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PAKISTAN RICE GENETIC RESOURCES–III: SDS-PAGE DIVERSITY PROFILE OF GLUTELINS (SEED STORAGE PROTEIN)

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Abstract

Rice grain quality characters pertaining to seed storage proteins profile for glutelin was evaluated for diversity within Pakistan local rice genetic resources using SDS-PAGE. Materials consisted of 475 accessions collected from 3-rice cultivation zones and other parts of the country. A wide variation was found in the glutelin fraction of rice protein at 57kD pro-glutelin and 40kD glutelin acidic subunit bands 3 and 4. The enriched glutelin variation at 57kD may be used in the development of improved protein cultivars with respect to quality and quantity.

Introduction

Even in this 21st century cereal crop is not only the most important energy source but also a main source of protein for mankind. Rice has 8-9% protein and is world's single most important food crop and primary food source for more than a third of the world's population (Khush, 1997). Rice also contributes 20-40% of the proteins in the Asian diet (Satoh *et al*., 2000). It plays a very significant role in providing protein to a large segment of the World's population, especially those in the developing countries where animal proteins are not affordable. Therefore, it is important to improve the quality and nutritional value of the protein in rice.

For the classification of plant storage proteins, Osborn`s method is still used, where it depends on the relative solubility of the target protein in standard solvents (Osborn, 1924). The major classes of storage proteins have been classified accordingly into albumins, globulins, prolamins and glutelins (Fig. 1). This classification has been very valuable in grain protein research for the improvement of their nutritional value. Juliano (1972) also found rice protein to be composed of these four types wherein the glutelin and prolamin being the major storage proteins.

Rice is an excellent sample to study the storage protein biosynthesis because it is one of the few plants that synthesize and accumulate both major classes of storage proteins, i.e. prolamins and glutelins. Moreover, stores these proteins in different subcellular compartments (Krishnan *et al*., 1986; Muench *et al*., 1998; Yamagata & Tanaka, 1986). The major seed storage protein in most of the cereals is the alcohol soluble protein. In contrast, rice stores a high content (about 75% of total proteins on a weight basis) of glutelins in starchy endosperm making itself unique among cereal crops, except for oat. Comparing to prolamins, glutelins are more easily digestible and contain higher amount of essential amino acid of lysine (Ogawa *et al*., 1987; Resurreccion *et al*., 1993; Tanaka *et al*., 1975), illustrating the reason of why glutelin is much better than prolamine in nutrition value. Tanaka & Ogawa (1986) also stated that in molecular and biological terms, we must try to breed rice seeds with an enhanced expression of glutelin gene(s).

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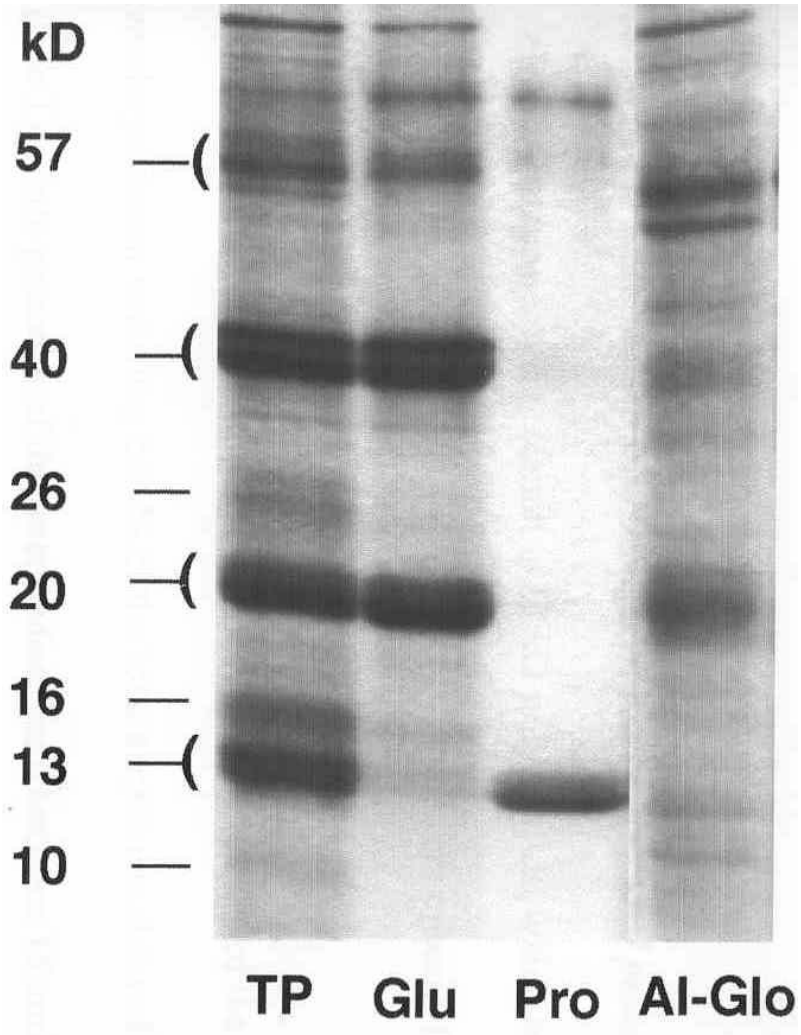

Fig. 1. Rice seed storage protein fractions of standard variety IR-36; TP- total proteins, Glu- glutelins, Pro- prolamins and Al-Glo- albumin globulins. 57kD bands represent pro-glutelin or glutelin precursor, 40kD bands are the glutelin acidic subunit of mature glutelin (α-1, 2, 3 &4). 20kD bands comprise the glutelin basic subunit of mature glutelin i.e., β-1, 2 &3. (The major classes of storage proteins have been classified according to their solubility properties into albumins, globulins, prolamins, and glutelins. Proteins which are soluble in water, 0.5 M sodium chloride and 70% ethyl alcohol are called albumin, globulin and prolamin, respectively. While the proteins which are soluble in 0.1 M acid or alkaline solution are called glutelin).

The successful improvement of rice storage proteins not only rely on a thorough understanding of its inheritance, characterization, biological and genetic regulation mechanism of biosynthesis and deposition, but also on the availability of the mutants to be used as material to study these mechanism and material incorporate by breeding or other technique. Investigations on the genetics and the effect of specific gene on qualitative and quantitative changes in carbohydrates, proteins or lipids in the rice endosperm started with the production of enormous amount of chemically induced mutants by Satoh & Omura (1979) using MNU. Later, mutants with high lysine content were reported for rice (Kumamaru *et al*., 1997). Satoh *et al*., (1990a, b; 1995) reported variation in seed storage proteins in rice collected from Tanzania, Madagascar and the North Asian countries.

In recent years variety discrimination and identification have been achieved in a range of agricultural crops by means of electrophoretic techniques (Moller & Spoor 1993),

according to them, the advantage of seed protein electrophoresis are the independence from the growing season, no need for plant cultivation, availability of material year round, ease of storing material, the relative speed of examination, the small size of sample needed, etc. More over, because proteins are the primary gene products they provide a valuable means of marking genetic system, this variation in protein composition is a reflection of genotypic variation. The detection and analyses of variation in the electrophoretic pattern of seed storage proteins is a useful method for establishing relationships among the plants accessions within a species (Gepts *et al*., 1986). Rice storage proteins have also been studied for their variation in composition and electrophoretic pattern (Hibino *et al*., 1989, Ogawa, *et al*., 1987, 1989, Resurreccion *et al*., 1993, Satoh 1985, Uemura *et al*., 1996).

Takeda (2000) is also of the view that, efficiency of plant breeding or selecting super genotype is dependent upon the efficiency of selection and magnitude of genetic variation existing in the breeding population. Thus the diversity of genetic resources is vitally important for the future plant breeding. Satoh *et al*., (2000) stated that mainly its storage proteins in a grain determine nutritional values of rice. Therefore, the development of improved germplasm with superior nutritional and cooking quality is one of the most important subjects on rice breeding. According to them, although, the artificial mutation technique is one of the useful methods to develop the novel genetic resources, spontaneous mutations preserved in the local rice germplasm are the most important genetic resources. Pakistan is one of the few countries which possess an enormous wealth of aromatic rices and old landraces (Rabbani *et al*., 2008).

The crop germplasm of Pakistan origin have been studied for variation of seed storage proteins in peas (Ghafoor & Arshad, 2008), wheat (Sultana *et al.*, 2007), lentil (Sultana *et al.*, 2006), black gram (Ghafoor & Arshad, 2005), groundnut (Javaid *et al.*, 2004) and cowpea (Iqbal *et al.*, 2003); but studies for rice seed storage proteins are scanty, though Siddiqui *et al.*, (2007a, b) reported rice grain morphological variation within Pakistani rice genetic resources. Rabbani *et al*., (2008) studied genetic diversity using RAPD markers; therefore the present study was carried out to determine the variation present in the glutelin (the major protein component of storage protein) for their utilization in grain quality improvement.

The research was conducted at the Laboratory of Plant Genetic Resources, Institute of Genetic Resources, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

Materials and Methods

Plant materials: Rice accessions (475) of Pakistan origin were obtained from the National Genebank of MAFF (Ministry of Agriculture, Food and Fisheries), Tsukuba, Japan. The seeds were collected from all over the country including the three rice cultivation zones.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

a. Extraction of Rice seed storage protein: All extraction procedures were carried out from powdered single de-hulled mature grain.

To extract total proteins, single grain powder was added with protein extraction buffer (0.125 M Tris, 4% SDS, 4 M urea and 5% 2-mercaptoethanol) vortex and gently shaken overnight, centrifuged at 10,000 rpm for 10 minutes.

b. Electrophoresis: The Laemmli's discontinuous buffer system was used (Laemmli, 1970) on a slab gel containing a linear gradient of 15-25% acrylamide / 0.5-0.67% N,N'-methylene- bis-acrylamide (BIS). 4 µl of the sample solution was used for each analysis. After electrophoresis the proteins were stained with 50% methanol, 7% acetic acid and 1% Coomassie brilliant blue (CBB) R-250. The observation was noted for presence or absence; slow or fast migration of band and their staining intensity.

Results

Pakistani varieties revealed 2 types of variation for pro-glutelin (57kD band) and 6 types of variation for glutelin subunit in the SDS-PAGE profile on the basis of apparent molecular mass.

The 57 kD polypeptide of the rice storage protein showed two types of variation (Fig. 2), i.e., enriched glutelin precursor (57H band) and normal intensity 57kD band (lane 1 and 2, respectively). The 57H spontaneous mutants were characterized by the remarkable increased content of 57 kD polypeptide with the markedly decreased content of 40 kD (glutelin acidic subunit) and 20 kD (glutelin basic subunit). The 84 variants for 57H character were distinctively observed as a separate group from the remaining 391 accessions. The frequency of 57H was 17.7% of the total germplasm.

The variation for α-3 band (Fig. 3A), i.e., low molecular mass (LMM) or the fast migration α-3 band type (lane 1), high molecular mass (HMM) or slow migration α-3 band type (lane 2), α-3 band having both LMM (fast) and HMM (slow migration) bands type (lane 3), and α-3 band deletion type (lane 4), were observed (Fig. 3A). Their respective frequencies were 82.7%, 15.6%, 1.3% and 0.4%, respectively (Table 1). The variation in α-4 band is shown in Fig. 3B revealing the variation for α-4 band, where LMM or fast migration (lane 1) and HMM or slow migration (lane 2) types were recorded. Their respective frequencies were 2% and 80%, respectively, for the total germplasm. In 17.7% of the 57H type rice cultivars that possesses low mature glutelin i.e., low α and β bands, the data for α and β bands were not recorded.

Discussion

Rice storage proteins are mainly composed of glutelin and prolamin. Rice glutelin consists of acidic (α) and basic (β) subunits which were composed of 4 and 3 bands, respectively (Uemura *et al*., 1996). From the screening of the available 475 Pakistan local rice cultivars on single seed bases, for total protein or glutelin fraction and compared to *indica* type IR36, a large variation was revealed for glutelin α3 band, α4 band and in pro-glutelin banding pattern. But only apparent intensity variation was observed for the glutelin β subunit in SDS-PAGE profile (Fig. 3).

In case of the proglutelin, two different types were identified (Fig. 2) differing in protein profile i.e., 57 kD high with 40 and 20 kD low type (lane 1) and normal types i.e. with 57kD low and 40 and 20 kD high type (lane 2). Similarly, Shiraishi *et al*., (1986) have also reported few rice varieties from foreign countries germplasm introduced into Japan to have high intensity of 57 kD polypeptide bands. Kumamaru *et al*., (1987) have stated that the enriched 57 kD and the reduced glutelin in (57H) CM1787 is supposed to be due to the abnormal processing of glutelin. Yamagata *et el.,* (1982) and Sarker *et al*., (1986) already showed that the 57kD polypeptide is a possible precursor of glutelin sub-units, which subsequently is cleaved into glutelin 22~23 kD β subunit and 37~39 kD α sub-units groups by proteolytic enzymes.

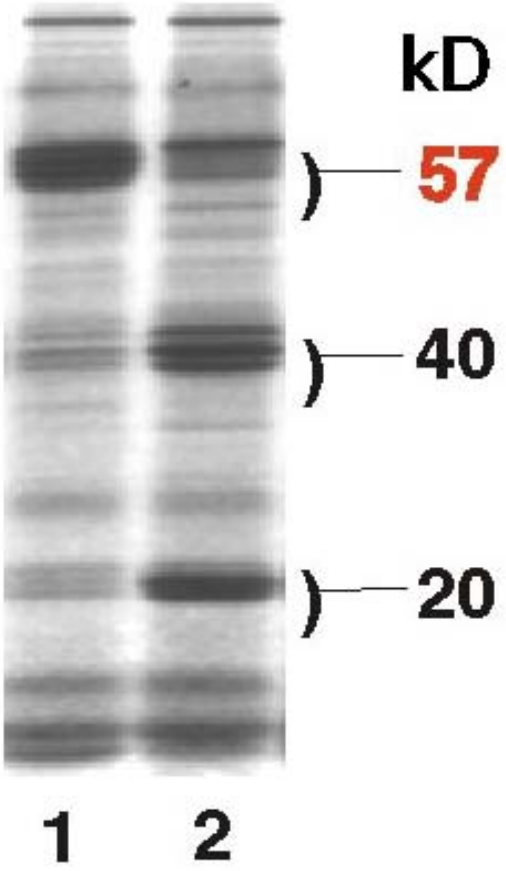

Fig. 2. Variation of the 57kD proglutelin polypeptide in Pakistan rice cultivars. (Lane 1- 57H or 57kD high, 40/20kD low; Lane 2- Normal or 57kD low, 40/20kD high).

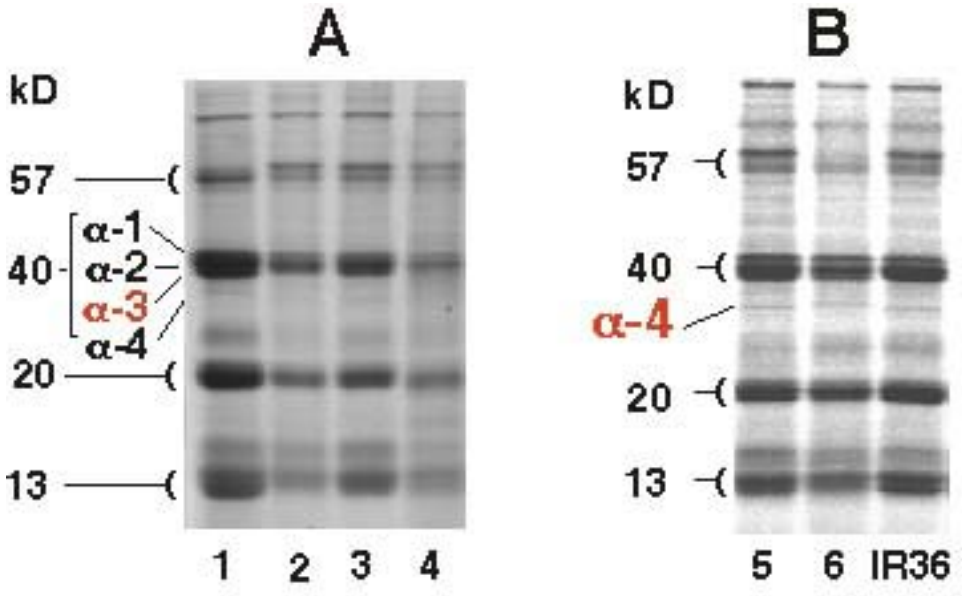

Fig. 3. Variation of acidic (α) glutelin band 3 (A) and band 4 (B) as revealed by SDS-PAGE analysis of seed storage glutelin, with rice genetic resources from Pakistan. (Lane 1- α3 fast migration; Lane2- α3 slow migration; Lane3- α3 two bands; Lane 4- α3 band absent; Lane 5- α4 fast migration; Lane 6- α4 slow migration band and Check variety IR36).

Table 1. Distribution of glutelin acidic subunit bands α3 and α4 variation among Pakistan rice genetic resources.

Location	α3 glutelin acidic band				α4 glutelin acidic band	
	LMM	HMM	L/H	α3L deletion	LMM	HMM
Azad Kashmir	23	5	1	2	26	5
Baluchistan	19	2	-	-	2	19
NWFP/NA	192	28	1	-	220	1
Punjab	51	4	8	-	56	7
No data	14	-	-	-	55	-
Total	326	63	10	2	356	35
% of total	68.6	11.2	2.1	0.4	91.0	9.0

LMM- low molecular mass or fast moving band, HMM- high molecular mass or slow moving band, L/H- both low and high molecular mass bands present, α3L deletion- low molecular mass band absent. Data for 84 accessions with low mature subunits could not be recorded.

According to Kumamaru *et al*., (1988), 57H mutants maybe useful in biochemical and genetical studies on the regulation, biosynthesis and accumulation mechanism of the rice glutelin. In another study Kumamaru *et al*., (1987) reported that 57H mutation is controlled by a single recessive gene. The presence of large number of 57H mutants in Punjab as compared to those reported by Satoh *et al*., (1995) is needed to be investigated. Its wide and frequent occurrence is possibly maybe due to some important agronomic trait, better adaptability, or some nutritional characteristic or maybe some disease resistance or stress tolerance, that made it possible to spread to that great extent.

Eggum *et al*., (1994) have also reported that the improved digestibility in *esp2* mutant compared to other mutants (*esp1, esp3,* and *Esp4*) and wild type Kinmaze was due to the higher glutelin precursor (57 kD band). Incorporation of these polypeptides will be an excellent achievement for improving the protein quality of rice grain or other cereals. Therefore the use of 57H mutant type recorded from Pakistan may be more nutritious for its biological value of protein.

The α 3 fast migration types were present along with slow migration types in SDS-PAGE (Fig. 3, lane 1 and 2, respectively). The distribution of both these variants was scored from all over the country, while accessions processing both these bands were not recorded from Baluchistan, which maybe due to low sample size (Table 1). The α 3 deletion type (Fig.3, lane 4) bearing rice cultivars may also be helpful in studying the genetics of glutelin α 3 band. This variation was only recorded from AJ-Kashmir although the sample size was low showing higher diversity for seed storage protein (glutelin). More collections of local landraces should be made from this area as all types of variants were recorded from this region.

The purpose of the research was to analyze the collected rice genetic resources from Pakistan to establish a basis for genetic improvement in seed storage protein. Large variation was observed for the major storage proteins i.e., glutelin. These variations maybe used in the improvement of grain nutritional quality by either increasing or decreasing the biological value of digestible proteins. The 57H variation seemed to be of very good utility in this regard.

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