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Update on low-molecular-weight glutenin subunit identification

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Abstract

Allelic variation for the low-molecular-weight glutenin subunits (LMW-GS) is a major determinant of differences in dough viscoelastic properties observed between cultivars of both bread wheat and durum. Technical difficulties in allelic identification due to the complexity of the protein profile produced by each cultivar and the use of different nomenclature systems in different laboratories has historically interfered with information exchange between research groups, a situation exacerbated by the vast number of possible profiles found in different cultivars due to the multi-allelic nature of the principal loci encoding LMW-GSs (*Glu-A3*, *Glu-B3* and *Glu-D3*). These various difficulties prompted research workers at CAAS, CIMMYT, INRA, NARO and UNCPBA to form an international collaborative group aimed at unifying criteria across laboratories and comparing four different methods of allelic identification (SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR). The current contribution summarizes progress to date made by this group in studies on bread wheat, seeks to address remaining challenges and places the findings in the context of the wheat gene catalog. We also propose the formation of a wider international group aimed at facilitating the resolution of the remaining problems in the field.

Introduction

Allelic identification of the low molecular weight glutenin subunits (LMW-GS), major determinants of genetic differences in quality in wheat, has historically been highly problematic, far more so than that of the other major group of glutenins influencing quality, the high molecular weight glutenin subunits (HMW-GS). Although both groups are encoded by series of complex homoeologous loci (*Glu-1* in the case of the HMW-GS and principally *Glu-3* in the case of the LMW-GS), the protein profile associated with each cultivar is considerably more complex for the LMW-GS than for the HMW-GS: multiple overlapping bands are observed in protein electrophoresis in which different alleles frequently produce bands of very similar mobility. Added to these technical difficulties is the fact that different nomenclature systems have been used in different laboratories and that the *Glu-3* loci are multi-allelic, the latter of which means there is an enormous number of protein profiles observed in germplasm collections. Indeed, these proteins were used to assist discrimination between cultivars in such collections; for example, Lerner et al. (2009) provisionally found 93 allelic combinations in a collection of 119 Argentinean cultivars for these and other protein groups, a figure that, due to the above technical difficulties, may well be an underestimation.

According to work published by our group (Liu et al. 2010), the following ambiguities have resulted from the different classification systems hitherto available: 1) at the *Glu-A3* locus, both *Glu-A3a* and *Glu-A3c* were reported for the same cultivar and *Glu-A3a*, *Glu-A3b*, *Glu-A3c* and *Glu-A3d* were reported to be identical to *Glu-A3e*; 2) at the *Glu-B3* locus, results differed for *Glu-B3b* and *Glu-B3g*, and for *Glu-B3f* and *Glu-B3g* in the same cultivars; and 3) at the *Glu-D3* locus, there was uncertainty for *Glu-D3a* and *Glu-D3c*, and for *Glu-D3a* and *Glu-D3b*, in some cultivars. As a consequence of these problems, reports of correlations between certain allelic forms of LMW-GS and quality parameters in common wheat have often been contradictory (see Results and discussion), which points towards the need for a simple and uniform classification system and for a set of standard cultivars for each LMW-GS allele. These various difficulties prompted research workers at CAAS, CIMMYT, INRA, NARO and UNCPBA to form an international collaborative group aimed at unifying criteria across laboratories and defining such a standard cultivar set, and the current contribution summarizes progress made by this group to date, seeks to address remaining challenges and places the findings in the context of the wheat gene catalog (McIntosh et al. 2011).

Materials and methods

Our studies to date have been based upon a worldwide collection of bread wheat cultivars widely used in glutenin subunit composition studies and relationships with processing quality, which were analyzed in different laboratories for LMW-GS allelic composition with each laboratory applying its own particular range of techniques. The collection consisted of 103 cultivars from 12 countries (21 from China, 19 from Argentina, 15 from Australia, 14 from France, 10 from Japan, 8 from Mexico, 7 from Canada, 3 from the USA, 2 from Italy, 2 from the Netherlands, 1 from Finland, and 1 from Germany).

Protein extraction was similar in all five laboratories and involved sequential extraction from wholemeal flour based upon, for gliadins, propanol-1-ol, and, for glutenins, a propanol-1-ol, Tris-HCl solution containing 1% w/v dithiothreitol (DTT) and subsequently 4-vinylpyridine. Details are found in Liu et al. (2010).

SDS-PAGE (one-dimensional sodium dodecyl sulphate - polyacrylamide gel electrophoresis) was performed in all five laboratories, with differences across them in three methodological aspects: (i) the concentrations of separation gel were 14.0% concentration (T) with 1.3% cross linker (C), 15.0% T with 1.3% C, 12.5% T with 0.97% C, 15.0% T with 1.4% C, and 13.5% T with 0.8% C in the laboratories of CAAS, CIMMYT, INRA, NARO and UNCPBA, respectively; (ii) the pH for separation gel was pH 8.8 in all laboratories except CIMMYT with pH 8.5; and (iii) running gel currents were 16, 12.5, 30, 30 and 40 mA in CAAS, CIMMYT, INRA, NARO and UNCPBA, respectively. LMW-GS compositions were identified according to Singh et al. (1991) and Jackson et al. (1996) and the gliadins were used as indicators of LMW-GS based on the linkage between LMW-GS and gliadin because the gliadin composition can be screened more readily than specific LMW-GS in some cases. The nomenclature system of LMW-GS followed Gupta and Shepherd (1990), Jackson et al. (1996), Branlard et al. (2003), Ikeda et al. (2008), Appelbee et al. (2009) and the catalog of gene symbols for wheat (McIntosh et al. 2011).

2-DE (two-dimensional gel electrophoresis by (IEF) isoelectric focusing x SDS-PAGE) was only performed at CAAS and NARO, with the protocols and differences described in Liu et al. (2010). At least three gel images of each sample were taken and compared. The LMW-GS compositions were identified by distinctive spot patterns according to Ikeda et al. (2006). The nomenclature system of the LMW-GS was the same as the above SDS-PAGE separation. In some cases the 2-DE was run without glutenin protein alkylation.

MALDI-TOF-MS (matrix assisted laser desorption/ ionization time-of-flight mass spectrometry) was performed at the State Agriculture Biotechnology Center, Murdoch University, Australia, using the protocol described by Liu et al. (2010); sample preparation was carried out according to the dried droplet method, using sinapinic acid as matrix and analyses performed on a Voyager DEPRO TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a 337 nm nitrogen laser and delayed extraction. Identification of LMW-GS alleles was established using a set of 19 near-isogenic lines of cultivar Aroona (Wang and Appels, unpublished data).

PCR (polymerase chain reaction) was performed only at CAAS; genomic DNA was extracted from seeds using a modified CTAB procedure. Details of allele-specific markers for the discrimination of Glu-A3 and Glu-B3 alleles and PCR conditions were reported previously by Wang et al. (2009, 2010).

Results and discussion

Results obtained from the different laboratories were compared in order to understand the basis of existing differences in interpretation. Minor differences in the conditions used during SDS-PAGE resulted in separations that were sufficiently different to result in contrasting conclusions regarding the allelic composition of the cultivars; hence the other three procedures (2-DE, MALDI-TOF-MS and PCR) were applied in order to refine the analyses. For example, in the collection of cultivars analyzed, all alleles observed at the *Glu-A3* locus could be distinguished by 2-DE or PCR, whereas there were specific cases where particular alleles could not be distinguished by 1-DE or MALDI-TOF-MS. At the *Glu-B3* locus, some alleles were clearly identified by all four methods, whereas only 2-DE was effective for others. At the *Glu-D3* locus, allelic identification was at times problematic for 1-DE, 2-DE and PCR; one of the marker bands detected by SDS-PAGE was not a LMW-GS, but a gliadin that contaminated the glutenin fraction; some of the alleles at this locus were detected only by SNPs in PCR; and it was found that MALDI-TOF-MS has considerable potential for allelic identification at this locus.

The following specific results were obtained in the set of cultivars studied:

SDS-PAGE

At *Glu-A3*, (a) alleles *Glu-A3a*, *Glu-A3b*, *Glu-A3c* and *Glu-A3f* could be readily distinguished, but it was difficult to distinguish *Glu-A3e* (null) and *Glu-A3f* since both tended to be identified as null; and (b) alleles *Glu-A3d* and *Glu-A3g* could only be distinguished by the gliadin encoded by *Gli-A1o* linked to *Glu-A3d*.

At *Glu-B3*, (a) alleles *Glu-B3d*, *Glu-B3h* and *Glu-B3i* each carried slow bands that were not always easy to distinguish; (b) allele *Glu-B3b* almost coincided with *Glu-B3a*, although the *Glu-B3b* band was usually lighter and thinner; (c) allele *Glu-B3f* could not be readily distinguished from *Glu-B3g*, although a gliadin band that differed between the two could be used to discriminate them; and (d) alleles classified as *Glu-B3b*, *Glu-B3g* and *Glu-B3i* were often identified as *Glu-B3ab*, *Glu-B3ac* and *Glu-B3ad* by 2-DE, Bands of *Glu-D3* can be faintly stained and not always easily distinguished, although technical improvements have often allowed discrimination of, for example, *Glu-D3a*, *Glu-D3b* and *Glu-D3d*.

As previously mentioned, ambiguities such as these imply that recourse to 2-DE, MALDI-TOF-MS or PCR is often required.

2-DE

At *Glu-A3*, alleles *Glu-A3d* and *Glu-A3g*, which could only be reliably distinguished by using linked gliadins in one dimensional electrophoresis (see above), could be distinguished from each other by 2-DE.

At *Glu-B3*, alleles *Glu-B3a* and *Glu-B3b*, which were difficult to distinguish in SDS-PAGE, could be distinguished in 2-DE. New alleles *Glu-B3ab*, *Glu-B3ac* and *Glu-B3ad*, which have an additional spot, were identified by Ikeda et al. (2009).

At *Glu-D3*, alleles *Glu-D3c* and *Glu-D3l* could be distinguished.

Nonetheless, there were *Glu-3* alleles that were not readily distinguished by this method. Some of them were gliadin alleles, which were contaminated in glutenin fractions.

MALDI-TOF-MS

At all three *Glu-3* loci, cases were identified of alleles difficult to distinguish in SDS-PAGE, but were readily distinguished by MALDI-TOF-MS; for example, at *Glu-A3*, alleles *Glu-A3e* and *Glu-A3f*; at *Glu-B3*, alleles *Glu-B3f* and *Glu-B3g*; and at *Glu-D3*, alleles *Glu-D3b* and *Glu-D3c*.

PCR

PCR made valuable contributions to allelic identification. For example, in this collection of cultivars, all alleles thought to be present at the *Glu-A3* locus could be distinguished (*Glu-A3a* to *Glu-A3g*); and of the *Glu-B3* alleles, only three (*Glu-B3ab*, *Glu-B3ac* and *Glu-B3ad*) do not yet have allele specific primers.

Liu et al. (2010) provide a table summarizing the allelic variants that can be distinguished by the four different methods. Furthermore, they give the following standard set of 30 cultivars that include all allelic variants identified in this study: at locus *Glu-A3*, allele *a*: Neixiang 188, Chinese Spring; *b*: Gabo, Pavon 76; *c*: Pitic, Seri 82; *d*: Nidera Baguette 10, Cappelle-Desprez; *e*: Amadina, Marquis; *f*: Kitanokaori, Renan; *g*: Bluesky, Glenlea; at locus *Glu-B3*, allele *a*: Chinese Spring; *b*: Renan, Gabo; *c*: Insignia, Halberd; *d*: Pepital, Ernest; *f*: Fengmai 27; *g*: Splendor, Cappelle-Desprez; *h*: Aca 303, Pavon 76; *i*: Norin 61; *j*: Grebe, Seri 82; *ab*: Nanbu-komugi; *ac*: Thesee, Aca 801; *ad*: Heilo, Opata 85; at locus *Glu-D3*, allele *a*: Chinese Spring, Neixiang 188; *b*: Gabo, Avocet; *c*: Insignia, Cappelle-Desprez; *m*: Darius; *l*: Amadina, Heilo; *n*: Fengmai 27. Of these, four cultivars (Chinese Spring, Opata 85, Seri 82 and Pavon 76) are recommended as a core set to be included in each SDS-PAGE gel when identifying LMW-GS alleles. The 30 cultivars have been placed in the germplasm banks of CIMMYT, Mexico, and INRA Clermont Ferrand, France, with the aim of making the set publicly available. Seeds of the cultivars are available by request.

Use of these tools should help to resolve the contradictions between different published reports of correlations between certain allelic forms of LMW-GS and quality parameters in bread wheat, referred to in the Introduction. Such contradictions can be discerned from the following summary of some of these reports.

In Australian cultivars (Gupta and Shepherd 1988; Gupta et al. 1989, 1990a,b, 1991, 1994; Gupta and MacRitchie 1991; Metakovsky et al. 1990), for R_{max} (maximum dough resistance), the *Glu-A3* alleles ranked *b>d>e>c*, the *Glu-B3* alleles ranked *i>b=a>e=f=g=h>c* and the *Glu-D3* alleles ranked: *e>b>a>c>d*. The allele *b* of both *Glu-A3* and *Glu-D3* seemed to be associated with more extensible genotypes. Cornish et al. (1993) found that the *Glu-3* allelic pattern *bbb* (at *Glu-A3*, *Glu-B3* and *Glu-D3*, respectively) gave the best extensibility, especially when combined with the *Glu-1* pattern *bba* (at *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively). *Glu-3 bbc* also had excellent extensibility. They also concluded that *Glu-A3e* was detrimental to extensibility by virtue of being null and that *Glu-B3 c*, *d* and *g* had medium to weak dough properties. They suggested that the best combinations for *Glu-3* were *bbb*, *bbc* and *cbc*. Branlard et al. (2001) also compared allelic effects on quality parameters, finding that, for dough strength, the rankings were as follows: at *Glu-A3*: *a=d=f≥e*, at *Glu-B3*: *b'≥d=c=c'=b=g>i>f≥j* and at *Glu-D3*: *a≥b=d=c*. For extensibility at *Glu-A3*: *d=a=f≥e*, at *Glu-B3*: *i≥b'≥c=c'=g>b=f=d>j*, and, at *Glu-D3*, no significant differences were found (see the wheat gene catalog for an explanation of the allelic terminology used in this study). Luo et al. (2001) found that, in New Zealand cultivars: (i) the *Glu-A3* alleles ranked: *d>c=e*, coinciding with Gupta et al. (1990a) for R_{max}; (ii) the *Glu-B3* alleles ranked: *b>g*, which coincides both with Gupta et al. (1990a) and Cornish et al. (1993); and (iii) the *Glu-D3* alleles ranked: *b>a*.

Thus it can be seen from these studies that not all the published allelic rankings are consistent, hence the need for the aforementioned tools and further work on this general research area.

Conclusions

In general, it was shown that the four methods of LWM-GS identification can be regarded as complementary and, together, provide powerful tools for allelic identification. As an aid to allelic identification across laboratories, a standard set of cultivars was defined to represent all allelic variants in the collection analyzed, and the results of this study have been coordinated with those presented in the wheat gene catalog (McIntosh et al. 2011) in order to ensure consistency in the availability of information to the wheat community.

Although there are currently over 90 described alleles at the three *Glu-1* loci, only a handful have been assessed for quality. We believe that only a true collaboration between many groups will provide sufficient resources to allow all or at least the majority of allelic variants to be evaluated, and hence we should also like to propose the formation of a wider International Gluten Research Group, as a part of the G20 Wheat Initiative (previously the International Research Initiative for Wheat Improvement). The group would be aimed at achieving the following:

- Sharing materials and methods, and studying together to improve wheat quality.
- Unifying the nomenclature of gluten protein alleles of bread and durum wheat (*Glu-1*, *Glu-3*, *Gli-1* and *Gli-2*, etc.).

- Unifying the standard methods to analyze gluten proteins and genes (SDS-PAGE, 2-DE, PCR and MS).
- Identifying standard lines and wheat cultivars from worldwide origins to form reference sets as genetic stocks representing diverse gluten protein alleles.
- Storing the reference sets in national and international seed banks to maintain and facilitate the availability of the existing range of biodiversity in wheat (near-isogenic lines, landraces and modern cultivars).
- Exchanging and producing materials having a unique gluten protein composition (near-isogenic lines and mapping populations).
- Studying the usefulness of particular gluten compositions for various food products by evaluating landraces and modern cultivars for quality under various environmental conditions considering the unpaired influence of drought and heat stress associated with climate change on wheat grain yield and quality attributes.

A flow diagram summarizing the project is provided as Fig. 1. We believe the project is important since, with increasing globalization, increasing urban populations, and the demand for more healthy and convenient wheat-based foods, there is a need for the development of wheat cultivars with more specific grain quality attributes.

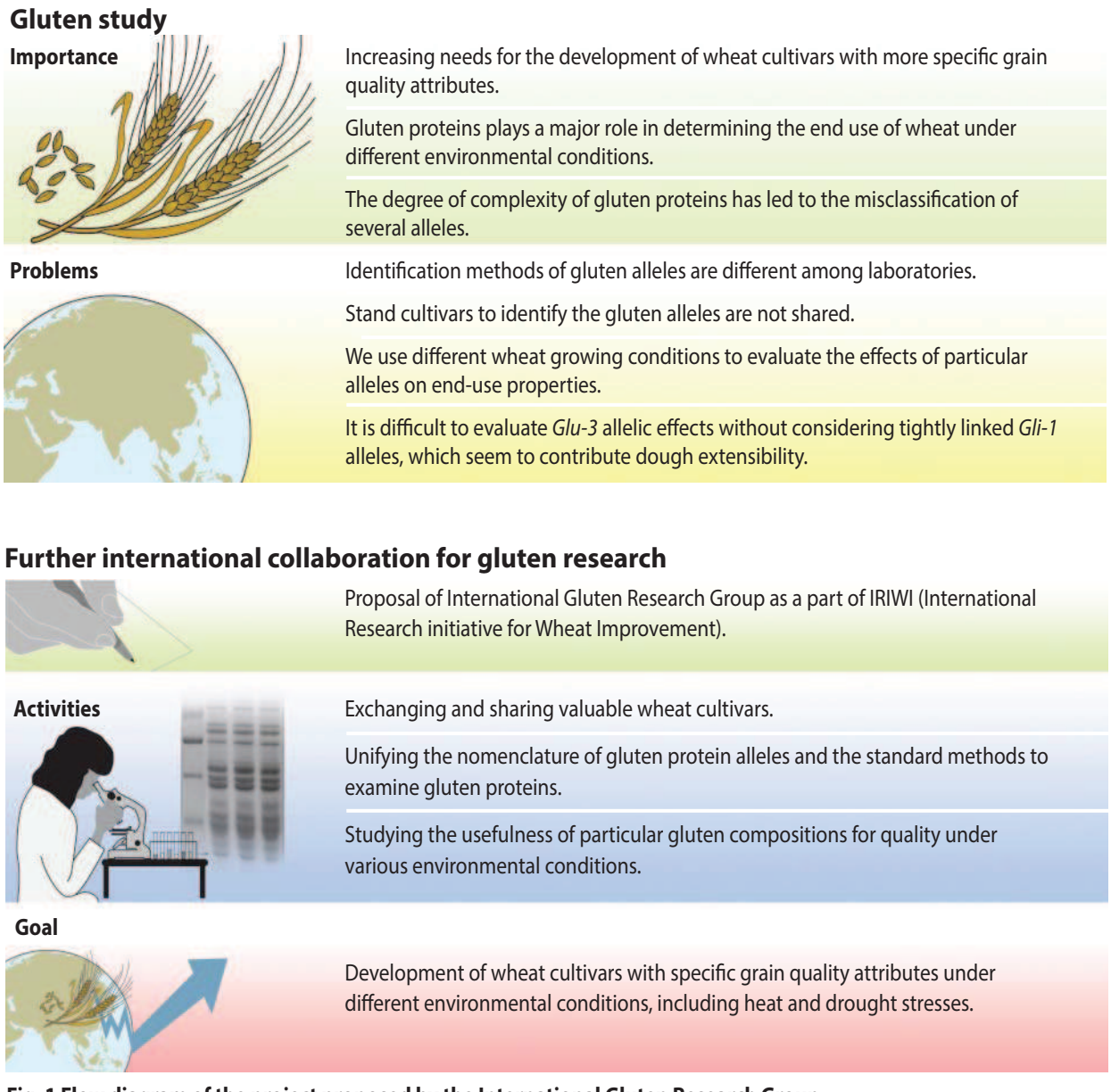

Fig. 1 Flow diagram of the project proposed by the International Gluten Research Group.

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