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DIVERSITY OF GLUTELIN ALPHA SUBUNITS IN RICE VARIETIES FROM PAKISTAN

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Abstract

The diversity of glutelin α subunits in thirty-two rice varieties from Pakistan was assessed using higher temperature sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Five variation types i.e. Type-I, Type-II, Type-III, Type-IV and Type-V on the basis of variation in molecular size and intensities of glutelin α subunits were identified. Six rice varieties, i.e., Khushboo-95, Shahkar, Sonahri-Sugdasi, Sugdasi-Bengalo, Sugdasi-Ratria and Sugdasi-Sadagulab exhibited Type-IV variation having comparatively higher glutelin content. These varieties may be important genetic resources for improving glutelin content of rice seed. Rice variety Jhona-349 exhibited Type-V variation type and displayed unique variation by showing the presence of unique bands. Accordingly, this variety might have some unique glutelin genes and need to be further explored for nutritional quality improvement of rice seed.

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crop which is staple food for half of humanity and also the dietary mainstay of mankind which provide more than one half of dietary protein especially to Asian peoples (Duff, 1991; Fresco *et al*., 2005). The future demand for rice as a dietary protein will increase as anticipated from projected world population increase (Mann *et al*., 1997).

Rice seed storage proteins are grouped into four classes based on solubility properties like albumins (water soluble), globulins (salt soluble), prolamins (soluble in aqueous alcohol solutions), and glutelins (soluble in dilute acid or alkali) (Osborne, 1964). Among the four classes, glutelin is the major part accounting for 80% of rice seed protein (Takaiwa & Oono, 1991). Glutelin is composed of heterogeneous disulfide linked α (acidic) and β (basic) polypeptides which are the product of posttranslational proteolytic cleavage of precursor form (Yamagata and Tanaka *et al*., 1986; Furuta *et al*., 1986; Katsube-Tanaka *et al*., 2004). As glutelin is the major component of rice seed, rice protein quality is mainly determined by this protein. Any change in glutelin will alter the seed protein and nutritional quality of rice seed.

Rice genetic resources are important reservoirs of genes and could be exploited to broaden the existing narrow genetic base and to enrich the existing varieties with important favorable traits (Zeng *et al*., 2007). The exploration concerning new superior genetic resources is widely suggested to broaden the genetic base for nutritional quality improvement of rice seed (Katsube-Tanaka *et al*., 2001, 2004ab, 2005). In our previous efforts to improve the nutritional quality of rice seed we assessed the inter-specific rice glutelin diversity in wild species (Khan *et al*., 2008ab). However, a thorough

understanding of intra-specific glutelin polypeptides diversity is also important for broadening the genetic base for nutritional quality improvement of rice seed. Some previous studies reported a wide variation of glutelin subunits in local rice germplasm and suggested their usefulness in nutritional quality improvement of rice seed (Kagawa *et al*., 1988; Bhowmik *et al*., 1990; Aung *et al*., 2003; Jahan *et al*., 2005). In Pakistan a large number of traditional and improved rice cultivars are available but to date no detailed report is available about the diversity of glutelin α subunits. Therefore, in the current study we assessed the diversity of glutelin α subunits in traditional and improved varieties from Pakistan which will greatly benefit our effort to find superior genetic resources for nutritional quality improvement of rice seed.

Materials and Methods

Plant materials: Rice traditional and improved varieties developed by various research institutes were used as plant materials in the current study. The detailed information about plant materials are presented in (Table 1).

Extraction of proteins: Seed proteins were extracted according to the method described by Iida *et al*., (1997). A single grain was crushed to powder and mixed with 700 µL of SDS-urea extraction buffer (0.125 M Tris pH6.8, 4% (w/v) SDS, 8 M urea, 20% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol and 0.01% (w/v) BPB) by vortexing. The samples were incubated overnight at room temperature. Then the samples were centrifuged at 12000 rpm at room temperature for 5 minutes. The resultant supernatant was subjected to SDS-PAGE analysis.

Higher temperature SDS-PAGE: SDS-PAGE analysis was carried out according to Khan *et al*., (2007) at 45^{o}C using 14%T acrylamide gel. Electrophoresis was performed at constant voltage of 200V with lowered concentration of running buffer (16.7 mM Tris, 127.9 mM glycin, 0.07% SDS). The temperature of the running buffer was equilibrated to 45^{o}C. Electrophoresis was continued for about 80 minutes or until the prolamin polypeptides were nearly reached to the bottom of gel.

Gel staining and de-staining: The gel was stained in staining solution (10% methanol, 6.6% acetic acid and 0.05% Coomassie Brilliant Blue R-250) for 1 hour. Then the gel was de-stained in de-staining solution (10% methanol, 6.6% acetic acid) for about 1 hour or until the color of background disappeared and electrophoresis protein bands were clearly visible.

Results

Seed proteins extracted from rice varieties were separated by the higher temperature sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and detected with CBB staining. Glutelin acidic subunits were mainly separated into three major bands (α1, α2, α3) and one minor (α4) band (Fig. 1). These results were inconformity with Jahan *et al*., (2005) who fractionated glutelin α subunits into four bands in Bangldesh germplasm. *Japonica* rice cultivar Kinmaze and *indica* rice cultivar IR36 were used as standard check. The α-3 band of Japanese rice cultivar Kinmaze is smaller in molecular size than that of rice cultivar IR36 (Uemura *et al*., 1996).

Table 1. List of rice varieties used in the present study.

Sr. No.	Name of variety	Type of variety	Breeding institute*	Year	Status
1.	Basmati-370	Aromatic	RRI, KSK, Lahore	1933	Cultivated
2.	Mushkan	Aromatic	RRI, KSK, Lahore	1933	Discontinued
3.	Basmati-C622	Aromatic	RRI, KSK, Lahore	1964	Discontinued
4.	Basmati-Pak	Aromatic	RRI, KSK, Lahore	1968	Cultivated
5.	Basmati-198	Aromatic	RRI, KSK, Lahore	1972	Cultivated
6.	PK-177	Aromatic	RRI, KSK, Lahore	1977	Discontinued
7.	Basmati-385	Aromatic	RRI, KSK, Lahore	1988	Cultivated
8.	Super-Basmati	Aromatic	RRI, KSK, Lahore	1996	Cultivated
9.	Kashmir-Basmati	Aromatic	NIAB, Faisalabad	1981	Cultivated
10.	IR8	Aromatic	RRI, KSK, Lahore	1969	Discontinued
11.	Shaheen-Basmati	Aromatic	SSRI, Pindi Bhatian	2001	Cultivated
12.	Rachna-Basmati	Aromatic	NARC, Islamabad	1996	Cultivated
13.	Sugdasi-Ratria	Aromatic	RRI, Dokri Sindh	1956	Discontinued
14.	Khushboo-95	Aromatic	NIA, Tandojam,Sindh	1996	Cultivated
15.	Dokri Basmati	Aromatic	RRI, Dokri, Sindh	1963	Discontinued
16.	Sugdasi-Bengalo	Aromatic	RRI, Dokri, Sindh	1942	Discontinued
17.	Sugdasi-Sadagulab	Aromatic	RRI, Dokri, Sindh	1945	Discontinued
18.	Sonahri-Sugdasi	Aromatic	RRI, Dokri, Sindh	1952	Discontinued
19.	Basmati-2000	Aromatic	RRI, KSK, Lahore	2000	Cultivated
20.	Pk-386	Non-aromatic	Unknown	-	Used as adulterant
21.	Sathra	Non-aromatic	RRI, KSK, Lahore	1934	Discontinued
22.	KS-282	Non-aromatic	RRI, KSK, Lahore	1982	Cultivated
23.	NIAB-IR9	Non-aromatic	NIAB, Faisalabad	2001	Cultivated
24.	IR6	Non-aromatic	RRI, Dokri, Sindh	1971	Cultivated
25.	DR-82	Non-aromatic	RRI, Dokri, Sindh	1982	Cultivated
26.	Pakhal	Non-aromatic	ARS, Mansehra	1993	Cultivated
27.	Dilrosh-97	Non-aromatic	ARI, Mingora, Swat	1997	Cultivated
28.	Mahlar-346	Non-aromatic	RRI, KSK, Lahore	1939	Discontinued
29.	Sonahri-Kangni	Non-aromatic	RRI, Dokri Sindh	1962	Discontinued
30.	KSK-133	Non-aromatic	RRI, KSK, Lahore	2006	Cultivated
31.	Shahkar	Non-aromatic	RRI, Dokri, Sind	2006	Cultivated
32.	Jhona-349	Non-aromatic	RRI, KSK, Lahore	1933	Discontinued

*RRI, KSK: Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan
NIAB: Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan
SSRI: Soil Salinity Research Institute, Pindi Bhattian, Hafizabad, Pakistan
NARC: National Agricultural Research Centre, Islamabad, Pakistan
NIA: Nuclear Institute of Agriculture, Tandojam Sindh, Pakistan
RRI: Rice Research Institute, Dokri, Sindh, Pakistan
ARS: Agricultural Research Station, Dhodial, Mansehra, Pakistan

In the present study glutelin subunits variation identical to *japonica* cultivar 'Kinmaze' was not observed. However, in addition to *indica* cultivar 'IR36' variation type, the variation observed in rice varieties were classified into five variation types i.e., Type-I, Type-II, Type-III, Type-IV and Type-V based on variation in molecular size and staining intensity (accumulation level) of glutelin α subunit (Fig. 1) and (Table 2). Type-I variation was characterized by the higher intensity of α3 subunit (α3HI). Type-II variation has higher molecular size of α3 subunit (α3HM). Type-III variation has higher intensity of α2 subunit (α2HI). Type-IV was characterized by the higher intensity of α1 subunit (α1HI), α2 subunit (α2HI) and α3 subunit (α3HI). Type-V variation was quite unique compared to other variation types. Type-V variation showed some unique bands of lower molecular size which were not observed in other variation types. These unique bands were tentatively designated as (α^a) and (α^b).

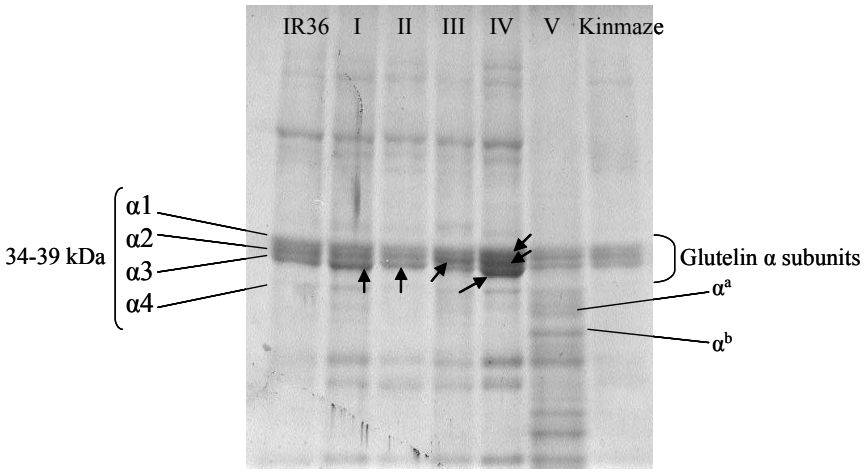

Fig. 1. Variation in glutelin α subunits in rice varieties assessed by the higher temperature SDS-PAGE. The numbers I-V represents the glutelin α subunits variation types observed in rice varieties. The arrow heads indicate the respective α subunit variation. α^a and α^b are tentatively named glutelin subunits.

Table 2. Glutelin variation types and their corresponding α subunit variation.

Glutelin variation types	Glutelin α subunit variation
Type-I	(α3HI)[a]
Type-II	(α3HM)[b]
Type-III	(α2HI)[c]
Type-IV	(α1HI)[d] , (α2HI) and (α3HI)
Type-V	(α^a and α^b)[e]

[a]Higher intensity of α3 subunit
[b]Higher molecular size of α3 subunit
[c]Higher intensity of α2 subunit
[d]Higher intensity of α1 subunit
[e]Tentatively designated glutelin subunits

Among varieties, 9 varieties exhibited Type-I variation, 6 varieties showed Type-II variation, 8 varieties exhibited Type-III variation, 6 varieties showed Type- IV variation, one variety has Type-V and 2 varieties showed standard IR36 variation (Table 3).

Discussion

Maximum separation is prerequisite for diversity assessment of rice glutelin subunits. Due to the close identity of glutelin subunits and unique solubility properties of glutelin, the distinct separation is very difficult by usual employed SDS-PAGE (Katsube-Tanaka *et al*., 2004). Higher temperature SDS-PAGE which was carried out at 45°C proved to be useful tool for maximum separation and diversity assessment of glutelin subunits in wild rice species (Khan *et al*., 2008a, 2008b). Higher temperature SDS-PAGE enabled us to categorize rice varieties into 5 variation types on the basis of variation in intensity and molecular size of glutelin α subunits. Glutelin α subunits are less conserved compared to β subunits so might be a noteworthy source for nutritional quality improvement of rice seed. Therefore, in this report we mainly focused on glutelin α subunits.

Table 3. Rice varieties and their corresponding glutelin variation types.

Sr. No.	Name of variety	Type of variety	Type -I	Type -II	Type -III	Type -IV	Type -V	IR36	Total
1.	Basmati-370	Aromatic	×	-	-	-	-	-	1
2.	Mushkan	Aromatic	-	×	-	-	-	-	1
3.	Basmati-C622	Aromatic	×	-	-	-	-	-	1
4.	Basmati-Pak	Aromatic	×	-	-	-	-	-	1
5.	Basmati-198	Aromatic	-	-	×	-	-	-	1
6.	PK-177	Aromatic	-	-	×	-	-	-	1
7.	Basmati-385	Aromatic	-	×	-	-	-	-	1
8.	Super-Basmati	Aromatic	×	-	-	-	-	-	1
9.	Kashmir-Basmati	Aromatic	-	-	×	-	-	-	1
10.	IR8	Aromatic	-	-	×	-	-	-	1
11.	Shaheen-Basmati	Aromatic	-	-	×	-	-	-	1
12.	Rachna-Basmati	Aromatic	×	-	-	-	-	-	1
13.	Sugdasi-Ratria	Aromatic	-	-	-	×	-	-	1
14.	Khushboo-95	Aromatic	-	-	-	×	-	-	1
15.	Dokri Basmati	Aromatic	×	-	-	-	-	-	1
16.	Sugdasi-Bengalo	Aromatic	-	-	-	×	-	-	1
17.	Sugdasi-Sadagulab	Aromatic	-	-	-	×	-	-	1
18.	Sonahri-Sugdasi	Aromatic	-	-	-	×	-	-	1
19.	Basmati 2000	Aromatic	×	-	-	-	-	-	1
20.	Pk-386	Non-aromatic	×	-	-	-	-	-	1
21.	Sathra	Non-aromatic	-	×	-	-	-	-	1
22.	KS-282	Non-aromatic	-	-	×	-	-	-	1
23.	NIAB-IR9	Non-aromatic	-	-	×	-	-	-	1
24.	IR6	Non-aromatic	-	-	×	-	-	-	1
25.	DR-82	Non-aromatic	-	×	-	-	-	-	1
26.	Pakhal	Non-aromatic	-	×	-	-	-	-	1
27.	Dilrosh-97	Non-aromatic	×	-	-	-	-	-	1
28.	Mahlar-346	Non-aromatic	-	×	-	-	-	-	1
29.	Sonahri-Kangni	Non-aromatic	-	-	-	-	-	×	1
30.	KSK-133	Non-aromatic	-	-	-	-	-	×	1
31.	Shahkar	Non-aromatic	-	-	-	×	-	-	1
32.	Jhona-349	Non-aromatic	-	-	-	-	×	-	1
	Total		**9**	**6**	**8**	**6**	**1**	**2**	**32**

× denotes the presence of variation type; - denotes the absence of variation type

Table 4. The frequency distribution of glutelin variation types in aromatic and non- aromatic rice varieties.

Type of variation	Aromatic varieties	Frequency (%)	Non-aromatic varieties	Frequency (%)
Type-I	7	36.84	2	18.18
Type-II	2	10.52	4	36.36
Type-III	5	26.31	3	27.27
Type-IV	5	26.31	1	9.09
Type-V	0	0	1	9.09
Total	**19**	**100**	**11**	**100**

In this study we used traditional and improved rice varieties of two different types i.e., aromatic (basmati) and non-aromatic (coarse type). Though both types share most of the glutelin variation types but when the frequency distribution of variation types of aromatic varieties were compared with non-aromatic varieties, the Type-I and Type-IV variations were mostly found in aromatic varieties, while Type-II variation was dominant in non-aromatic varieties (Table 4). Rice variety Jhona-349 exhibited Type-V variation type and displayed unique variation by showing the presence of unique bands which were not observed in other varieties. An extensive analysis of the complete rice genome sequence indicates that there are at least 11 glutelin genes (Katsube-Tanaka *et al*., 2004). However, only 6 glutelin genes GluA1, GluA2, GluA3, GluB1, GluB2 and GluB4 had been isolated so far (Katsube-Tanaka *et al*., 2004). The rice variety Jhona-349 might have some unique glutelin genes and need to be further explored for nutritional quality improvement of rice seed. Similarly, these results support the finding of Rabbani *et al*., (2008) who on the basis RADP analysis observed that rice variety Jhona-349 displayed unique banding pattern in comparison to other genotypes and clustered apart from other varieties in dendrogram tree. On the other hand, the rice varieties which exhibited Type-IV variation having higher intensity of α1, α2 and α3 subunits which reflect the comparatively higher glutelin content in these varieties. These varieties may be important genetic resources for improving glutelin content of rice seed.

Rice glutelin is encoded by a small multigene family, which can be classified into two subfamilies, that is, A-type (GluA) and B-type (GluB) glutelin, according to the degree of nucleotide sequence similarity (Takaiwa & Oono, 1991). B-type glutelin is more nutritious compared to A-type because the major subunits of B-type contain 20% more lysine in average compared to A-type (Takaiwa & Oono, 1991). The diversity of glutelin α subunits observed in this study reflects the quality differences that might exist among rice varieties. However, from the current study it rather seems difficult to differentiate the glutelin α subunits into A-type and more nutritious B-type glutelin. For such subunit sensitive study subunit-specific antibodies against A-type and B-type glutelin subunits are needed. Such subunit specific antibodies have been successfully used for identification and assessment of relative accumulation level of A-type and B-type glutelin subunits in our previous studies (Khan *et al*., 2008ab). After this preliminary evaluation of glutelin α subunits diversity in rice varieties, in our next study the A-type and B-type glutelin will be identified and its relative accumulation level will be assessed by our combine proteomic approach of two-dimensional gel electrophoresis and immuno detection using subunit-specific antibodies (Khan *et al*., 2008ab).

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