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Genetic Variations of Gliadin and HMW Glutenin Subunits in Spelt Wheat

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Abstract

Gliadin and HMW glutenin subunit variations in 162 spelt wheat (*Triticum spelta* L.) accessions were detected by A-PAGE and SDS-PAGE. Higher gliadin variation was observed, and 121 gliadin patterns were detected. A total of 14 HMW-GS alleles were found. There were 2, 5 and 2 alleles at *Glu-A1, Glu-B1* and *Glu-D1* loci, respectively, resulting in 8 HMW-GS combinations. HMW-GS combination (1, 6+8, 2+12) was the dominant phenotype, which was found in 83.00% of the spelt wheat accessions. These results suggested that the polymorphism of spelt wheat on *Gli-1* loci was higher than that on *Glu-1* loci.

Key-words: gliadin, HMW glutenin subunit, spelt wheat.

1. Introduction

Spelt wheat (*Triticum spelta* L.) is well recognized as one of the well-known wheat, which possesses the same genome (AABBDD) as bread wheat. The morphological character of spelt is well defined, typically characterized by a narrow, lax and pyramidal spike with a brittle rachis and adherent glumes, generally long spike internodes and non-spherical seeds (Winzeler et al., 1994). Spelt as a crop appears to quality for cultivation in less tolerant environmental condition like the high altitude regions as well as for low-input farming. Moreover, the ever-increasing demand for natural or organically grown foods creates a niche market for spelt and stimulates research into their utilization in traditional and new nutritious (Cubadda et al., 1996; Abdel-Aal et al., 1998), attractive foods such as breakfast cereals and extrude cooked products (Auricchio et al., 1982; Strehlow et al., 1991). In addition, spelt has other excellent characters like disease resistance, flooding tolerance (Burgos et al., 2001). The spelt wheat also is an important source of genetic diversity for endosperm proteins that are associated with bread-making quality in wheat. In a word, the interest in this crop is growing gradually.

Biochemical and genetic aspects of wheat storage proteins had received great attention due to their importance in determining the nutritional and technological properties of cultivated wheats. Gliadin and glutenin are the main storage proteins in wheat endosperm and are encoded by genes at *Gli-1* and *Glu-1* loci, respectively (Payne et al., 1982; Payne et al., 1983). Variations at each *Glu-1* locus have been extensively characterized in the past two decades, mainly due to their relationships with bread-making quality (Payne et al., 1987). On the other hand, storage protein polymorphisms are also useful genetic markers for crop origin (Shewry et al., 1992) and evolutionary studies (Fernandez-Calvin and Orellana, 1990). A-PAGE and SDS-PAGE have been used frequently to fractionate wheat gliadins and glutenin for identification of genotypes, establishment of chromosomal control, selection of desirable wheat lines in breeding programs, and/or the correlation of individual proteins with end-use quality.

The objective of this study was to detect the gliadin and glutenin variations in 162 spelt wheat accessions derived from various countries.

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2. Materials and methods

2.1 Plant materials

The gliadins and HMW-glutenin variations in 162 spelt wheat accessions, derived from various countries, were analyzed in this study (Table 1).

2.2 Electrophoresis

Gliadin proteins were extracted from single seeds with a solution of 70% (V/V) ethanol and 0.01% (W/V) methyl green, and fractionated by a standard acid polyacrlamide gel electrophoresis (A-PAGE) at pH 3.1 according to Wei et al. (2000).

HMW-glutenins were extracted from individual grains using the sequential procedure of Ng and Bushuk (1987). Electrophoresis of HMW-glutenins was performed on vertical gel according to the SDS-PAGE protocol described by Ng and Bushuk (1987). Common wheat variety Chinese Spring was used as a reference, and HMW-glutenin subunits were identified according to Payne and Lawrence (1987) and Ciaffi et al. (1991).

3. Results and discussion

3.1 Gliadin variations

One hundred and twenty-one gliadin patterns were detected in 162 spelt wheat accessions (Fig. 1). It was obvious that there were differ-

Table 1. Origin of 162 spelta wheat accessions.

Country	Accesions
United States	CItr1772, CItr1774, CItr2986, CItr3264, CItr13959, CItr13967, CItr15071, CItr17764, PI168682, PI294576, PI355656, PI355595
Spain	PI190960, PI190962, PI190963, PI191100
Ethiopia	PI191392
Portugal	PI191617
Poland	PI192717, PI286060
Yugoslavia	PI221404, PI221419, PI221420
Iran	PI225271, PI225295
United Kingdom	PI266848, PI289607, PI330558, PI330559
Hungary	PI272529, PI272573, PI272574, PI272576, PI27257, PI272578, PI272579, PI290513, PI290514, PI290515, PI290516
Bulgaria	PI295056, PI295059, PI295060, PI295061, PI295062, PI295063, PI295064, PI295066, PI295067, PI295068, PI295069
Germany	PI286048, PI355611, PI355612, PI355613, PI355614, PI355615, PI355618, PI355622, PI355623, PI355624, PI355626, PI355633, PI355636, PI355639, PI355640, PI355641, PI355648, PI355649, PI355657, PI355658, PI355660
Ethiopia	PI297861
Romania	PI306550, PI306551, PI306553, PI306554, PI306555, PI306556
Austria	PI323438
Belgium	PI338366, PI338367, PI228368, PI355619, PI355620, PI355621, PI355625, PI355634, PI355635, PI355650, PI355659
Argentina	PI346853
Switzerland	PI347850, PI355591, PI355592, PI355593, PI355594, PI355596, PI355597, PI355598, PI355599, PI355600, PI355601, PI355602, PI355603, PI355604, PI355605, PI355606, PI355607, PI355608, PI355609, PI355610, PI355616, PI355617, PI355627, PI355628, PI355629, PI355630, PI355631, PI355632, PI355637, PI355638, PI355643, PI355644, PI355645, PI355646, PI355647, PI355652, PI355653, PI355654, PI355655, PI347851, PI347852, PI347853, PI347854, PI347855, PI347856, PI347857, PI347858, PI347859, PI347860, PI347861, PI347862, NGB4495
Austria	PI355651
Denmark	NGB4798, NGB5148, NGB5149, NGB9004, NGB9005, NGB9027, NGB9028, NGB9047, NGB9055, NGB9056, NGB9680, NGB9700, NGB10883
Unknown	CItr14138, As327, As328

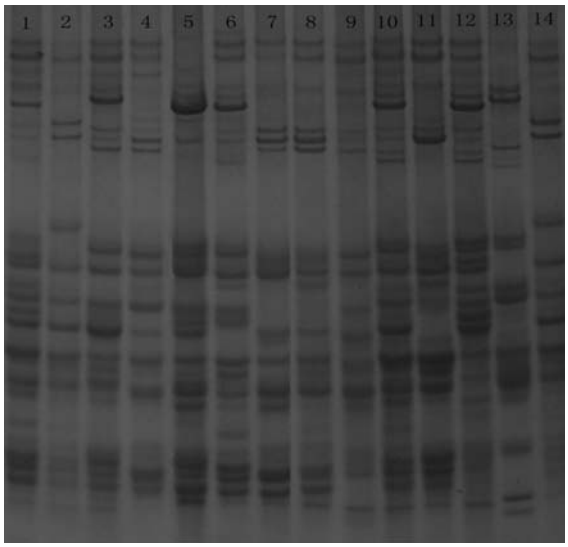

Figure 1. Gliadin patterns of the spelt wheat accessions CItr1774 (1), CItr2968 (2), CItr3264 (3), CItr14138 (4), CItr17764 (5), PI168682 (6), PI225271 (7), PI266848 (8), PI272529 (9), PI272573 (10), PI272574 (11), PI272576 (12), PI27257 (13) and PI27258 (14).

ences in number of ω/γ-gliadin bands and their relative motilities among these spelt wheats. Seventeen gliadin bands were found in the ω-gliadin zone, resulting in 48 ω-gliadin patterns. The number of ω-gliadin bands varied from 4 to 8 in each accession. Diagrammatic of ω-gliadin patterns and its frequency were shown in figure 2. The patterns 1 and 2 were the most frequent patterns, and observed in 20 and 15 accessions, respectively. A few patterns also had relatively higher frequency, such as 3, 4, 5 and 6, whereas 20 ω-gliadin patterns were unique. A total of 10 gliadin bands were detected in the γ-gliadin zone, ranging from to 1 to 5 bands per accession (Fig. 3). There were 24 γ-gliadin patterns, among which 10 patterns were unique. However, the relatively lower variations were detected in α- and β-gliadin zones. There were only 4 and 8 patterns in α- and β-gliadin zones, respectively (data not shown). Most of these spelt wheat accessions shared the identical α- and β-gliadin bands. It was found that ω- and γ-gliadins had higher diversity than that of α- and β-gliadins (Abdel-Aal et al., 1996). In spelt wheat, it was reported that the gliadins encoding by 1A, 1B and 1D (i.e. ω- and γ-gliadins) had higher genetic variability than that of encoding by 6A, 6B and 6D (i.e. α- and β-gliadins) (Baker and Bushuk, 1978; Caballero et al., 2004b). In this study, the results were in agreement with that of Baker and Bushuk (1978) and Caballero et al. (2004b).

3.2 HMW-glutenin variations

A total of 14 HMW-GS alleles were found among the 162 spelt wheat accessions (Fig. 4). There were 2, 5 and 2 alleles at G*lu-A1, Glu-B1* and *Glu-D1 l*oci, respectively, resulting in 8 HMW-GS combinations (Table 2). It was obvious that HMW-GS combination (1, 6+8, 2+12) was the dominant phenotype, which was found in 83.00% of the spelt wheat accessions. Ni (2002) reported that *Glu-B1d* (6+8) was the preponderant subunits of spelt wheat (57.1%), whereas Caballero et al. (2001, 2004) and Rodriguez-Quijano et al. (1990) found that *Glu-B1f* (13+16) was the dominant subunits (87.8%). In this study, we observed that *Glu-B1d* (6+8) was the preponderant subunits.

In this study, the *Glu-A1c* allele was rare (frequency 2.5%) and found in only 4 accessions (i.e. PI295056, PI355640, NGB9004 and NGB9027), whereas higher frequency of *Glu-*

Table 2. HMW-glutenin subunits combinations in 162 spelt accessions.

Glu-A1	*Glu-B1*	*Glu-D1*	Accessions	Frequency (%)
1	6+8	5+10	PI355608	0.62
1	20	2+12	PI355594	0.62
1	6*+8*	2+12	PI355612	0.62
1	6.1+22.1	2+12	PI347850	0.62
null	6+8	2+12	PI355640; NGB9004	1.24
null	7+8	2+12	NGB9027; PI295056	1.24
1	7+8	2+12	NGB9700; PI355615; As328; NGB5148; As327; PI295060; PI272576; PI190960; PI355621; PI355622; PI221420; PI355605; NGB9680; PI355597; PI191717; PI355623; PI272579; NGB9028; NGB10883; NGB9047	12.04
1	6+8	2+12	The remaining accessions	82.72

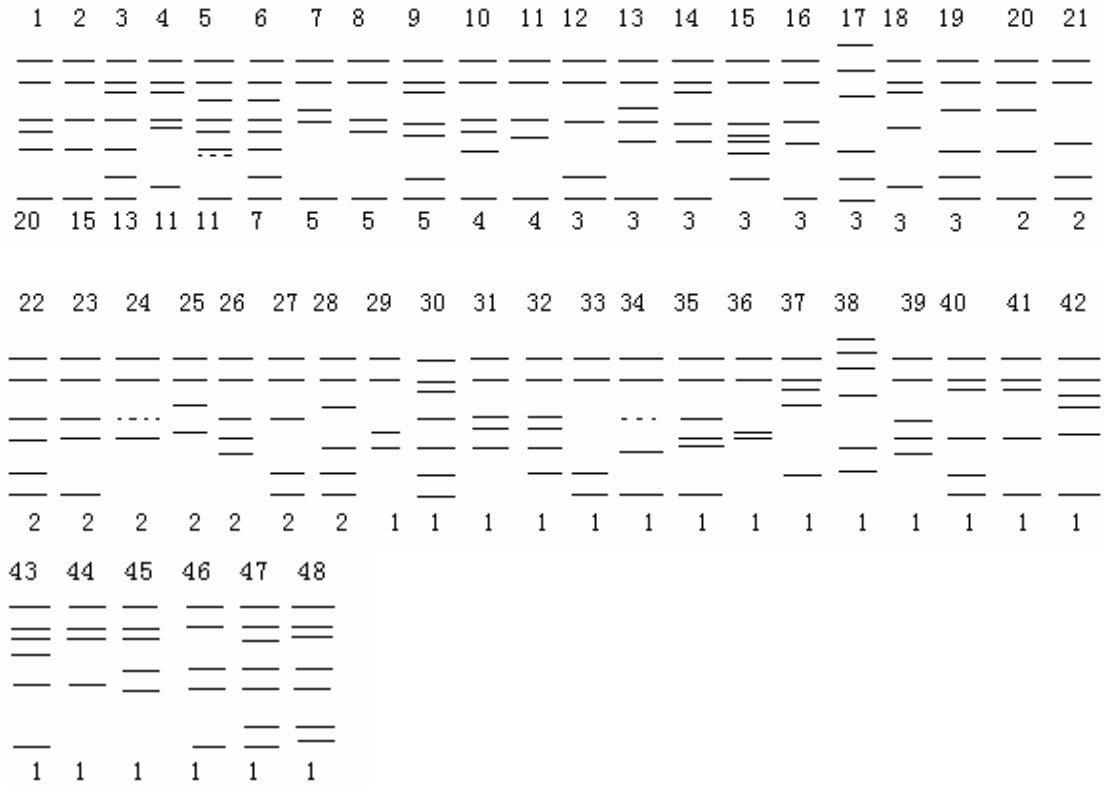

Figure 2. Diagrammatic representation of the patterns in the ω-gliadins zone. The bands with light trace are shown with discontinuous line. The patterns are numbered from 1 to 48, in decreasing order to the presence among all accessions, and the number under the patterns indicated the number of accessions that the patterns present.

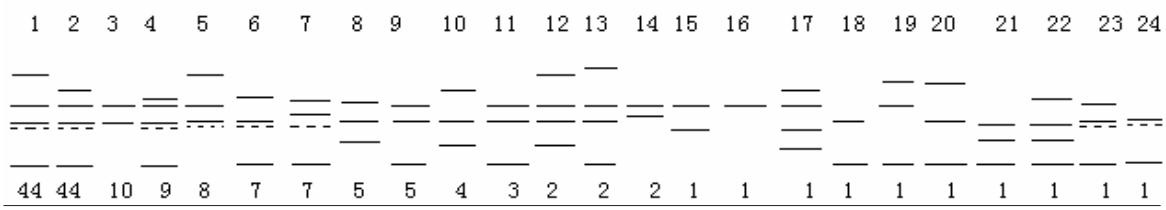

Figure 3. Diagrammatic representation of 24 patterns of γ-gliadins zone. The patterns are numbered similar to figure 2.

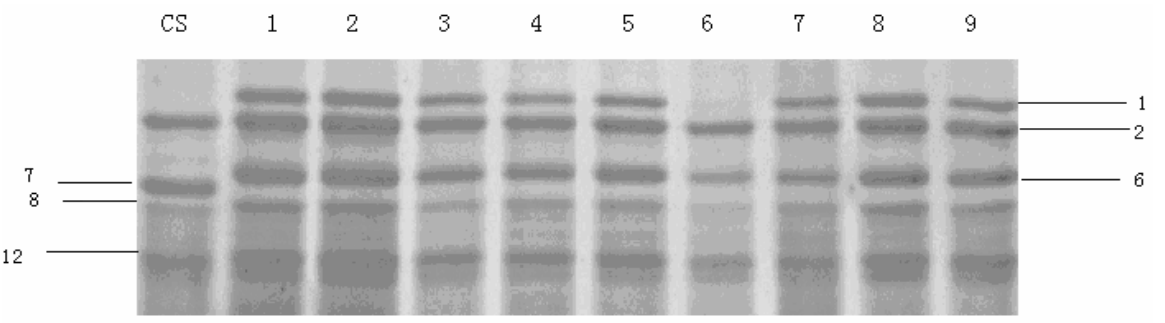

Figure 4. HMW-glutenin subunits of spelt wheat accessions PI355635 (lane 2), PI355636 (lane 3), PI355637 (lane 4), PI355638 (lane 5), PI355639 (lane 6), PI355640 (lane 7), PI355641 (lane 8), PI355642 (lane 9) and PI355643 (lane 10). Chinese Spring (lane 1) (subunits: null, 7+8, 2+12) was used as a reference.

A1c allele was found by Rodriguez-Quijano et al (1990). The subunit 2* coded by the *Glu-A1b* allele, which appeared with higher frequency in the findings of Rodriguez-Quijano et al. (1990) and Caballero et al (2001, 2004), were not found in this study. In addition, we found that the allele of *Glu-A1* in PI190963 was subunit 1 other than "null" as reported by Caballero et al. (2001).

The allele *GluB1b* (subunits 7+8) associated with good bread-making quality (Payne and Lawrence1983), were rare in these analyzed spelt wheat accessions. The subunit 20 *(Glu-B1e)* was only found in one accession (PI355594), being classified as rare with a local distribution, and all these alleles had been observed in drum and bread wheat.

In 162 spelt wheat accessions, a higher homogeneity for the *Glu-D1* locus was observed, and 99.4% of spelt wheat accessions had the *Glu-D1a* allele (subunits 2+12). The subunits 5+10 (*Glu-D1*d), associated with good quality, was only detected in one a*ccession PI355614.*

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