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Variation of High-Molecular-Weight Glutenin Subunits and Gliadin in *T. aestivum* ssp. *macha*

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Abstract: In order to exploit new genetic resources and provide fundamental materials for the breeding improvement of bread wheat quality, genetic variation of high-molecular-weight glutenin subunits and gliadin in 29 *macha* wheat accessions were observed by acidic polyacrylamide-gel electrophoresis and sodium dodecyl sulphate polyacrylamide-gel electrophoresis. Nine HMW-glutenin subunits alleles and 9 combinations were identified. Subunit null (82.8%), 7+8 (53.3%) and 2+12 (82.8%) scored the highest frequency at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. In addition, subunits 7+9 (23.3%) was also found at higher frequency. A total of 49 gliadin bands and 28 patterns were detected and the polymorph among all accessions was identified in most of bands (97.96%). Furthermore, all materials could be clustered into three major groups based on genetic similarity coefficient. The results indicated that the variations of gliadin among *macha* accessions were not associated with their geographic origins.

Key words: *T. aestivum* ssp. *macha*, gliadin, glutenin, genetic diversity

INTRODUCTION

In the endosperm of wheat (*Triticum aestivum* L.), the main storage protein classes are glutenin and gliadin. The wheat baking quality is controlled by the content and composition of wheat endosperm proteins. Specific HMW-glutenin subunits have been closely associated with bread making quality (Payne, 1987; He *et al*., 1999; Barro *et al*., 2003; Edwards *et al*., 2007; Lefebvre and Mahmoudi, 2007). Glutenin and gliadin are generally considered to contribute to the viscosity and extensibility of gluten. Glutenins were composed of high-molecular-weight (HMW) subunits and low-molecular-weight (LMW) subunits. HMW-glutenin subunits are controlled by three gene loci, located on the long arm of chromosomes 1A, 1B and 1D and identified as *Glu-A1*, *Glu-B1* and *Glu-D1* (Payne, 1987). Gliadins on the other hand are encoded by the complex gene family, which located on the short arms of group 1 and 6 chromosomes (Lafiandra *et al*., 1984; Hassani *et al*., 2007).

In order to improve wheat quality breeding, it is essential to exploit the valuable gene resource from wheat relative species. One of the oldest hexaploid cultivated species, *T. aestivum* ssp. *macha* (2n = 6x = 42, AABBDD), that was grown mainly in the west of Gelugia in the past. It possessed stronger tillering ability, resist smut and moisture tolerance. Because it has the same genome structure as the common wheat, the useful genes of *T. aestivum* ssp. *macha* could be easy transferred into common wheat. Datiashvili (1983) found that there was better ratio between gliadin and glutenin

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in *T. aestivum* ssp. *macha*. Fang *et al*. (1997) suggested that moisture tolerance of *macha* wheat was controlled by a pair of dominant gene and further scored a SSR marker Xgwm148 (Fang and Cai, 2003). Zhang *et al*. (2003) further demonstrated that these genes are located on 5A chromosome based on RAPD marker. Xiong *et al*. (2006) also reported that macha wheat had higher plant height, strong tillering ability and more spikelets. However, little information for *macha* was investigated. This confined the utility of *T. aestivum* ssp. *macha* in wheat improvement breeding. In the present study, were report on genetic diversity of HMW-glutenin subunits and gliadin as evaluated in 29 *T. aestivum* ssp. *macha* accessions from 11 countries.

MATERIALS AND METHODS

Plant Materials

A total of 29 accessions from different countries were kindly provided by Dr. Harold Bockelaman, USDA-ARS, National Small Grains Collection (Table 1). This study was conducted in 2007. Electrophoresis was performed at the Triticeae Research Institute, Sichuan Agriculture University.

A-PAGE

According the standard protocol of ISTA acidic polyacrylamide-gel electrophoresis (pH 3.1) (A-PAGE) (Draper, 1987), the gliadin electrophoresis was carried out. Common wheat cultivar Chinese Spring was used as reference.

SDS-PAGE

According to the procedures of Ng and Bushuk (1987), the HMW-glutenin subunits were separated by sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE). HMW-glutenin subunits were identified according to Payne and Lawrence (1983). Common wheat cultivar Chinese Spring (null/7+8/2+12) and Chuanyu12 (1/7+8/5+10) were used as reference.

Statistic Analysis

The presence or absence of gliadin bands was carefully recorded. The present bands were recorded as 1 and the bands absent as 0. Based on the gliadin bands data, the Genetic Similarity (GS) was calculated according to Nei's (1973) method as $GS = 2N_{ij}/(N_i + N_j)$, where N_i is the number of the band

Table 1: A list of accessions and their country of origin

No.	Accession	Country	No.	Accession	Country
1	PI272554	Hungary, Pest	16	PI428177	Sweden, Uppsala
2	PI272555	Hungary, Pest	17	PI428178	Italy, Latium
3	PI278660	United Kingdom	18	PI428179	Iran, Esfahan
4	PI290507	Hungary, Pest	19	PI542466	United States, Oregon
5	PI352466	Former Soviet Union	20	PI572905	Georgia
6	PI355508	Former Soviet Union	21	PI572906	Georgia
7	PI355509	Former Soviet Union	22	PI572907	Georgia
8	PI355510	Former Soviet Union	23	PI572908	Georgia
9	PI355511	Russia Federation, Lening	24	PI572909	Georgia
10	PI355512	Former Soviet Union	25	PI572910	Georgia
11	PI355513	Former Soviet Union	26	PI572911	Georgia
12	PI355514	Switzerland	27	PI572912	Georgia
13	PI361862	Denmark	28	PI572913	Georgia
14	PI428146	Sweden, Uppsala	29	PI611470	Georgia
15	PI428148	Russian Federation, Stavro			

in the material i; N_j is the number of the gliadin band in the material j; N_{ij} is the number of the gliadin band shared by material i and j. Using the unweighted pair-group method, a clustering analysis was carried out based on GS index under NTSYS-pc (version 2.1) software.

RESULTS

Gliadin Variations

A total of 49 gliadin polymorphic bands and 28 patterns were detected in 29 *T. aestivum* ssp. *macha* accessions. The number of bands ranged from 19 to 34 per accession, with the mean of 25. Ten bands were detected in α-, β- and γ-region, respectively and 19 bands in ω-region. Twenty-two, nineteen, twenty-two and twenty-six gliadin patterns were identified in α-, β-, γ- and ω-region, respectively (Fig. 1). The genetic similarity (GS) among *T. aestivum* ssp. *macha* ranged from 0.29 to 0.95, with a mean of 0.61. Based on the gliadin GS coefficient, the genetic relationships among *macha* wheat accessions were further investigated by UPGMA program. The result indicated that all accessions could be divided into three groups at the 0.58 level of GS (Fig. 2). Group 1 contained 19 accessions, including all accessions from Hungary, Iran, Italy, United Kingdom and most of accessions from the Former Soviet Union and Georgia. It could be further divided into two subgroups at the 0.63 level of GS. Group 3 only contained one accessions (PI355513) from Former Soviet Union. Group 2 contained 9 accessions, including 5 accessions from Switzerland, United States, Denmark, Sweden and Russian Federation, respectively and 4 accessions from Georgia. Group 2 also could be further divided into two subgroups. One accession (PI572909) from Georgia formed subgroup 2, whereas other three accessions from Georgia were clustered in another subgroup. Therefore, the clustering of *macha* wheat is not associated with their geographic origin. These results also indicate that the *macha* wheat accessions from Former Soviet Union and Georgia had higher genetic variation than material from other sources.

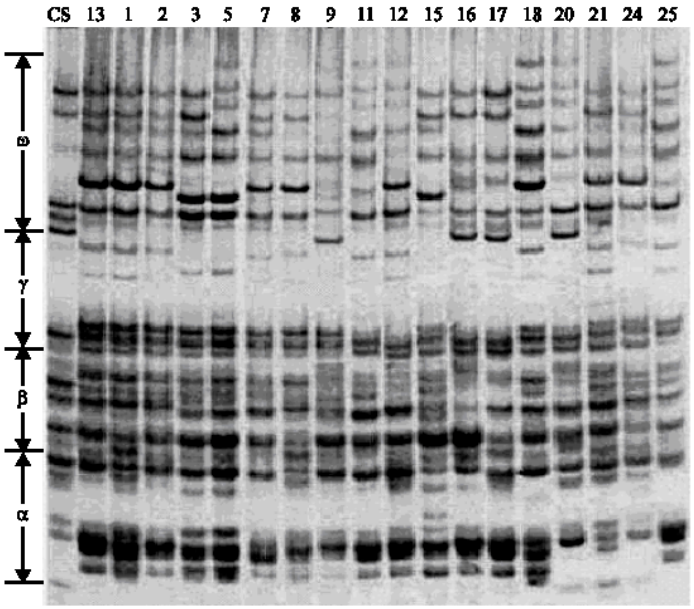

Fig. 1: Gliadin patterns for 17 accessions of *macha* wheat in APAGE profile Note:The name of material was listed in Table 1, CS: Chinese spring, α, β, γ and ω refer to the corresponding gliadin region

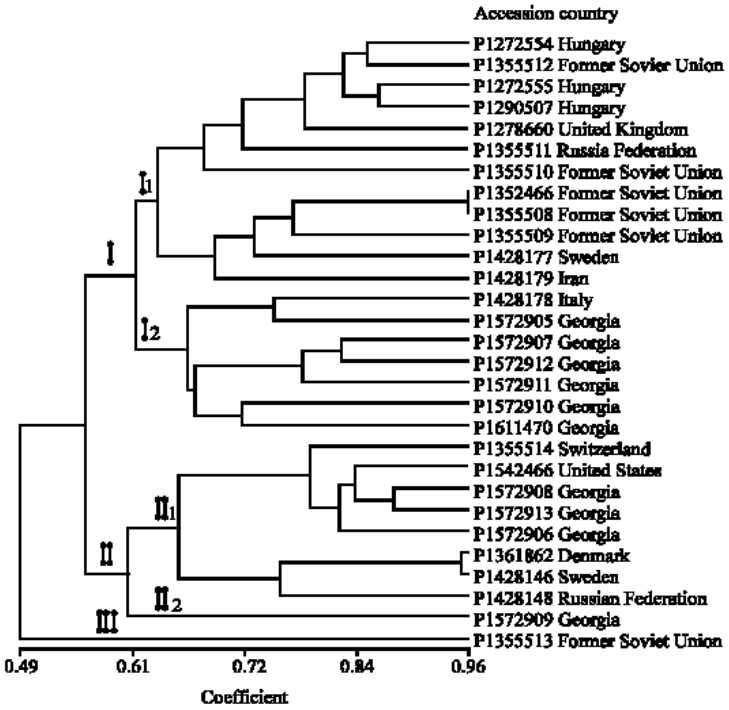

Fig. 2: A dendrogram generated from gliadin

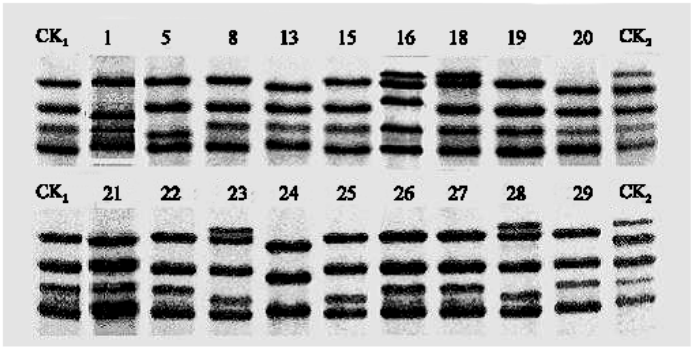

Fig. 3: HMW-GS of *macha* wheat accessions after SDS-PAGE Note: The name of material was listed in Table 1, CK_1: Chinese spring, CK_2: Chuanyu12

HMW-GS Glutenin Variations

A total of 9 HMW-GS subunits were identified in all accessions, including 2, 5 and 2 subunits at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (Table 2) (Fig. 3). At the *Glu-A1* locus, subunit null had the highest frequency (82.8%), whereas subunit 1 had low frequency (17.2%). At *Glu-B1* locus, subunits 7+8 was the predominant frequency 55.2%, followed by subunit 7+9 (24.1%) and the subunits 8, 20 and 6+8 with low frequency were also detected. At *Glu-D1* locus, only subunits 2+12 and 5+10 were identified and the former subunit had the highest frequency (82.8%). Nine HMW-GS patterns were identified among all accessions. The distribution of HMW-glutenin patterns are listed

Table 2: The distribution frequency of HMW-GS alleles in *macha* wheat accessions

		Accessions	
Locus	Bands	No. of accessions	Frequency (%)
Glu-A1	1	5	17.2
	null	24	82.8
Glu-B1	7+8	16	55.2
	7+9	7	24.1
	6+8	1	3.4
	8	3	10.3
	20	5	17.2
Glu-D1	5+10	5	17.2
	2+12	24	82.8

Table 3: The combination and distribution frequency of HMW-GS in *macha* wheat accessions

Subunit combination	*Glu–A1*	*Glu–B1*	*Glu–D1*	No. of accessions
1	null	8, 20	2+12	1, 3, 4
2	null	7+8	2+12	2, 8, 9, 10, 15, 19, 22, 26, 27, 29
3	null	7+9	2+12	5, 6, 7, 25
4	null	20	2+12	11, 12
5	null	7+8	5+10	13, 14, 20, 21
6	1	6+8	2+12	16
7	1	7+8	2+12	17, 18
8	1	7+9	2+12	23, 28
9	null	20	5+10	24

in Table 3. The pattern null/7+8/2+12 was detected in 10 accessions and had the highest frequency. Four patterns, including null/7+9/2+12, null/7+8/5+10, 1/7+8/2+12 and 1/7+9/2+12, had higher frequency. The HMW-GS pattern 1/6+8/2+12 and null/20/2+12 were only observed at low frequency.

DISCUSSION

The gliadin patterns with a great diversity of variations have been used as fingerprints, genotype identification, quality markers and so on (Yan *et al*., 1992; Liu *et al*., 1999; Lang *et al*., 2001). Zhang *et al*. (1995) suggested that A-PAGE could provide an available method for identifying wheat germplasm. In this study, the higher genetic diversity of gliadin was found, a total of 28 gliadin patterns were identified in 29 *T. aestivum* ssp. *macha*. This indicates that gliadin could provide a valuable and exact diversity marker for *T. aestivum* ssp. *macha* accessions. These data is supported by the results of Khachidze (1989) who also observed a great diversity of variations in *T. aestivum* ssp. *macha* and found some bands in the patterns that is species specific. Yakobashvili and Naskidashvili (1988) reported that the greatest polymorphism (11 variants of gliadin blocks) was found for the gliadin locus on chromosome 6A. One of its variants was identical to block *Gld* 6A20 of bread wheat. Six variants of blocks associated with chromosome 1B, 3 were identical to blocks in bread wheat (*Gld-1B4*, *Gld-1B8* and *Gld-1B10*). Metakovsky and Iakobashvili (1990) further reported an additional component mapped between *Glu-B1* and *Gli1B* in the gliadin spectrum of *T. aestivum* ssp. *macha*. The analyses of seed storage protein components of the gliadin and glutenin in *T. aestivum* ssp. *compactum*, *T. aestivum* ssp. *sphaerococcum*, *T. aestivum* ssp. *macha*, *T. aestivum* ssp. *spelta* had revealed limited variation at the tightly linked coding loci *Gli-D1*/*Glu-D3* and *Glu-D1*, located respectively on the short and long arm of chromosome 1D and at the *Gli-D2* locus, positioned on the short arm of chromosome 6D (Lafiandra *et al*., 1992).

In this study, a great diversity of variations on gliadin loci in *T. aestivum* ssp. *macha* accessions, while the relationship between genetic variety and other properties traits need further study. Meanwhile, two accessions with the same gliadin bands had no diversity in all properties, but had

further close relative relations, such as PI352466 and PI355508 used in this study. So the genetic varieties between accessions need other methods, like by molecular marker technology to detect and analyses.

In the tree of the cluster analysis, accessions from Georgia were clustered together, but only few accessions were grouped with other counties accessions. From the clustering dendrogram, the Georgia accessions could be divided into two groups. Accessions PI572908, PI572913, PI572906 and PI572909 formed a group, while others were another group. This indicates that there were two allelic variety types on gliadin locus in Georgia accessions. In this study, the clustering analysis indicated that the gliadin variations among *T. aestivum* ssp. *macha* are not associated with their geographic origin. High level of HMW-GS polymorphism was observed in the 29 *T. aestivum* ssp. *macha* by SDS-PAGE method. The main HMW-glutenin patterns was null (*Glu-A1*), In all ten Georgia accessions, the pattern (null, 7+8 and 2+12) had the highest frequency, the pattern (null, 7+9, 2+12) had the similar performance in Former Soviet Union accessions. The consistent efforts have been made to improve wheat quality by accumulating good HMW-glutenin subunits, such as 1, 5+10. Yakobashvili's *et al.* (1988) indicated that the species included *T. aestivum* ssp. *macha*, *T. aestivum* ssp. *spelta* and *T. aestivum* ssp. *aestivum* were closely related through comparison with HMW glutenins of 3 species by SDS-PAGE. The greatest polymorphism in *T. aestivum* ssp. *macha* and *T. aestivum* ssp. *spelta* was for *Glu-1B*, for which the greatest difference between them was also found suggesting that they may had different donors of genome B.

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