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Comparative Study of Physicochemical Properties of Mutant Rice Varieties Cultivated in Sindh

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Abstract: Physicochemical Characterization of the individual rice seed and protein analysis by SDS-PAGE electrophoresis provides useful information for the isolation and utilization of seed proteins. Rice is highly consumable food with a variety of proteins and a popular subject to mutagenesis. Four mutant varieties with their parental varieties were collected from Nuclear Institute of Agriculture (NIA) Tandojam. Physicochemical analysis including grain weight, length, width, L/B ratio, Amylose and protein content were carried out for screening of comparatively better quality characters. After quantification, proteins were separated by SDS-PAGE Electrophoresis. Analysis of results of SDS-PAGE was performed by using gel documentation system (Biorad) to find out the molecular weight of different protein fractions present in mutant rice varieties.

Keywords: Physicochemical analysis, SDS-PAGE, seed storage proteins, mutagenesis.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crop feeding more than 2 billion people in Asia (FAO 1995). It is no longer a luxury food but has become the cereal that constitutes a major source of calories for the urban and the rural population (Sasaki and Burr, 2000). Rice has been a popular subject to mutagenesis because it is the world's leading food crop (McKenzie and Rutger, 1986). The use of induced mutation has been considered as an appropriate approach for developing short statured mutants accompanied by high yield and excellent grain quality. A number of traditional varieties and improved cultivars have been released for cultivation in different regions of Pakistan since the early nineteen century (Rabbani *et al.*, 2008).

2. MATERIALS AND METHODS

Sample collection:

Four mutant rice varieties Shadab, Shua-92, Sarshar and Khushboo-95 including their parental varieties IRRI-6, IRRI-8 and Jajai-77 were obtained from Nuclear Institute of Agriculture (NIA) Tandojam.

Physicochemical analysis:

Physical test:

The grain was dehulled and physico chemical characteristics including weight, grain length, width, length width ratio, were measured by using micrometer (veneer calliper).

Extraction and quantification of protein:

The grain was milled to get a fine powder. The total protein was extracted for quantification by suspending overnight 0.5 gram in 1ml extraction buffer. The suspension was sonicated for 30 min to optimize solublization. Then centrifuged for 20 min at 14000 rpm for 10 min. Supernatant was collected for quantification of proteins. Quantification was performed by following the procedure of Lowry *et al.*, 1951. Supernatant was stored at -40°C for further analysis.

Amylose content:

The formation of a helical complex between Amylose and iodine gives rise to the typical deep blue color of starch dispersions stained with iodine and forms the basis for quantitative determination of Amylose content. Amylose content was determined following the procedure of Juliano, 1971.

SDS- PAGE electrophoresis:

Protein samples were solubilized in the sample buffer (0.5M 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 5µl of the standard(fermentas prestain 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 electrophesis . (Naomi *et al.*, 1996) SDS-PAGE

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electrophoresis was performed, following the procedure of Leammli,1970. Using 10% acrylamide gel slabs overlaid with a 4% staking gel at a constant range of 30 mA for approximately 3 hours at 20°C. After completion of electrophoresis, the gel slabs were stained with 0.25% Coomassie Brilliant Blue R250/2-propanol :Acetic acid: H_2O 5:1:5 and destained in 10% acetic acid and 30% methanol until the background became transparent & 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.

3. RESULTS

The rice variety Shadab, evolved by treating the seeds of *IR6* 0.5%, with chemical mutagen, ethyl methane sulphonate (EMS 0.5%), approved for release in 1987.Comparative physiochemical analysis **(Table 1).** with improved grain length 7.5 mm, Amylose 30.70 % and protein 9.57 of Shadab shows a significant difference to its parental variety IR6.

Table 1.Comparative study of IR-6 and mutant rice variety Shadab

Characteristics	IR-6	Shadab
weight of 100 seeds	2.11g	1.87g
Length	6.95mm	7.5mm
Width	1.9mm	1.8mm
L/B ratio	3.65	4.16
Amylose%	28.90%	30.70%
Protein%	8.59%	9.57%

Table 2.Comparative study of IR-8 and mutant rice varieties Shua-92 and Sarshar

Characteristics	IR-8	Shua-92	Sarshar
weight of 100 seeds	2.39g	2.01g	2.19g
Length	6.5mm	7.3mm	8mm
Width	2.5mm	2.1mm	2.5mm
L/B ratio	2.6mm	3.47	3.2
Amylose%	26.20%	29.40%	27.20%
Protein%	6.86%	7.76%	7.74%

(**Table 2)** shows physiochemical characteristics of IR-8, Shua-92 and Sarshar. Rice varieties Shua-92 and Sarshar were evolved by irradiating the seeds of IR-8 with Gy fast neutrons and 150 Gy gamma rays were approved for Sindh province in 1993 and 2001 respectively. Both mutant varieties show improved characteristics including higher content of Amylose and protein comparatively than that of parental variety IR-8

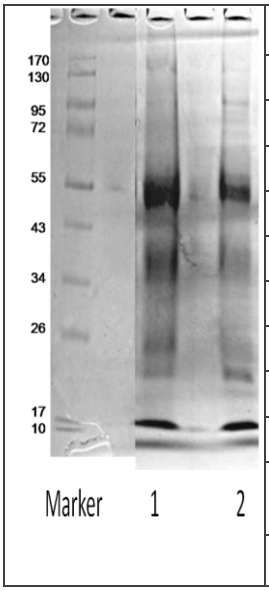
Table 3.Comparative study of Jajai-77 and mutant rice varieties Khushboo-95

Characteristics	Jajai-77	Khushboo-95
weight of 100 seeds	1.77g	1.73g
Length	7.5mm	7.5mm
Width	2.5mm	2.26mm
L/B ratio	3	3.31
Amylose%	20.92	20.8
Protein%	9.7	8.21

(Table 3) showws the comparative analysis of jajai-77 and its mutant variety khushboo-95 is in aromatic variety developed by exposing the seeds of Jajai-77 to 200 Gy Gamma rays was approved for released as a new variety in 196 for Sindh province. This variety contains less Amylose and protein value. However, it has strong aroma than its parental variety.

SDS-PAGE electrophoresis:

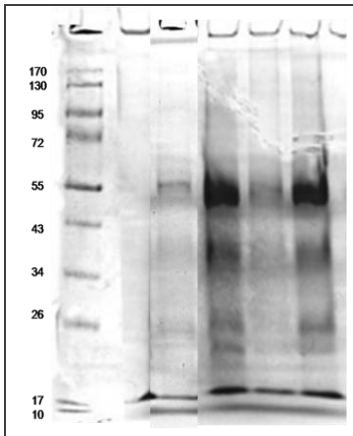
SDS-PAGE electrophoresis is the most effective as well as one of the simplest methods for separating proteins.



IR- 6 MW/ KDa	Shadab MW/ KDa
140	140
-	94
55	55
37	37
36	36
33	-
24	24
-	21
20	20
-	18
10	10

Fig. 1: Electrophoregram of 1. IR-6 and mutant rice variety 2. Shadab.

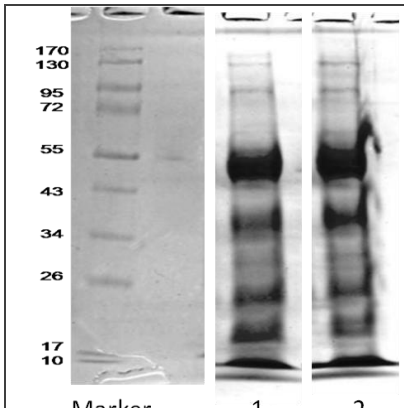
(Fig 1). Shows the electrophoregram of IR6 and its mutant variety Shadab with their banding pattern of different molecular weight protein fractions.IR6 contain 8 protein fraction 10,20,24, 33,36,37,55,and 140 KDa while in banding pattern of Shadab 10 subunits were observed including 8, 21, and 94 KDa protein which are not present in its parental variety. 33 KDa protein is found in IR6 is absent in Shadab variety.



IR 8 MW/ KDa	Sashar MW/ KDa	shua-92 MW/ KDa
-	140	140
-	103	-
94	-	-
-	67	67
55	55	55
-	37	37
36	-	36
-	-	33
-	26	-
24	24	24
-	21	21
20	-	-
-	17	-
-	-	16
10	-	-

Fig. 2. Electrogram of 1. IR-8, and mutant rice varieties 2. sarshar and3. shua-92.

The protein pattern of IR8, with its mutant varieties ,Sarshar and Shua-92 after SDS PAGE are shown in **(Fig 2).** A total of 15 bands were obtained in three varieties. A difference of 3 protein fractions was observed in mutant rice varieties as compare to IR8 which contain 6 bands 10, 20, 24,36, 55, and 94 KDa. Sharshar contain 17, 21, 24, 26, 37, 55, 67, 103, and 140 KDa protein fractions and Shua-92 shows 16, 21, 24, 33, 36, 37, 55, 67, and 140 KDa protein fractions in its banding pattern. 55 KDa protein is common among three varieties.



jujai-77 MW/ KDa	khushboo-95 MW/ KDa
140	140
94	94
55	55
37	37
36	36
33	33
26	26
24	24
-	21
20	20
18	18
17	17
10	10

Fig. 3: Electrogram of 1. Jajai-77 and mutant rice variety 2. Khushboo-95

(Fig 3). Represent the electrophoretic pattern of seed proteins of Jajai-77 and Khushboo-95. These varieties have considerable homogeneity in their banding pattern.Khushboo-95 contain 13 fractions 10, 17, 18, 20, 21, 24, 26, 33 36, 37, 55, 94 and 140 KDa with difference of only one 21 KDa protein fraction from its parental variety Jajai-77.

4. DISCUSSION

Physiochemical characteristics are of prime importance while developing a variety. The characteristic include grain weight, length, width, L/B ratio Amylose and protein content. The ratio of length to width is an important aspect to determine the shape of the variety (Normita and Cruz, 2002) and grain weight gives the information about the size and density of the grain. The density of different rice grains effect the cooking quality. So grain weight should be uniform because it determines the quality of grain Amylose content varies from 20 to 24% in basmati varieties while in non-basmati varieties 21.6 to 30.7% (Khurram *et al*, 2007). Proteins contents varies greatly in mature rice grain (Juliano *et al*, 1968)

The results indicated that the use of induce mutation has a good impact in the development of different varieties which are differ significantly in their properties. There were significant differences in physical characteristics, protein content and Amylose content as compare to parental rice varieties. Proteins could be reliably fractionated by SDS-PAGE. All of the varieties under study showed variability in their protein pattern except 55 KDa protein fractions which is common in all varieties.

The results of SDS PAGE of seven varieties compare favourably with those reported by Sachiko Furukawa *et al*., 2003. Donald and Shridhar, 1995 and QU Le-Qing *et al*.**,** 2001.

REFERENCES:

Donald F. S., and K.S. Shridhar (1995) Characterization and Digestibility of Basmati Rice *(Oryza sativa* L. var. Dehraduni) Storage Proteins. Cereal Chem. 72 (3), 275-280.

Food and Agriculture Organization Of The United Nations, (1995) FAO quarterly bulletin of statistics 8, 1-2.

Juliano B.O., C.C Ignacio., V.M., Panganiban and C.M. Perez (1968) Screening for high protein varieties. Cereal Sci. Today. Vol. **(13):** 299-301.

Juliano B.O. (1971) A simplified assay for milled rice amylase. *Cereal Science Today* Vol. **(6):** 334-338.

Khurram B., N. M. Khan, S. Rasheed, M. Salim. (2007) Indica rice varietal development in Pakistan: an overview. Paddy Water Environ**. (5):** 73–81.

Leammli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage. T4.Nature. 227: 680-685.

Lowry O. H., N.J. Rosebrough, A.L. Farr, R. J. Randall (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193 **(1):** 265-275.

Malik A. R.., Z. H. Pervaiz., M. S. Masood. (2008) Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. Electronic J. of Biotechnology. 11 **(3):** 1-10.

McKenzie K.S. and J. N. Rutger. (1986) A new semi dwarf mutant in a long grain rice cultivar crop. 26: 81-84.

Naomi J. C. Pollard. W. Wrigley. F. Bekes, A. A. Finlay and Mac. Ritchie (1996) Distinction between genotypes of Lupinus species by sodium dodecyl sulphate-gel electrophoresis and by capillary electrophoresis. Electrophoresis. **(17):** 221-223.

Normita M. and D. Cruz. (2002) *Rice Grain Quality Evaluation Procedures*. Graham R. A Proposal for IRRI to establish a grain quality and nutrition research centre: IRRI discussion paper series No. 44 Los Banos, Philippines: International rice research institute 15Pp.

QU Le Qing., H. Satoh, M. Ogawa, W. Xiao Li. (2001) Improved electrophoretical analysis for rice seed storage glutelin. Acta Genetica Sinaca. 28 **(8):** 730-737.

Sachiko F., T. Mizuma., Y. Kiyokawa., T. Masumura., K. Tanaka and Y. Wakai. (2003) Distribution of Storage Proteins in Low-Glutelin Rice Seed Determined Using a Fluorescent Antibody. Journal of Bioscience And Bioengineering. 96 **(5):** 467–473.

Sasaki T. and B. Burr (2000) International rice genome sequence project: the effort to complete the sequence of rice genome. *Current Opinion in Plant Biology*. 3 **(2):** 138-14