

Study of Glutelin (Storage Protein of Rice) in Al-Hassawi Rice Grains

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ABSTRACT. SDS-Polyacrylamide gel electrophoresis of glutelin fraction reveals that dry grains of American, Basmati and Al-Hassawi rice varieties tested have the same pattern of the major polypeptides of 14, 15-18, 26-31 and 45-51 KD. However, three major polypeptides vary in their degree of expression. These cultivars vary in the number of minor high molecular weight polypeptides. American and Basmati rice have a 75 KD polypeptide which is not expressed in Al-Hassawi rice varieties. However, Al-Hassawi rice varieties have a polypeptide with a MW of 59 KD which is not expressed in American and Basmati rice.

Glutelin is the major storage protein in rice grain, and accounts for 80% of the total protein in the endosperm. This protein is composed of two disulphide linked acidic and basic polypeptide subunits with molecular weight of 18-22 and 33-36 KD typically of legumin like globulin (Robert *et al.* 1985, Zhao *et al.* 1983). These major polypeptides are subunits arising from posttranslational cleavage of a precursor of 51 KD (Robert *et al.* 1985 and Takaiwa *et al.* 1986). The coding region was 95% homologous to each other at the nucleotide and amino acid level. It is specifically synthesized and deposited in the endosperm of a developing grain, and is used as a nitrogen source during germination.

When reduced glutelin is resolved on SDS-PAGE it is found to be composed of four major groups of polypeptides having molecular sizes of 51, 38, 34, 21-22 and

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14 KD. The 14 KD polypeptide is found to be a prolamin formed in the main constituent as a major contaminant of glutelin fraction (Krishnan and Okita 1986). Under reduction conditions, one major subunit with MW of ca 20 and two minor subunits with MW of ca 35 and 48 have been separated. In contrast to these findings, reduced glutelin, from corn, oat, wheat and sorghum have two or more major polypeptide subunits. Reduced and alkylated glutelin from different cereals yields several major components which are not completely resolved on SDS-PAGE, six with corn, three with wheat and five with sorghum (Juliano and Boulter 1976).

S-cyanoethyl glutelin (Ce-glutelin) account for 9.5% of protein. IR-480-5-9 rice gave three major subunit with MW 38, 25 and 16 KD. The MW 38 KD subunit was unique to globulin and was not present in C3-albumin-globulin or prolamin (Juliano and Boulter 1976).

The amino acid composition of Ce-glutelin from IR-480-5-9 rice and their three major subunits showed high levels of aspartic and glutamic acid and low level of sulphur-amino acid. The 16 KD subunit is relatively lower in glutamic acid and arginine and higher in methionine. The MW 38 and 16 KD had lower lysine than the Ce-glutelin. In contrast the MW 25 KD subunit had higher content of lysine, aspartic acid and glycine and lower content of threonine, proline and methionine than whole glutelin (Juliano and Boulter 1976).

Since the rice glutelin fraction conforms to the general properties of cereal glutelin in being soluble in non-denaturing media it is difficult to characterize. Immunoprecipitation of *in vitro* translated product resulted in the synthesis of only the precursor indicating that the β (36 KD) and α (22 KD) subunit are the proteolytic products of the 57 KD precursor protein (Krishnan and Okita 1986, Yamagata and Tanaka 1986). The larger subunit group (30-36 KD) displays both molecular weight and charge heterogeneity and displays a relatively acidic *pI* value that all these characteristics are typical of 12S globulin. Western blot with antibody raised against 12S globulin bound strongly to both subunit group of rice glutelin and rice globulin fraction (Robert *et al.* 1985). Western blot analysis of the 34 and 21 to 22 KD polypeptides revealed distinct patterns indicating that these proteins are structurally unrelated. Partial amino acid sequence of the purified Mr 22 KD glutelin subunit was found to be homologous to the β -subunit of pea legumin, a storage protein which also contains disulphide-linked subunit pairs (Mr 38 KD and Mr 22 KD). Zhao *et al.* (1983) proposed that the major component of rice glutelin is a legumin like protein. Glutelins are synthesized at about 4 to 6 days post pollination whereas prolamin accumulation was detected several days later. The increase in glutelin was noted 10 days after pollination (Krishnan and Okita 1986).

Several lines of evidence indicate that rough endoplasmic reticulum (RER) is most likely the site of storage protein synthesis in endosperm of developing corn (Larkins and Dalby 1975), barley, wheat and oat seed (Yamagata and Tanaka 1986). The synthesis of glutelin and prolamin is not coordinate during seed development, glutelin and prolamin are both synthesized on the RER, but are then sequestered into distinct protein bodies by different cellular pathways. Glutelins are transported to a vacuolar compartment via the golgi complex whereas prolamin like maize zeins, are retained within the RER lumen, which eventually form the prolamin protein bodies. (Li *et al.* 1993).

Takaiwa *et al.* (1987) has reported that two dimensional gel analysis of acidic and basic subunits of rice glutelin could be resolved into at least 12 and 9 polypeptides respectively. One interpretation of the high heterogeneity is the existence of *in vitro* posttranslational modification. The other is that there are other types of glutelin genes. The presence of two or three subfamilies differing in size and sequences has been reported in *Vicia faba*, *Arabidopsis thaliana* 12S globulin and sunflower 12S globulin soybean glycinin and pea legumin (Takaiwa *et al.* 1987, 1991b).

Rice glutelin has many characteristics in common with 12S globulin of dicotyledon plants and the oat globulin. Amino acid and nucleotide sequences confirmed the structural homology between rice glutelin and those of other plants (Takaiwa *et al.* 1987, 1991a and b).

The rice glutelin is coded for by a small multigene family consisting of about 10 members per haploid genome. Expression of these genes is under the strict tissue-specific and temporal control. Five glutelin genes have been sequenced at the cDNA and genomic level. These glutelin genes are grouped into two major subfamilies (GluA and GluB) based on their amino acid sequence sharing 50-60% homologies to each other. Sequences of cDNA and two genomic counterparts of glutelin were determined (Takaiwa and Oono 1991). The nucleotide sequence of one gene (Glu A-3) was completely identical with the new cDNA while the other Glu A-4 was found to be a pseudogene. These glutelin genes were closely related to each other and belong to the subfamily A containing the type I (Glu A-1) and II (Glu A-2) glutelin genes. The degree of homology among all subfamily A members is above 80% to 88% in their coding region and it appears that the 2 subfamilies are coordinately expressed, whereas the recent identity between members of different subfamilies were less than 65% (Takaiwa *et al.* 1991b).

Glutelin A subfamily are encoded by at least three classes of genes each present

at about 5-8 copies per haploid genome (Kim *et al.* 1993). The major 15 KD prolamin appears to be encoded by 80 to 100 genes. Posttranscriptional level mRNA stability and cytoplasmic transport play a significant role in affecting the steady state level of mRNA. Glutelin mRNA are enriched on the cisternal-endoplasmic reticulum (Li *et al.* 1993). Glutelin is accumulated at 5-8 fold greater level than prolamin, depending on the stage of grain development. Northern blot analysis indicates that glutelin and prolamin mRNA are present at the same abundance levels during endosperm development but glutelin transcripts are more efficiently recruited into membrane bound polysomes. These observations suggest that both transcriptional and posttranslational events control storage protein biosynthesis in rice endosperm (Okita *et al.* 1989).

Okita *et al.* (1989) reported that glutelin are encoded by a complex gene group consisting of at least three subfamilies, G1, G2 and G3 each containing five to eight copies. Gt1 and Gt2 were closely related and evolved by more recent gene duplication events. Gt3 shows significant divergence from the other glutelin genes (Takaiwa and Oono 1991). The three glutelin genomic clones showed significant homology to the legume IIS storage proteins indicating a common gene origin. The relative constancy of the number of gene copies per glutelin subfamilies would be consistent with the hypothesis that genes encoding the glutelin protein, already existed as a multigene family which was then duplicated. Divergences by segmental mutations and subsequent homogenization by gene conversion would account for the diversity of Gt1/Gt2 and Gt3 gene subfamilies. More recent rounds of duplication and divergence of Gt1/Gt2 would result in the generation of Gt1 and Gt2 classes sharing closer sequence similarity (Okita *et al.* 1989).

Glutelin genes are expressed only in the endosperm during seed development and are regulated at the transcriptional level (Takaiwa and Oono 1991).

Rice is used as the major source of protein in the diets of tropical Asia, (Juliano and Boulter 1976). Because of the importance of rice as the major source of carbohydrate (and a major daily nutrient) in the diet of Arabian Gulf countries citizens, breeding program are under way in Hofuf experimental station with the cooperation of the Regional Center of Research on Plants and Animals to improve Al-Hassawi rice. This paper describes the characteristics of the major and minor subunits of rice glutelin from Al-Hassawi rice, and attempts to establish the value of protein in this variety.

Materials and Methods

A number of rice (*Oryza sativa* L.) cultivars including, American, Basmati (commercial products favored by Saudi Arabia citizen in Riyadh and Al-Hassa area respectively), local Al-Hassawi variety (Land race) and Al-Hassawi-1 (Al-Hassawi hybrid from crosses between Al-Hassawi and a Chinese inbred variety) and Al-Hassawi-2 (Al-Hassawi hybrid from crosses between Al-Hassawi and a Chinese inbred variety) all genotypes were hand planted during the summer seasons of 1992-93 and 1993-94 at Al-Hassa, Irrigation and Drainage Research Center, Al-Hassa, Saudi Arabia. The experimental design was conducted in a randomized complete block design with three replicates. Spikes were tagged at flowering and developing seed were harvested at the appropriate time after flowering or complete maturation. The whole grain were either used immediately or stored in liquid nitrogen. All plants were self pollinated and spike samples were harvested at maturity unless otherwise indicated.

Total protein determination and protein fractionation

The extraction procedure was basically a modified Osborne methods as described by Tsai (1979) and Lee (1981). Grains were ground in Waring blender for about 30 seconds. A 1g sample was then powdered in miniature ball mill (Wig-L-Bug Crescent Dental Mfg. Co., Chicago Illinois) for 5 min. Samples were defatted for 24 hr with a n-hexane in Soxhlet apparatus (Lee 1981). Material was used for total protein and nitrogen determination. For electrophoretic studies, approximately 1 g of defatted samples were sequentially fractionated into albumin, globulin, prolamin, and glutelin protein (Tsai 1979). The sample was extracted with 1 ml of distilled water in a 0-4°C water bath for 30 min., followed by centrifugation at 18,800 xg for 10 min. This was repeated twice and the supernatant, from this treatment, were combined and considered to be albumin proteins. The residue after the third centrifugation was suspended two times in 1 ml 5% NaCl in 0-4°C water bath for 30 min. and the combined NaCl-soluble fraction was referred to as globulin proteins. The residue for the NaCl treatment was suspended in 1 ml of water, then centrifuged to lower the salt concentration. 0.25 ml of the 95% ethanol containing 1 mM 2-mercaptoethanol was added to the residue and centrifuged. The suspension was shaken for 30 min at 60°C, then centrifuged and supernant saved. The pellet was shaken with 0.5 ml of 70% ethanol containing 1 mM 2-marcopethanol for 60 min. at 60°C and centrifuged. The two supernatant were combined and referred to as prolamin proteins. The residue from the alcohol treatment was suspended in 1 ml water and centrifuged to lower the alcohol concentration, then the residue was treated two times with 0.25 ml of 0.1N NaOH and centrifuged. The suspension was

shaken for 30 min at 50°C, then centrifuged at the same speed for 10 min, and the supernatant was saved. The two supernatants were combined and considered as glutelin proteins. Protein in these fractions, was determined colorimetrically using the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Total protein content was measured by micro-Kjeldahl method (AOAC 1970).

Gel electrophoresis

Extracted glutelin was dialyzed overnight against the sample buffer (0.025 M tris-glycine, pH 8.3, 1% SDS, 1% 2-mercaptoethanol to lower the alkaline concentration. The samples were dissolved in sample buffer containing, 0.004 % bromophenol blue and 10% glycerol and analyzed on slab gel by SDS-polyacrylamide gel electrophoresis system similar to that of Laemmli (1970) and Tsai (1979). Samples containing approximately 10-30 µg of glutelin were loaded in one of the sample slot of the slab gel. Gels were 1 mm thick and consisted of a 15 cm running gel of 12% acrylamide/bis acrylamide = 75/1) in a 0.375 M tris-HCl (pH 8.9), 0.058 mM TEMED, and 0.05% SDS. The running gel was overloaded with a 4 cm stacking gel of 4% acrylamide. Freshly prepared ammonium persulfate solution was added to a final concentration of 0.35% immediately before gel layer was poured. Electrophoresis was done at room temperature by applying 10 µAmp constant current through the stacking gel and 20 µAmp for the running gel until the tracking dye reached the bottom of the gel. Gels were stained in solution of 0.1% coomassie blue, 45% methanol and 9% acetic acid and destained in 15% methanol-7% acetic acid.

Results

Nitrogen and protein determination

The mean nitrogen and protein content of varieties were compared and the analysis of variance of the means pooled across years showed a significant difference among rice varieties for nitrogen and protein (Table 1). The nitrogen content ranged from 1.69 percent for Basmati and Al-Hassawi-2 to 2.12 percent for local Hassawi while protein content ranged from 9.6 percent in Basmati and Al-Hassawi-2 to 12.19 percent in local Al-Hassawi.

Characterization of normal storage components in rice glutelin

Glutelin was isolated according to Tsai (1979) and Lee (1981), and its quantity in dry seed weight was determined spectrophotometrically according to the method

of Lowry *et al.* (1951). Glutelin quantity (Table 2) was the same in American and Basmati rice which amount nearly to 1.00 mg. However glutelin quantity was the same (1.3 mg) in local Al-Hassawi (land race) and Al-Hassawi-2, but Al-Hassawi-1 showed a relatively higher quantity compared to all other varieties tested. The quantity was 25% more than local Al-Hassawi and Al-Hassawi-2, and double that of American and the Basmati rice (Table 2).

Table 1. Total nitrogen and protein in rice cultivars

No.	Variety	Nitrogen %	Protein %
1	Al-Hassawi 1	1.96b	11.27b
2	Al-Hassawi 2	1.67d	9.6d
3	Local Al-Hassawi	2.12a	12.19a
4	American rice	1.76c	10.12c
5	Basmati rice	1.69d	9.71d

Means within each column followed by the same letter are not significantly different ($p = 0.05$) based on Duncan's multiple range test.

Table 2. Total protein fractions (mg) in some rice cultivars in 100 mg of dry weight

No.	Variety	Enzymatic protein		Storage protein		Total
		Albumin	Globulin	Prolamin	Glutelin	
1	American rice	0.875c	1.54a	0.41c	0.97c	3.795c
2	Basmati rice	0.894c	1.47b	0.12d	0.97c	3.80c
3	Local Al-Hassawi	1.5b	1.58a	0.58ab	1.32b	4.90b
4	Al-Hassawi 1	1.9a	1.44bc	0.60a	1.73a	5.67a
5	Al-Hassawi 2	1.4b	1.40c	0.54b	1.28b	4.62b

Means within each column followed by the same letter are not significantly different ($p = 0.05$) based on Duncan's multiple range test.

SDS polyacrylamide gel electrophoresis pattern of rice glutelin prepared from dry grain.

SDS-polyacrylamide gel electrophoresis (PAGE) has been developed as a mean for characterizing, identifying and comparing the glutelin polypeptides pattern of different rice (*Oryza sativa*) varieties (Tsai *et al.* 1980). Typical SDS-PAGE of glutelin protein reveals that American and Basmati rice have the same pattern of glutelin polypeptides. Both have major polypeptides of 17-14 KD, < 10 KD of Class A component and 45-51 and 26-31 KD of Class B components, and a minor polypeptides of 84, 74.5 and 68 of Class C components and a number of minor bands whose molecular weight are more than 100, 110 and 130 KD of Class D. In most cases these bands separated as a doublet (Fig. 1 and 2). All the major polypeptides are of relatively low molecular weights which may be classified into Class A and B while most of the minor polypeptides are of high molecular weights of Class C and D components. (Fig. 1 and Table 3).

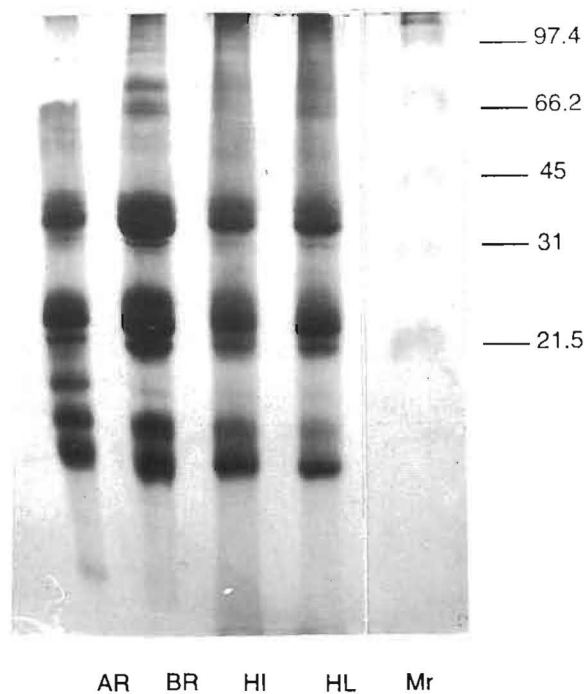


Fig. 1. SDS-Polyacrylamide gel electrophoresis pattern of glutelin extracted from rice (*Oryza sativa*) dry seed of American rice (AR), Basmati rice (BR), Al-Hassawi cultivars (HI), Al-Hassawi Land race (HL), and molecular weight marker (Mr).

Al-Hassawi-1, Al-Hassawi-2 and Local Al-Hassawi rice reveal the same pattern of glutelin polypeptides on SDS-PAGE. All show a number of major and minor bands. The major polypeptides are the same as those expressed in American and Basmati rice (Fig. 1 and 2) except that the intensity of these polypeptides in all Al-Hassawi varieties are relatively less. The minor polypeptides in all-Al-Hassawi varieties are of > 100 KD of Class D, 84 KD of Class C, 59 KD of Class B components expressed at low level than their counterpart in American and Basmati rice. One of the Class B component (59 KD) is not expressed in American or Basmati rice. The bands of 80, 75, 68 KD are not expressed in any one of the Al-Hassawi varieties. The minor doublet high molecular bands (110-130 KD) observed in American and Basmati rice are not observed in Al-Hassawi cultivars but most high molecular weight bands > 100 KD are observed at low intensities (Fig. 1 and 2).

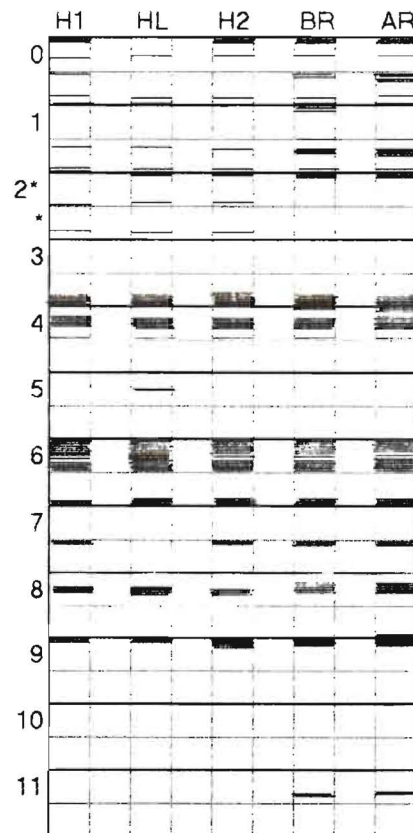


Fig. 2. SDS-Polyacrylamide gel electrophoresis from rice (*Oryza sativa*) dry seed of American rice (AR), Basmati rice (BR), (H1) Al-Hassawi 1, (H2) Al-Hassawi 2 and (HL) Hassawi land race.

Discussion

Glutelin constitutes the major fraction 70-75% of storage proteins in all varieties (Table 2). Al-Hassawi-1 showed the highest content of glutelin among varieties. We found a positive correlation between glutelin content and the total storage proteins in all Al-Hassawi cultivars. These results were in agreement with previous observation (Juliano and Boulter 1976) suggesting that difference in the level of rice endosperm protein were mainly due to difference in their glutelin content.

The only difference that we detected between the American and Basmati rice in the intensity of the Class A polypeptides of 14-17 KD. It is faint in the Basmati and sharp in American rice. This means that this band is highly expressed in American rice more than in Basmati rice suggesting a differential regulation in the two varieties.

Table 3. The calculated molecular weight of major and minor rice (*Oryza sativa*) glutelin components from SDS-PAGE

No.	A.R.	B.R.	H.R.I.	H.L.R.
1	> 100, 110, 130	> 100	> 100	> 100
2	84	84	84	84
3	80*	80	59*	59
4	74.5*m	74.5	45-51	45-51
5	68*	68	26-31	26-31*m
6	45-51*	45-51	17.5-14.5*	17.5-14.5*
7	26-31*	26-31	< 14	< 14
8	17.5-14.5*	17.5-14.5*	< 10	< 10
9	< 14	< 14		
10	< 10	< 10		

* Major band.

The similarity between American, Basmati and Al-Hassawi rice cultivars in the major polypeptides suggest that these polypeptides are encoded by the same number of genes and the relative difference between these polypeptides in the degree of expression indicated that their mode of regulation may be different either at the level

of translation, posttranslation or both. The absence of some of the high molecular weight polypeptides in Al-Hassawi rice and the low degree of expression of other high molecular weight polypeptides in compared to American and Basmati, suggest that polypeptides in Al-Hassawi rice are either encoded by small number of genes controlled by a relatively large number of genes, some of these genes may be repressed.

Kim and Okita (1988a) reported that reduced glutelin had four major subunits of 51, 38-34, 21-22 and 14 KD. In this study the subunits with 51 and 14 KD are also found beside the polypeptides 18-15 and 26-31 KD, which are some what lower but very close to those one reported by Juliano and Boulter (1976). Kim and Okita (1988b) have suggested that glutelin 14 KD polypeptides was a major contaminant of prolamin fraction. In other independent study, we had not found the 14 KD polypeptides in the prolamin SDS-PAGE, but only found in the glutelin fraction (Al-Mssallem 1996).

This study indicates that local Al-Hassawi rice contains all the polypeptides reported for other rice cultivars studied. However, the local cultivars appear to be unique in possessing high MW polypeptide bands which may have been conserved.

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دراسة على الجلوتلين (البروتين التخزيني للأرز) في حبوب الأرز الحساوي

إبراهيم صقر المسلم و منيرة قاسم المسلم

كلية العلوم الزراعية والأغذية - جامعة الملك فيصل
الهفوف - ص.ب (٤٢٠) - الاحساء ٣١٩٨٢ - المملكة العربية السعودية

الرحلان الكهربائي للجلوتلين عبر هلام عديد الإكريلاميد - كبريتات دوديسيل الصوديوم يوضح بأن الحبوب الجافة للأرز الأمريكي والبسمتي وأصناف الأرز الحساوي (المحلية أو المرباة) تعطي نفس النموذج من عديدات الببتيد الرئيسة ١٤، ١٥-١٨، ٢٦-٣١، ٤٥-٥١ كيلو دالتون. ورغم ذلك تتباين ثلاثة من عديدات الببتيد الرئيسة في درجة تعبيرها. وتتباين هذه الأصناف في عدد عديدات الببتيد الثانوية عالية الوزن الجزيئي. الأرز الأمريكي والبسمتي يملكان عديدا الببتيد ذات الوزن الجزيئي ٧٥ كيلو دالتون التي لم يُعبر عنها في أصناف الأرز الحساوي (المحلية أو المرباة)، في حين أن أصناف الأرز الحساوي (المحلية أو المرباة) تملك عديدا الببتيد ذات الوزن الجزيئي ٥٩ كيلو دالتون والتي لم يُعبر عنها في الأرز الأمريكي أو البسمتي.