

Short Communication

Analysis of high-molecular-weight glutenin subunits in five amphidiploids and their parental diploid species *Aegilops umbellulata* and *Aegilops uniaristata*

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Abstract

Amphidiploids serve as a bridge for transferring genes from wild species into wheat. In this study, five amphidiploids with AABBUU and AABBNN genomes were produced by spontaneous chromosome doubling of unreduced triploid F₁ gametes from crosses between diploid *Aegilops* (*A. umbellulata* accessions CIAe 29 and PI 226500, and *A. uniaristata* accession PI 554419) and tetraploid *Triticum turgidum* (ssp. *durum* cultivar Langdon and ssp. *dicoccum* accessions PI 94668 and PI 349045) species. The composition of high-molecular-weight glutenin subunits (HMW-GS) in these amphidiploids and in their parental *A. umbellulata* and *A. uniaristata* species was analysed. As expected, the amphidiploids from *T. turgidum* ssp. *dicoccum* accession PI 944668 or PI 349045 and *A. umbellulata* accession CIAe 29 or PI 226500 and *A. uniaristata* accession PI 554419 showed the same HMW-GS patterns as those of their *Aegilops* parents, because HMW-GS genes were all silenced in the *T. turgidum* ssp. *dicoccum* parents. The amphidiploids from CIAe 29 and Langdon inherited all of the HMW-GS genes from their parents except for the Uy type. Using 10 and 15% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and 10% urea/SDS–PAGE, 11 Ux and ten Uy types in 16 combinations were observed in 48 *A. umbellulata* accessions, and two Nx and two Ny types in two combinations were detected in six *A. uniaristata* accessions. These novel HMW-GS variants may provide new genetic resources for improving the quality of wheat.

Keywords: *Aegilops umbellulata*; *Aegilops uniaristata*; amphidiploids; HMW-GS; synthetic hexaploid species; *Triticum turgidum*

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Introduction

In wheat, high-molecular-weight glutenin subunits (HMW-GS) represent about 10% of the total endosperm protein. They are encoded by three sets of paralogous loci on the long arms of group 1 chromosomes (Payne *et al.*, 1980). At each locus, two tightly linked *x* and *y* genes encode a larger *x* and a smaller *y* subunit. Owing to the varying degrees of gene silencing, the numbers of expressed HMW-GS genes range from 3 to 5 among hexaploid wheat cultivars (Payne *et al.*, 1980, 1981). The types and numbers of HMW-GS are critical determinants of the baking and processing quality of wheat flour (Payne *et al.*, 1984).

Aegilops umbellulata ($2n = 2x = 14$, UU) and *A. uniaristata* ($2n = 2x = 14$, NN) are diploid donors of many polyploid *Aegilops* species (Kilian *et al.*, 2011). However, their HMW-GS and qualities are not well documented (Castilho *et al.*, 1996, 1997; De Bustos and Jouve, 2006; Rodríguez-Quijano *et al.*, 2001; Liu *et al.*, 2003). One way to evaluate the quality of HMW-GS in these *Aegilops* species is to produce hexaploid amphidiploids from crosses between *Aegilops* species and *T. turgidum*. The most successful examples of using HMW-GS in the D genome of diploid *A. tauschii* involved synthetic hexaploid wheats (Peña *et al.*, 1995; Mujeeb-Kazi *et al.*, 1996; Pflüger *et al.*, 2001; Rasheed *et al.*, 2012; Hu *et al.*, 2013).

In the present study, five amphidiploids were produced by spontaneous chromosome doubling of unreduced triploid F_1 gametes ($2n = 3x = 21$, ABU or ABN) from the crosses between *T. turgidum* ssp. *dicoccum* (accessions PI 94668 and PI 349045) or ssp. *durum* (cultivar Langdon) and *A. umbellulata* (accessions Clae

29 and PI 226500) or *A. uniaristata* (accession PI 554419). The composition of HMW-GS in these amphidiploids and their parental diploid species was investigated.

Experimental

The composition of HMW-GS in amphidiploids and their parental species was analysed by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) gels (Yan *et al.*, 2002). Amphidiploids with AABBNN genomes derived from *A. uniaristata* accession PI 554419 and *T. turgidum* ssp. *dicoccum* accession PI 94668 produced the same HMW-GS patterns as those of the accession PI 554419, because all of the HMW-GS genes in accession PI 94668 were silenced (Fig. 1; Hu *et al.*, 2013).

Amphidiploids with AABBUU genomes derived from *A. umbellulata* accession PI 226500 and *T. turgidum* ssp. *durum* cultivar Langdon and from two *T. turgidum* ssp. *dicoccum* accessions (PI 94668 and PI 349045) and *A. umbellulata* accession Clae 29 inherited all of the HMW-GS genes from both parents (Fig. 1, lanes 10, 4 and 6). However, amphidiploids derived from Clae 29 and Langdon inherited exactly the same HMW-GS patterns as those of their parents except for the Uy type (Fig. 1, lane 8). The Ux and Uy types in these amphidiploids were very similar to the Dx 2.2 and Dy 12 types in the wheat cultivar Shinchunaga (with the HMW-GS combination 7 + 8, 2.2 + 12).

HMW-GS in diploid *A. umbellulata* and *A. uniaristata* species were preliminarily grouped by 10% SDS–PAGE and further differentiated by 15% SDS–PAGE and 10% urea/SDS–PAGE (Yan *et al.*, 2003; Hu *et al.*, 2013).

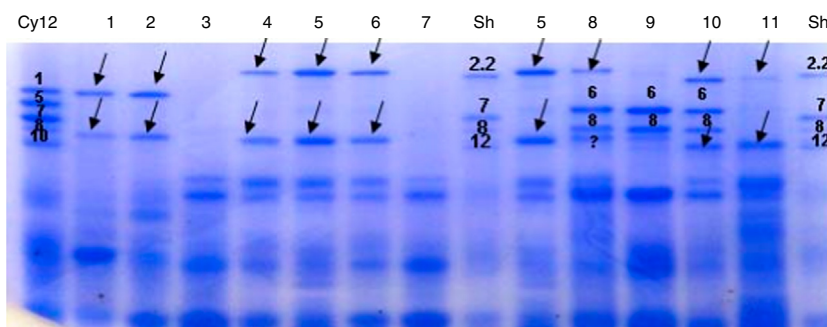


Fig. 1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis pattern of high-molecular-weight glutenin subunits (HMW-GS) in five amphidiploids from the crosses of *Aegilops umbellulata* and *A. uniaristata* with *Triticum turgidum* ssp. *durum* and ssp. *dicoccum*. Lane 1, PI 554419 (*A. uniaristata*); lane 2, PI 94668/PI 554419 amphidiploids; lane 3, PI 94668 (*T. turgidum* ssp. *dicoccum*); lane 4, PI 94668/Clae 29 amphidiploids; lane 5, Clae 29 (*A. umbellulata*); lane 6, PI 349045/Clae 29 amphidiploids; lane 7, PI 349045 (*T. turgidum* ssp. *dicoccum*); lane 8, Langdon/Clae 29 amphidiploids; lane 9, Langdon (*T. turgidum* ssp. *durum*); lane 10, Langdon/PI 226500 amphidiploids; lane 11 PI 226500 (*A. umbellulata*). Two hexaploid wheat varieties (ChuanYu 12 (Cy12) and Shinchunaga (Sh)) with the HMW-GS combinations 1, 7 + 8, 5 + 10 and 7 + 8, 2.2 + 12 were used as references. The arrows indicate the HMW-GS that originate from the *A. uniaristata* and *A. umbellulata* parents. The question mark indicates the unconfirmed source of the band.

A total of eight Ux and seven Uy types were initially detected in 48 *A. umbellulata* accessions by 10 and 15% SDS–PAGE gels (Table 1; Figs. S1(a) and (b) and S2(a) and (b), available online). Subunits with equal electrophoretic mobility were differentiated by 10% urea/SDS–PAGE. The Ux 3, Ux 6 and Ux 8 proteins in 2, 3 and 25 accessions, respectively, were further divided into two subunits each: Ux 3-1 (one accession) and Ux 3-2 (one accession), Ux 6-1 (one accession) and Ux 6-2 (two accessions), and Ux 8-1 (three accessions) and Ux 8-2 (22 accessions) (Fig. S1(c), available online; Table 1). The Uy 2 and Uy 5 proteins in 3 and 33 accessions, respectively, were differentiated as the following subunits: Uy 2-1 (one accession) and Uy 2-2 (two accessions), and Uy 5-1 (25 accessions), Uy 5-2 (one accession) and Uy 5-3 (seven accessions) (Fig. S2c, available online; Table 1). In the 48 *A. umbellulata* accessions, 22 accessions with the HMW-GS combination Ux 8-2 + Uy 5-1 represented the predominant HMW-GS type (Table 1).

In six accessions of *A. uniaristata*, two Nx, two Ny and two combinations of HMW-GS were observed by 10 and 15% SDS–PAGE and 10% urea/SDS–PAGE (Fig. S3(a)–(c); Table 1).

Discussion

Amphidiploids are considered as bridges for transferring genes from *T. turgidum* and *Aegilops* species, or even more distantly related wild species, into hexaploid wheat (Sharma and Gill, 1983; Jiang *et al.*, 1994). The quality of HMW-GS specific to *A. tauschii* was effectively evaluated in amphidiploid crosses between *T. turgidum* and *A. tauschii* (Peña *et al.*, 1995; Hu *et al.*, 2013). However, the quality of HMW-GS in amphidiploids derived from *A. uniaristata* and *A. umbellulata* has not been evaluated due to a lack of materials. In this study, five amphidiploids from crosses between *T. turgidum* ssp. *durum* or ssp. *dicoccum* and *A. uniaristata* or *A. umbellulata* were produced, and HMW-GS from two *A. umbellulata* accessions (Clae 29 and PI 226500) and one *A. uniaristata* accession (PI 499516) were introduced into hexaploid species with AABBUU and AABBNN genomes, respectively. They provide ideal materials for evaluating the quality of HMW-GS. Quality tests will be performed when enough seeds are produced.

Table 1. Composition of high-molecular-weight glutenin subunits in *Aegilops umbellulata* and *A. uniaristata*

Subunit combinations	Accessions	No. of accessions
<i>A. umbellulata</i>		
Ux 1 + Uy 1	PI 554405	48
Ux 2 + Uy 5-1	PI 298905, PI 542369, PI 554414	1
Ux 2 + Uy 5-3	PI 542375, PI 554411	3
Ux 3-1 + Uy 5-2	PI 486261	2
Ux 3-2 + Uy 5-3	PI 486256	1
Ux 4 + Uy 1	PI 226500, PI 276994, PI 428569, PI 542365, PI 542366, PI 542367, PI 554415	1
Ux 4 + Uy 2-1	PI 227436	7
Ux 4 + Uy 2-2	PI 542377, PI 542378	1
Ux 5 + Uy 5-3	PI 554385	2
Ux 6-2 + Uy 5-3	PI 542376, PI 554399	1
Ux 6-1 + Uy 7	PI 542370	1
Ux 7 + Uy 3	PI 542379	1
Ux 8-1 + Uy 4	Clae 29	1
Ux 8-1 + Uy 5-3	PI 554416	1
Ux 8-1 + Uy 6	PI 542374	1
Ux 8-2 + Uy 5-1	PI 116294, PI 204546, PI 486252, PI 542362, PI 542363, PI 542364, PI 542381, PI 542383, PI 542384, PI 554386, PI 554389, PI 554390, PI 554393, PI 554394, PI 554395, PI 554396, PI 554397, PI 554398, PI 554400, PI 554412, PI 554413, PI 560557	22
<i>A. uniaristata</i>		
Nx 1 + Ny 1	PI 554419	6
Nx 2 + Ny 2	PI 276995, PI 276996, PI 554418, PI 554420, PI 554421	1
		5

Polymorphisms were identified in the HMW-GS of the diploid parental species *A. uniaristata* and *A. umbellulata*. Preliminary identification with 10 and 15% SDS–PAGE detected eight Ux and seven Uy types. SDS–PAGE gels containing 4M urea are generally used to differentiate polymorphic HMW-GS with similar electrophoretic mobilities in SDS–PAGE. Using such methods, five novel Dx and Dy types were distinguished from a collection of 43 synthetic hexaploid wheat lines (Xu *et al.*, 2010). In this study, three additional Ux and three Uy proteins were differentiated from *A. umbellulata* accessions by 10% urea/SDS–PAGE gels. Therefore, we identified a total of 11 Ux and 10 Uy proteins in 16 combinations among the 48 accessions of *A. umbellulata*. We also analysed the composition of HMW-GS in six accessions of *A. uniaristata*, but only two Nx and two Ny types in two combinations were detected as only a few accessions were used. These HMW-GS variants may provide new genetic resources for improving the quality of wheat.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1479262114000719>

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