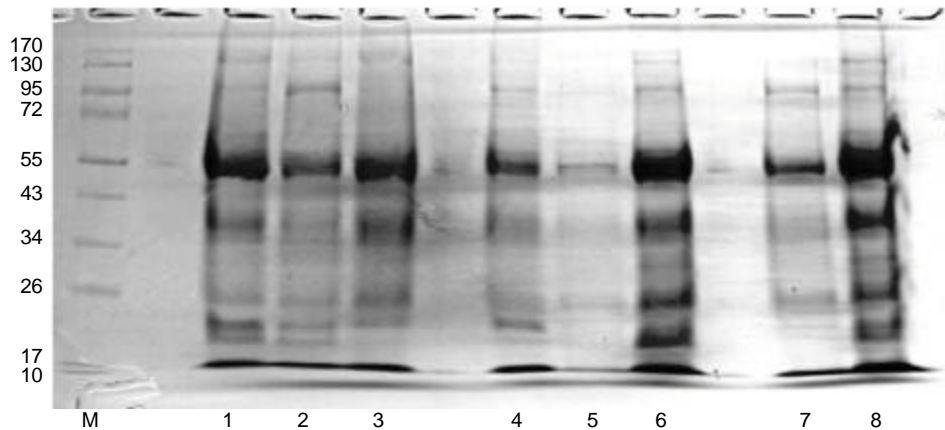


Fig. 1: Electrogram showing banding pattern of different molecular weight protein fractions. 1. Basmati, 2. Garah sagdasi, 3. IR-6, 4. Shadab, 5. IR-8, 6. Jujai-77, 7. Khara gunja, 8. Khushboo-95

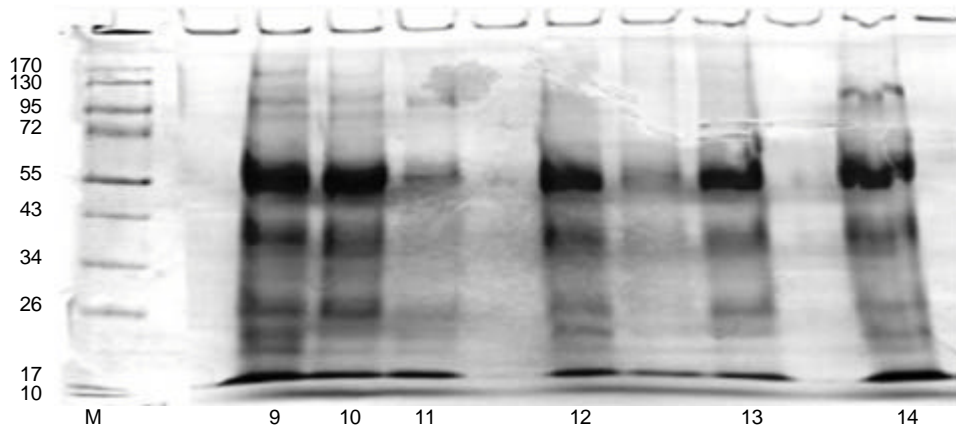


Fig. 2: Electrogram showing banding pattern of different molecular weight protein fractions. 9. Mahak, 10. Pokali, 11. Sada gulab, 12. Sarshar, 13. Shua-92, 14. Super basmati

RESULTS

Genetic diversity: Among numerous techniques available for assessing the genetic variability and relatedness seed proteins electrophoresis is reliable for measuring genetic diversity and studying evolutionary relationship (Oppong-Konadu *et al.*, 2005) and relatively inexpensive way of developing genetic markers for identification and genetic analysis of seed storage proteins in several grains (Bushuk and Zillman, 1978; Wrigley *et al.*, 1982).

Figure 1 and 2 show the electrophoretic patterns of seed storage proteins of fourteen rice varieties cultivated in Sindh.

A total of 17 bands were obtained among which 55 KDa protein is common in all varieties, 24 KDa protein is found in 13 varieties except basmati, while 37 KDa protein is observed in 12 varieties except in Garah sagdasi and IR-8. 64 KDa protein is present only in Basmati and Super basmati rice. The result shows that Basmati, Garah sagdasi, Sarshar and Shua-92 contain

9 subunits ranging from 16-140 KDa while other varieties have slightly variations in their protein patterns. Variety Jujai-77 and Khushboo, Mahak and Pokali show similar protein subunits except 21 and 18 KDa protein fraction appeared in Khushboo and in Mahak respectively.

Cluster analysis: The data of genetic similarities obtain from electropherogram were used to create a cluster diagram. Unambiguous resolution of protein banding pattern depending on their absence and presence were coded for 0 and 1 respectively. Similarity index and simple matching coefficient for percent genetic similarities between varieties were derived by Nei and Li (1979). The diversity of different varieties represented in a dendrogram (Fig. 3) constructed on the basis of linkage distance (Euclidian distance) (Romesburg, 1990) (Table 1) with arithmetic averages (UPGMA) cluster analysis for calculating the genetic resemblance by using software SPSS 16.0.

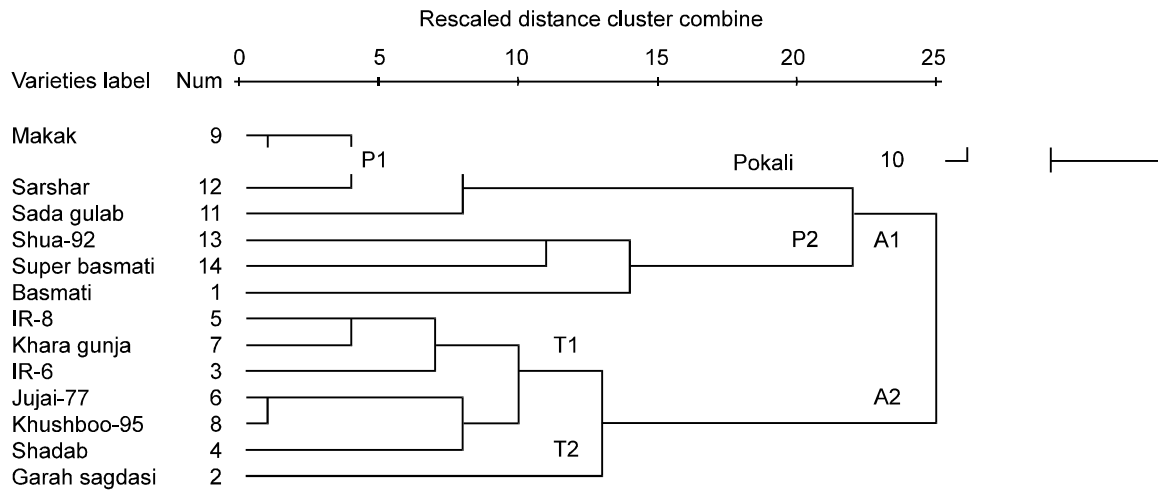


Fig. 3: Dendrogram using average linkage within group

Table 1: Molecular weight analysis of different rice varieties cultivated in Sindh Pakistan

MW/Kda	1	2	3	4	5	6	7	8	9	10	11	12	13	14
140	1	1	1	1	0	1	0	1	1	1	0	1	1	1
103	0	1	0	0	0	0	0	0	1	1	1	1	0	1
94	0	1	0	1	1	1	1	1	1	1	0	0	0	0
67	0	0	0	0	0	0	0	0	1	1	0	1	1	1
64	1	0	0	0	0	0	0	0	0	0	0	0	0	1
55	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	0	1	1	0	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	0	0	0	0	1	1
33	1	0	1	0	0	1	1	1	1	1	0	0	1	1
26	1	0	0	0	0	1	0	1	1	1	0	1	0	1
24	0	1	1	1	1	1	1	1	1	1	1	1	1	1
21	0	1	0	1	0	0	0	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	0	0	0	0	0	1
18	0	1	0	1	0	1	0	1	1	0	0	0	0	0
17	0	0	0	0	0	1	0	1	1	1	1	1	0	0
16	1	0	0	0	0	0	0	0	0	0	0	0	1	1
10	0	0	1	1	1	1	1	1	0	0	0	0	0	0

1. Basmati, 2. Garah sagdasi, 3. IR-6, 4. Shadab, 5. IR-8, 6. Jujai-77, 7. Khara gunja, 8. Khushboo, 9. Mahak, 10. Pokali, 11. Sada gulab, 12. Sarshar, 13. Shua-92, 14. Supper basmati

At Euclidian distance of 2.5 all the varieties show similarity with another and distributed into two main groups A1 and A2 shown in dendrogram. Each group comprised 7 varieties. A1 is further divided into two subgroups P1 and P2. P1 contain Mahak, Pokali, Sarshar and Sada gulab while Shua-92, Super basmati and Basmati are appeared in P2. In the same way A2 is further divided into two subgroups T1 and T2. Six varieties IR-8, Kharagunja, IR-6, Jujai-77, Khushboo-95 and Shadab are observed in T1 and T2 include only one variety Garah sagdasi. The varieties Mahak and Pokali of P1 and Jujai-77 and Khushboo-95 show 99% similarity.

DISCUSSION

In order to screen out the molecular weight of different protein fraction for characterization of rice protein and

genetic diversity SDS-PAGE electrophoresis of rice varieties cultivated in Sindh was done. Documentation by gel DOC system provide the data about banding pattern and molecular weight (MW) of different protein fraction ranging from 10-140 KDa according to standard protein marker (ferment as page ruler 10KDa-170KDa pre stained protein marker).

Rice glutelin is synthesized in rough endoplasmic reticulum as proglutelin (59 KDa) then pass Golgi body forming a group of proglutelin with the molecular mass 55~57 Kda (Qu Le-Qing *et al.*, 2001). Figure 1 and 2 show the variation in number of bands in which 55 KDa is common in all varieties but other bands are different. Low Molecular Weight (LMW) protein fraction of Sindh rice varieties were comprised of 11 different MW fractions in which 10, 16, 26 and 37 KDa compare favourably with those reported by Sachiko Furukawa *et*

al. (2003). Donald and Shridhar (1995) observed 16, 18, 20, 21, 26 and 33 KDa protein fraction correspond with Similar molecular mass in Sindh rice varieties.

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