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Allelic Identification and Genetic Diversity at *Gli-A1* and *Gli-A2* Loci in Einkorn Wheat

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Abstract: The allelic compositions at *Gli-A1* and *Gli-A2* loci were investigated in 113 accessions of einkorn wheat by using Acid Polyacrylamide Gel Electrophoresis (APAGE). A total of 42 alleles were identified and the mean genetic variation index was 0.9418 at *Gli-A1* and *Gli-A2* loci in 113 accessions of einkorn wheat. A total of 37, 33 and 38 alleles were identified and the mean genetic variation indexes were 0.9378, 0.9314 and 0.9251 at *Gli-A1* and *Gli-A2* loci in the accessions of *T. monococcum* ssp. *boeoticum*, *T. monococcum* ssp. *monococcum* and *T. urartu*, respectively. In some accessions, the gliadin genes can't be identified at *Gli-A1* or *Gli-A2* loci. It indicated that there might exist some new gliadin genes in these accessions. The results indicated that higher gliadin diversity was present in einkorn wheat. In addition, these accessions analyzed could also be used as genetic resource to improve the quality and to broaden the genetic base of common wheat.

Key words: Einkorn wheat, Gliadin, *Gli-A1*, *Gli-A2*, genetic diversity

Introduction

In the endosperm of wheat, gliadins and glutenins are the main seed storage proteins. Due to its related to the end-use quality of bread and durum wheat, more attentions have been paid on the characterization of the seed storage proteins (Waines and Payne, 1987; Metakovsky and Baboev, 1992a, b). Gliadins, which are alcohol-soluble seed storage proteins, show the highest level of intervarietal polymorphism when they were separated by a standard method of acid polyacrylamide gel electrophoresis (APAGE) (Zillam and Bushuk, 1979). Most gliadins are controlled by the *Gli-1* and the *Gli-2* loci, on the short arms of the homoeologous group 1 and 6 chromosomes in wheat, respectively (Payne, 1987). Gliadins are inherited as blocks or linked groups (Mecham *et al.*, 1978) and vast multiple allelism has been established at each of these loci (Sozinov and Poperely, 1980). Combinations of gliadin alleles at the main loci ensure a great diversity of APAGE patterns and, therefore, make it possible to distinguish a number of wheat genotypes and to interpret the genetic diversity in wheat (Metakovsky, 1991a; Metakovsky and Branlard, 1998).

Einkorn wheat, including *T. monococcum* L. and *T. urartu*, was diploid wheat (AA = 14) though their assignment to species taxa is still controversial (Castagna *et al.*, 1994). They have been regarded as a promising genetic resource to improve cultivated wheat (D'Egidio *et al.*, 1993; Borghi *et al.*, 1996; Rodriguez-Quijano *et al.*, 1997). The allelic compositions at *Gli-A1* and *Gli-A2* loci in *T. monococcum*

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ssp. *monococcum* (Metakovsky and Baboev, 1992a; Saponaro *et al.*, 1995) and *T. monococcum* ssp. *boeoticum* (Metakovsky and Baboev, 1992b) had investigated. However, to our knowledge, little information is available for the gliadin allelic composition in *T. urartu*. The objective of the present study was to detect the gliadin allelic compositions at *Gli-A1* and *Gli-A2* loci in 113 accessions of einkorn wheat and to determine the genetic diversity of the gliadin alleles among the species and subspecies of einkorn wheat.

Materials and Methods

A total of 113 accessions of einkorn wheat, including 30 *T. monococcum* ssp. *boeoticum*, 41 *T. monococcum* ssp. *monococcum* and 42 *T. urartu* accessions, were kindly provided by Dr. Harold Bockelmen of American Germplasm Resources Information Network (GRIN). They were collected from various countries such as Turkey, Armenia, Iraq, Asia Minor, Iran and so on (Table 1). For brevity, *T. monococcum* ssp. *boeoticum*, *T. monococcum* ssp. *monococcum* and *T. urartu* were referred as “boeoticum”, “monococcum” and “urartu” in the article, respectively.

Gliadin proteinins were extracted with a solution of 70% ethanol and 0.01% methyl green and fractionated by a standard APAGE at PH3.1 according to the procedure of Cooke (1987). Two common wheat varieties, Chinese Spring and Bezostaya, were used as references. The *Gli-A1* and *Gli-A2* alleles were identified according method described by Metakovsky (1991b). The genetic diversity at each loci was calculated according to Nei (Kudryavtsec *et al.*, 1988) as:

$$H = 1 - \sum P_i^2,$$

where, H is Nei's genetic variation index and P_i is the frequency of a particular allele at that locus.

The experimentations were conducted in the Triticeae Research Institute of Sichuan Agricultural University and the study was finished in Aug. 2005.

Results

Gliadin allelic composition

As a whole, a total of 18 and 24 alleles (from 14 to 22 per locus) were identified at *Gli-A1* and *Gli-A2* loci in 113 accessions of einkorn wheat, respectively (Fig. 1. and Table 2). In 30 accessions of ‘boeoticum’, 18 and 20 alleles were identified at *Gli-A1* and *Gli-A2* loci, respectively. Fourteen and 19 alleles were identified at *Gli-A1* and *Gli-A2* loci in 41 accessions of ‘monococcum’, respectively. In 42 accessions of ‘urartu’, 15 and 22 alleles were identified at *Gli-A1* and *Gli-A2* loci, respectively. Therefore, considering *Gli-A1* and *Gli-A2* loci, a total of 42 gliadin alleles were identified in 113 accessions of einkorn wheat; total 38, 33 and 37 gliadin alleles were observed in ‘boeoticum’, ‘monococcum’ and ‘urartu’, respectively. However, several gliadin genes can't be identified in some accessions of einkorn wheat because no identical block bands with the reported genes' (Table 1).

In 113 accessions of einkorn wheat, the alleles in higher frequency were *Gli-A1b* (9.73%), *Gli-A1e* (9.73%), *Gli-A1d* (7.96%), *Gli-A1l* (7.96%), *Gli-A2l* (7.96%), *Gli-A1q* (7.08%), *Gli-A2a* (7.08%) and *Gli-A2r* (7.08%). In 30 accessions of ‘boeoticum’, the alleles in higher frequency were *Gli-A1b* (10%), *Gli-A1l* (10%), *Gli-A2n* (10%) and *Gli-A2r* (10%). In 41 accessions of ‘monococcum’, the alleles in higher frequency were *Gli-A2h* (12.20%), *Gli-A2m* (12.20%),

Table 1: List of accessions used in the study. B = *T. monococcum* ssp. *boeoticum*; M = *T. monococcum* ssp. *monococcum*; U = *T. urartu*

| Species | Country | Accession | Species | Country | Accession | Species | Country | Accession |
|---------|--------------|------------|----------|-----------|-----------|---------|----------|-----------|
| B | Asia Minor | PI352266 | M | Macedonia | PI345242 | U | Armenia | PI428258 |
| | | PI352273 | | | Iraq | | PI428253 | |
| | PI352277 | Romania | PI192063 | Iran | PI428316 | | | |
| | PI352501 | | Russian | PI428317 | | | | |
| | Azerbaijan | PI352502 | Spain | PI94743 | Lebanon | | PI538738 | |
| | | Citr1 7672 | | PI191095 | PI538740 | | | |
| | | PI418580 | | PI191096 | PI428266 | | | |
| | | PI427463 | | PI191097 | PI428268 | | | |
| | Balkans | PI352272 | | PI191098 | PI428269 | | | |
| | England | PI427461 | | PI94740 | PI428276 | | | |
| | Iraq | PI428007 | | PI191094 | PI428262 | | | |
| | | PI538510 | Sweden | PI428149 | PI428260 | | | |
| | | PI538556 | Georgia | PI591871 | PI428261 | | | |
| | | PI538557 | Greece | PI225164 | PI428281 | | | |
| | | PI538606 | Iraq | PI427927 | PI428288 | | | |
| | | PI538613 | | PI427959 | PI428289 | | | |
| | | PI428005 | Turkey | PI428154 | PI428328 | | | |
| | | PI428006 | | PI428173 | PI538736 | | | |
| | | | | PI428174 | Syria | | PI487270 | |
| | | Iran | PI401416 | | PI428175 | | PI487272 | |
| | | | PI401415 | | PI428176 | | PI487266 | |
| | | | PI538578 | | PI119435 | | PI487267 | |
| | | | PI227669 | | PI170196 | | PI487268 | |
| | | Lebanon | PI503309 | | PI428160 | | PI487269 | |
| | | | PI428001 | | PI428161 | | Turkey | PI428186 |
| | | | PI428002 | | PI428165 | | PI538730 | |
| | Soviet Union | PI554514 | | PI428166 | PI428232 | | | |
| | | PI554515 | | PI428167 | PI428220 | | | |
| | Unkown | PI542475 | | PI428168 | PI428221 | | | |
| | | Citr1 7741 | | PI428170 | PI428209 | | | |
| | | | | PI428171 | PI428233 | | | |
| | | | | PI428172 | PI428208 | | | |
| | M | Armenia | PI349049 | | PI596286 | | PI428188 | |
| | | Bulgaria | PI428152 | | PI428180 | | PI428197 | |
| | Bosniaand | PI265008 | U | Armenia | PI428181 | | PI428200 | |
| | England | PI428156 | | | PI428182 | | PI428207 | |
| | Ethiopia | PI191381 | | PI428183 | PI428186 | | | |
| | | PI191383 | | PI428257 | | | | |
| | | Morocco | PI307984 | | | | | |

Gli-A1a (9.76%), *Gli-A1b* (9.76%), *Gli-A1d* (9.76%), *Gli-A1e* (9.76%), *Gli-A1l* (9.76%) and *Gli-A2a* (9.76%). In 42 accessions of ‘urartu’, the alleles in higher frequency were *Gli-A1e* (11.90%), *Gli-A2l* (11.90%), *Gli-A1d* (9.52%) and *Gli-A2s* (9.52%).

Genetic diversity

In 113 accessions of einkorn wheat, no identical gliadin patterns were found in the gliadin spectra. The genetic variation indexes at *Gli-A1* were higher than that of *Gli-A2*. The mean Nei’s genetic variation indexes (*H*) were much high either within or among the species and/or subspecies, with the mean genetic variation indexes 0.9418 at the *Gli-A1* and *Gli-A2* in 113 accessions of einkorn wheat. In the accessions of ‘boeoticum’, ‘urartu’ and ‘monococcum’, the mean genetic variation indexes were 0.9378, 0.9314 and 0.9251 at the *Gli-A1* and *Gli-A2*, respectively (Table 3). The results indicated that there was higher genetic diversity in einkorn wheat, among which the highest genetic diversity existed in the accessions of ‘boeoticum’, whereas the lowest genetic diversity existed in the accessions of ‘monococcum’.

Table 2: *Gli-A1* and *Gli-A2* allelic compositions in 113 accessions of einkorn wheat. M = *T. monococcum ssp. monococcum*; B = *T. monococcum ssp. boeoticum*; U = *T. urartu*

| Species | Accession | Gli-A1 | Gli-A2 | Species | Accession | Gli-A1 | Gli-A2 | Species | Accession | Gli-A1 | Gli-A2B | |
|----------|------------|----------|--------|----------|-----------|----------|----------|----------|-----------|----------|---------|----|
| B | PI352266 | k | n | M | PI345242 | f | I | U | PI428258 | n | o | |
| | PI352273 | o | t | | PI362610 | d | w | | PI428253 | j | k | |
| | PI352277 | c | ? | | PI192063 | b | p | | PI428316 | j | s | |
| | PI352501 | h | o | | PI428150 | b | a | | PI428317 | b | s | |
| | PI352502 | d | m | | PI94743 | q | r | | PI538738 | p | w | |
| | Citr1 7672 | f | r | | PI191095 | e | a | | PI538740 | l | q | |
| | PI418580 | r | n | | PI191096 | q | o | | PI428266 | q | o | |
| | PI427463 | m | r | | PI191097 | a | m | | PI428268 | b | l | |
| | PI352272 | g | f | | PI191098 | n | l | | PI428269 | r | i | |
| | PI427461 | p | c | | PI94740 | l | r? | | PI428276 | n | u | |
| | PI428007 | i | e | | PI191094 | f? | n | | PI428262 | p | s | |
| | PI538510 | ? | k | | PI428149 | o | m | | PI428260 | f | r | |
| | PI538556 | g | u | | PI591871 | e | h | | PI428261 | q | u | |
| | PI538557 | l | a | | PI225164 | d | v | | PI428281 | f | x | |
| | PI538606 | f | ? | | PI427927 | b | h | | PI428288 | b | f | |
| | PI538613 | l | n | | PI427959 | i | n | | PI428289 | h | p | |
| | PI428005 | j | w | | PI428154 | m | a | | PI428328 | d | m | |
| | PI428006 | m | p | | PI428173 | h | x | | PI538736 | e | r | |
| | PI427928 | q | q | | PI428174 | i | m | | PI487270 | d? | t | |
| | PI401416 | i | l | | PI428175 | n | b | | PI487272 | r | h | |
| | PI401415 | a | j | | PI428176 | c | l | | PI487266 | n | l | |
| | PI538578 | h | t | | PI119435 | d | o | | PI487267 | a | s | |
| | PI227669 | e | I | | PI170196 | r | g | | PI487268 | o | ? | |
| | PI503309 | b | I | | PI428160 | e | s? | | PI487269 | d | d | |
| | PI428001 | b | c | | PI428161 | a | v | | PI428186 | l | f | |
| | PI428002 | k | o | | PI428165 | j | g | | PI538730 | a | f | |
| | PI554514 | b | l | | PI428166 | b | r | | PI428232 | j | b | |
| | PI554515 | n | a | | PI428167 | d | h | | PI428220 | o | x | |
| | PI542475 | e? | s | | PI428168 | l | f? | | PI428221 | n | p | |
| | Citr1 7741 | r | d | | PI428170 | q | d | | PI428209 | e | l | |
| | M | PI349049 | a | | c | PI428171 | e | | d | PI428233 | f | a |
| | | PI428152 | g | | m | PI428172 | j | | h | PI428207 | d | n |
| | | PI265008 | a | | p | PI596286 | l | | m | PI428208 | m | g? |
| PI428156 | | h | o | PI428180 | b | l | PI428188 | c | j | | | |
| PI428157 | | l? | s | PI428181 | e | a | PI428197 | o | b | | | |
| PI191381 | | r | a | U | PI428182 | r | I | PI428200 | e? | l | | |
| PI191383 | | h | w | | PI428183 | e | ? | PI428222 | l | f | | |
| | PI307984 | c | h? | PI428257 | h | t | | | | | | |

Note: '?' indicated the most possible or unidentified allele

Table 3: Genetic variation indexes (H) and the number of allele at *Gli-A1* and *Gli-A2* in einkorn wheat. M = *T. monococcum ssp. monococcum*; B = *T. monococcum ssp. boeoticum*; U = *T. urartu*, E = 113 accessions of einkorn wheat in the study

| Species | Genetic variation indexes (H) | | | The number of allele | | |
|---------|-------------------------------|--------|--------|----------------------|--------|-------|
| | Gli-A1 | Gli-A2 | Mean | Gli-A1 | Gli-A2 | Total |
| M | 0.929 | 0.9292 | 0.9251 | 14 | 19 | 33 |
| B | 0.9356 | 0.94 | 0.9378 | 18 | 20 | 38 |
| U | 0.9229 | 0.9399 | 0.9314 | 15 | 22 | 37 |
| E | 0.9338 | 0.9498 | 0.9418 | 18 | 24 | 42 |

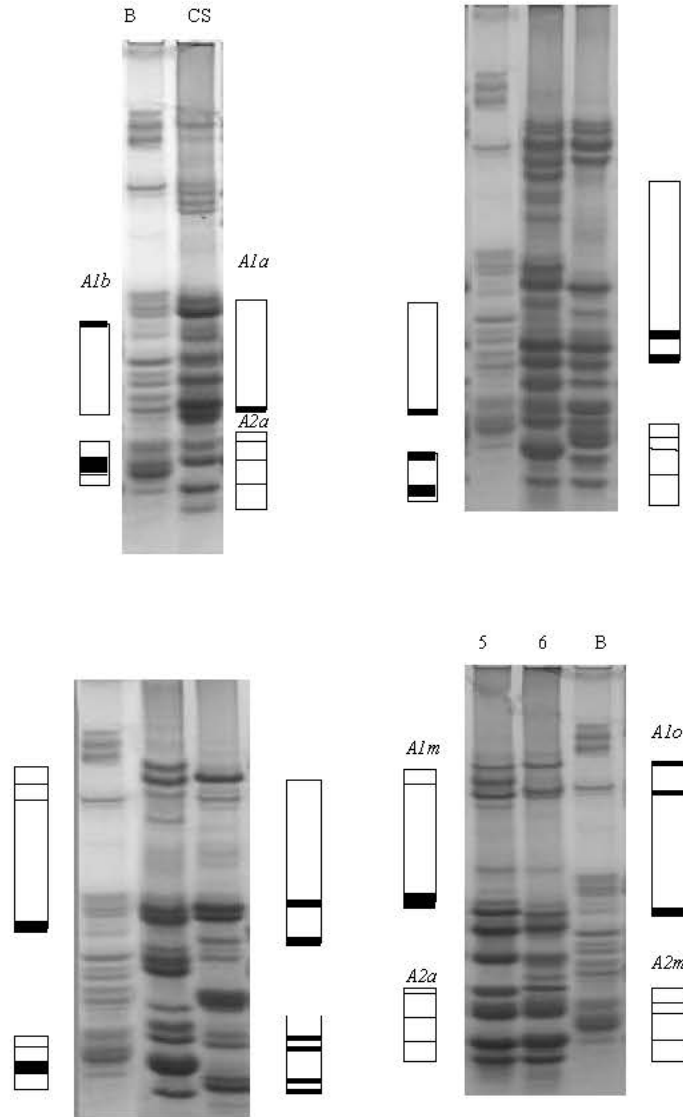


Fig. 1: The Gliadin allelic identification at Gli-A1 and Gli-A2 loci of Bezostaya, Chinese Spring and partial einkorn wheat. B = Bezostaya ; CS = Chinese Spring ; 1= PI487267 ; 2 = PI428328; 3 = PI227669 ;4 = CItr17672; 5 = PI428154; 6 = PI428149

Discussion

Einkorn wheat was the A-genome donor of all polyploid wheat (Castagna *et al.*, 1994). A total of 42 gliadin alleles, including 18 alleles at *GliA1* loci and 24 alleles at *GliA2*, had been identified in the A genome of common wheat (Metakovsky, 1991b). In this study, all of 42 gliadin alleles at *Gli-A1* and *Gli-A2* loci were detected in 113 accessions of einkorn wheat. However, neither *T. monococcum* (including 'monococcom' and 'boeoticum') nor *T. urartu* had all the known alleles at *Gli-A1* and *Gli-A2* loci of the A genome. Furthermore, there still existed several gliadin genes couldn't be identified in some accessions of einkorn wheat because no identical block bands with the reported genes'. It indicated that there might exist some new gliadin genes in these accessions (Metakovsky *et al.*, 1991a) and this need to be further study.

It has been found that the A genome of polyploid wheat was complex: obviously more than one diploid wheat was involved in its formation (Kudryavtsec *et al.*, 1988; Metakovsky *et al.*, 1989; Metakovsky and Baboev, 1992b). Therefore, a comparative intraspecific study of polymorphism is needed for a better understanding of the phylogenetic relationships between wheat species (Metakovsky and Branlard, 1998). In the present study, the results of gliadin allelic compositions in 'monococcom' and 'boeoticum' were similar with those described by Metakovsky and Baboev (1992a, b) and Saponaro *et al.* (1995). It was suggested that *T. urartu* was the A-genome donor of common wheat on the basis of the analysis of DNA markers (Dovrak *et al.*, 1988). Therefore, the information of the gliadin allelic composition in *T. urartu* is important for the breeder to utilize this species. To our knowledge, it is the first time that the gliadin allelic composition was investigated in *T. urartu*.

It had been confirmed that the variation of gliadin allele was significant relevantly to wheat quality and some protein bands or blocks of gliadin relevant to good quality, such as *Gli-A2b*, had been identified (Wrigley *et al.*, 1982; Saponaro *et al.*, 1995; Metakovsky *et al.*, 1997). The high-quality gliadin alleles at *Gli-A1* and *Gli-A2*, such as *Gli-A2b*, were found in einkorn wheat, which might be very useful to improve the quality of common wheat varieties.

The genetic diversity in einkorn wheat had been estimated. Very low amounts of genetic diversity within populations of 'boeoticum' or 'urartu' had been described by isoenzymes (Smith-Huerta *et al.*, 1989; Moghaddam *et al.*, 2000). However, high polymorphism was found by RFLP analysis (Castagna *et al.*, 1994; Corre and Bernard, 1995). In this study, the number of different gliadin patterns was nearly equal to the number of accessions studied and high genetic diversity was observed in einkorn wheat. It was in agreement with that of Metakovsky and Baboev (1992a, b), who found a large number of gliadin patterns when studying several accessions of 'monococcom' and 'boeoticum'. Genetic diversity in breeding material might be monitored by means of analysis polymorphic markers (Metakovsky and Branlard, 1998). Therefore, the gliadin patterns of einkorn wheat could be used as helpful markers in selecting breeding parents from einkorn wheat germplasm collection.

Much less gliadin alleles were found at *Gli-A1* loci and *Gli-A2* loci (from 2 to 4 per locus) of common wheat in China (Wei *et al.*, 2000). The narrow genetic base of common wheat in a country could be caused both by breeders' activities and natural selection (Metakovsky and Branlard, 1998). Obviously, only the breeders' activities could be controlled and reduced by breeding with the relative species of wheat. In 113 accessions of einkorn wheat analyzed, 14 to 22 alleles were found at *Gli-A1* and *Gli-A2* loci within each species or subspecies of einkorn wheat, which the mean Nei's genetic variation indexes (*H*) were much high either within or among the species and subspecies. It indicated

that the higher genetic variations existed in the species and subspecies of einkorn wheat. Therefore, einkorn wheat could be used as good genetic resource to broaden the genetic base of common wheat.

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