International collaboration for unifying *Glu-3* nomenclature systems in common wheat

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ABSTRACT

Low-molecular-weight glutenin subunit (LMW-GS) composition in common wheat is one of the critical determinants of gluten properties. However, the nomenclature of *Glu-3* encoding LMW-GSs has not been consistent among laboratories, due to the complexity of the LMW-GSs and the distinct separation methods used by different researchers. It is very important to unify the nomenclature systems in current use, to facilitate the sharing of information about the effects of individual LMW-GS on gluten properties. We therefore shared 103 cultivars having various *Glu-A3*, *Glu-B3* and *Glu-D3* alleles from Argentina, China, France, Japan and Mexico. Using 1D SDS-PAGE and 2D analyses, we found differences in nomenclature particularly for *Glu-A3* and *Glu-B3*, including new *Glu-3* alleles among laboratories. We propose a new list of standard cultivars representing *Glu-3* alleles.

INTRODUCTION

It has been shown that allelic variation for the high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs) affects the properties of dough made with different wheat cultivars. LMW-GS composition in common wheat is one of the critical determinants of gluten properties[1, 2, 3, 4, 5]. Gupta and Shepherd assigned the individual LMW-GSs to *Glu-A3*, *Glu-B3* and *Glu-D3* loci and selected standard cultivars that covered the allelic variation observed[6]. However, subsequent use of *Glu-3* nomenclature has not been consistent among laboratories, due to the complexity of LMW-GSs, different separation methods and different standard cultivars used by researchers[7, 8, 9]. It is very important to unify the various *Glu-3* allelic nomenclature systems in use, to allow information to be shared regarding the effects of individual alleles on gluten properties and to be applied in breeding programs aimed at improving gluten properties. Although European groups once proposed a LMW-GS nomenclature system[10], it has not been used internationally, partly due to the limited availability of the cultivars used in their analysis. In the current study, four laboratories plus an international institution shared cultivars and compared results.

MATERIALS AND METHODS

A total of 103 cultivars were shared among the participating groups; 19, 20, 23, 10 and 31cultivars from Argentina, France, Japan, CIMMYT (China) and CIMMYT (Mexico), respectively. The methods of glutenin extraction and running conditions of 1D SDS-PAGE were based on Singh *et al.* [9]. The modifications by individual groups are shown in Table 1. Two-dimensional (2D) gel electrophoresis was carried out by Ikeda's group according to Ikeda *et al*[8]. The *Glu-3* nomenclature system used is that described in the "Catalogue of gene symbols for wheat" (http://wheat.pw.usda.gov/ggpages/awn/53/Textfile/WGC.html).

RESULTS AND DISCUSSION

Using SDS-PAGE and 2D analyses, we shared the same complete identification of *Glu-3* alleles for only 21 cultivars among laboratories, implying we found differences in the identification of the alleles among the rest of cultivars. An example of a SDS-PAGE profile is shown in Fig. 1. The amount of discrepancy is similar for *Glu-A3* and *Glu-B3*, and less for *Glu-D3*. These differences may be partly related to the number of alleles present at each locus and partly by band resolution (overlapping of bands) due to the differing methods applied. We also found new *Glu-3* alleles present in a number of the cultivars analyzed. Considering each locus in turn, the results of allele identification are summarized and discussed below:

Glu-A3 alleles

1) *Glu-A3b* and *Glu-A3d* alleles were frequently identified.

2) Branlard's group did not differentiate *Glu-A3a* from *Glu-A3c*.

3) *Glu-A3f* was hard to identify by SDS-PAGE. By 2D analysis, the spot corresponding to *Glu-A3f* was clearly

identified, although it overlapped with other LMW-GSs (Fig. 2). This means that, as well as the number of bands, band intensity in SDS-PAGE analysis should also be considered,

4) *Glu-A3g* was hardly differentiated from *Glu-A3b* and *Glu-A3d* by SDS-PAGE, partly due to similar molecular weights of the corresponding LMW-GSs.

5) In the cultivars that initially appeared to carry *Glu-A3c*, the band intensity was low in some cultivars. We provisionally named this as a separate allele, *Glu-A3s*.

Glu-B3 alleles

1) *Glu-B3a*, *Glu-B3c*, *Glu-B3d*, *Glu-B3g* and *Glu-B3i* were frequently identified.

2) Branlard's group differentiated *Glu-B3m* from *Glu-B3b*, and *Glu-B3n* from *Glu-B3c*, as reported in Branlard *et al*[7]. Since these alleles are differentiated by small molecular weight differences, other groups did not confirm these alleles.

3) No groups identified *Glu-B3e*. It might not be included in our samples.

4) *Glu-B3f* was identified by Branlard's and Peña's groups. However, other labs tended to consider this allele as *Glu-B3b*, because the differences between these two alleles are very subtle.

5) Ikeda's group found a new spot by 2D analysis in some cultivars apparently carrying *Glu-B3b*, *Glu-B3g* or *Glu-B3i*. We provisionally named as *Glu-B3ab, Glu-B3ac* and *Glu-B3ad*, respectively.

6) Branlard's group differentiated five new alleles from *Glu-B3h*. We provisionally named them as *Glu-B3ae*, *Glu-B3af*, *Glu-B3ag*, *Glu-B3ah* and *Glu-B3ai* respectively.

7) Branlard's group differentiated a new allele from *Glu-B3i*. We provisionally named it as *Glu-B3aj*.

8) Comparing SDS-PAGE and 2D analysis, it seems that *Glu-B3ai* and *Glu-B3aj* are the same allele as *Glu-B3ad*.

Glu-D3 alleles

1) *Glu-D3a*, *Glu-D3b*, *Glu-D3c* and *Glu-D3d* were frequently identified.

2) Ikeda's group differentiated a new allele from *Glu-D3c*. This allele was characterized in 2D analysis by the absence of a spot corresponding to group 8/9[8]. We provisionally named it as *Glu-D3j*.

3) Peña's group and Ikeda's group identified a new *Glu-D3* allele in Ernest. We provisionally named it as *Glu-D3k*.

4) Branlard's group identified a new allele in Fengmai 27 and Ikeda's group confirmed it. We provisionally named it as *Glu-D3l*.

5) He's group identified a new allele in Jing 411, Yumai 63, Zhongyou 9507, Chopin, Clément, Pavon and Klein Jabalí. We provisionally named it as *Glu-D3m*.

6) Although Orca is listed to have *Glu-D3e* in the "Catalogue of gene symbols for wheat", it was classified as *Glu-D3c* by all groups.

Inconsistency in the allele identification among groups appears to be partly due to the differences in the methods applied and partly due to interpretational differences: which bands are considered or neglected. Fig. 1 shows the bands used to identify *Glu-3* alleles. Although identification of LMW-GSs by 2D analysis is better than that achieved by SDS-PAGE, SDS-PAGE analysis is better at identifying small molecular weight differences between the LMW-GSs than 2D analysis. Combining data by these methods should help to identify these alleles in detail. *Glu-3* allele specific DNA markers should also help to identify known alleles[11], but further analysis is necessary to confirm the new alleles by proteomic analysis and/or other molecular techniques.

CONCLUSIONS

In this collaboration, using the same materials, we showed that, although we shared the same identification for some alleles, there were many discrepancies among researchers, including candidates for new alleles. These discrepancies should be resolved by further analysis with other methods. We summarized our results and listed cultivars representing individual alleles in Table 2. We expect this list to provide a useful basis for reaching consensus over *Glu-3* allelic designation and to provide an opportunity to renew the *Glu-3* section of the wheat gene catalogue.

ACKNOWLEDGEMENTS

NOTE

The provisional list of *Glu-3* alleles in this study is available from TMI (tmikeda@affrc.go.jp).

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Table 1. Methods applied to identify *Glu-3* alleles among participating groups.

Research group	Condition		
	Gel concentration of separation gel	pH of separation gel	Current of running gel
Branlard	12.5% T , 0.97% C or 13.0% T, 1.7% C	8.8	30mA
He	14.0% T, 1.3% C	8.8	16mA
Ikeda	15.0% T, 1.4% C	8.8	30mA
Pena	15.0% T, 1.3% C	8.5	12.5mA
Rogers	13.5% T, 0.8% C	8.8	40mA

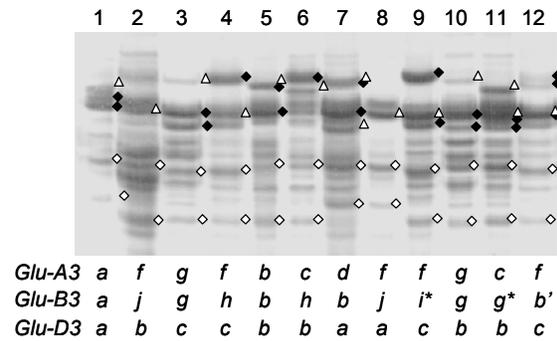| Glu-A3	a	f	g	f	b	c	d	f	f	g	c	f
Glu-B3	a	j	g	h	b	h	b	j	i*	g	g*	b'
Glu-D3	a	b	c	c	b	b	a	a	c	b	b	c

Fig. 1. SDS-PAGE profile of glutenin fractions run by Rogers' group. 1. Chinese Spring, 2. Klein Martillo, 3. Klein Jabalí, 4. ACA 303, 5. ProINTA Isla Verde, 6. Klein Chaja, 7. ProINTA Colibrí, 8. ProINTA Amanecer, 9. Buck Pingo, 10. Klein Proteo, 11. ACA 801, 12. ACA 601. White triangles, black squares and white squares indicate bands corresponding to *Glu-A3*, *Glu-B3* and *Glu-D3*, respectively.

Table 2. Recommended standard cultivar set for *Glu-3*

Locus	Allele	Cultivar
Glu-A3	a	Chinese Spring
	b	Gabo, Pavon
	c	Thesee, Seri, Cheyenne*
	d	Cappelle-Desprez, Orca
	e	Marquis, Neepawa
	f	Clément, Insignia, Heilo
	g	Glenlea, Klein Proteo
	s	Spear, Buck Pingo
Glu-B3	a	Chinese Spring
	b	Gabo, Marquis
	c	Insignia, Halberd
	d	Pepital, Orca
	e	Cheyenne*
	f	Magali Blondeau
	g	Brimstone, Cappelle-Desprez
	h	Petrel, Pavon
	i	Demai3, Norin61
	j	Clément, Seri
	m	Soissons
	n	Courtot
	ab	Nanbukomugi, Klein Proteo
	ac	Thesee, ACA 801
	ad	Ruso, Opata, Heilo
	ae	CA9722, Huaimai16,
	af	Spear, Neepawa
	ag	Shinchunaga,
	ah	Jing411
	ai (*ad?*)	Heilo, Buck Pingo
	aj (*ad?*)	Shiranekomugi, Carnamah
	ak	Ernest
Glu-D3	a	Chinese Spring
	b	Gabo, Wilgoyne, Seri
	c	Cappelle-Desprez, Insignia
	d	Brimstone, Buck Brasil
	f	Cheyenne*
	j	Pepital, Thesee, Heilo
	k	Ernest
	l	Fengmai 27
	m	Jing 411, Clément

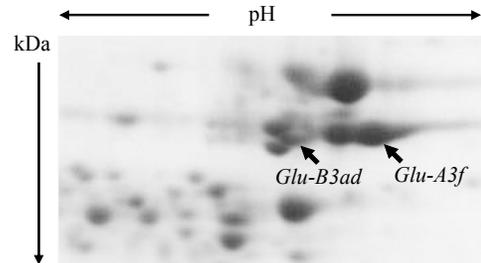*:Cheyenne is not included in our samples.

Fig. 2. 2D profile of glutenin fractions of Heilo run by Ikeda's group. Arrows indicate spots corresponding to *Glu-A3f* and *Glu-B3ad*, respectively.