Transfer of HMW glutenin subunits from *Aegilops kotschy* to wheat through radiation hybridization

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Abstract High molecular weight glutenin subunits (HMWGS) are responsible for dough elasticity and bread making quality of bread wheat. Related wild non-progenitor species, *Aegilops kotschy* possesses higher molecular weight *x* and *y* glutenin subunits than the bread wheat cultivars. A wheat-*Aegilops* substitution line with 1U chromosome was used for the transfer of (HMWGS) of 1U to wheat by using pollen radiation hybridization approach. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiling showed different patterns of allelic variations with either the presence or absence of HMWGS, *Glu-1A* (1, null), *Glu-1B* (7, 7 + 8, 17 + 18) and *Glu-1D* (5 + 10, 2 + 12, null). The pollen irradiated wheat-*Aegilops* derivatives, B-56-1-4-2, B-56-1-4-3, B-14-1 and B-14-2 with *Glu1Ux* and 1Uy and absence or presence of some *Glu-1A* and *Glu-1B* HMWGS showed high micro SDS sedimentation test (MST) values while B-16-1 and B-16-2 had moderate MST values and high protein content. However, B-58-3 with transfer of *Glu-1Ux* + 1Uy for *Glu-*

1D showed very low MST values indicating that *Glu-1Ux* + 1Uy enhance MST value only in the presence of *Glu1D* HMWGS. The transfer/substitution of alien HMWGS for *Glu-1A* and or *Glu-1B* loci only can lead to improved bread making quality of wheat.

Keywords *Aegilops kotschy* · High molecular weight glutenin subunits · Micro-sedimentation test · Pollen radiation hybridization · *Triticum aestivum* L.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops followed by maize and rice. The production and consumption of wheat is increasing world-wide. According to FAOSTAT (2014), the global wheat production in 2014–2015 was 723 million tons with India being the second largest wheat producing country contributing 13.01 % (94 million metric tons) to the total world wheat production. In India, Punjab and Uttar Pradesh are the largest wheat producing states which contribute 52 % of the total wheat production in India and 7 % to the global wheat production. Bread wheat has more than 25,000 different cultivars developed by wheat breeders worldwide (Sapone et al. 2012) and majority of the world population consumes wheat flour in different forms of baking products such as chapatti, bread, pasta, biscuits and noodles. Wheat grain comprises of an embryo, an endosperm, testa and bran. The starchy endosperm is packed with insoluble starch and proteins which make up to 80 % of the grain mass. Seed storage proteins such as gluten proteins are responsible for the bread making quality of wheat (Shewry 2003). These gluten proteins comprise 78–85 % of the total wheat endosperm proteins which play major role in the elasticity and

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extensibility of wheat dough. Gluten proteins belong to a group of seed storage proteins namely prolamins which contain high contents of proline and glutamine (Payne 1987). The presence of such amino acids in gluten proteins is not ideally suitable for human consumption, so the only role of gluten is to provide viscoelasticity to the dough. The elasticity and extensibility of dough is genetically controlled, differs greatly among wheat varieties and has major influence on the type of products that can be prepared from wheat flour (Payne 1987). Hence, in most of the countries the wheat varieties that produce strong elastic dough with some extensibility are used to make bread. The varieties that produce strong extensible dough with some elasticity are used to make biscuits and the varieties that produce dough with properties intermediate between the two are used to make chapatti which is the staple food in Asian countries like India and Pakistan. *T. aestivum* L. is allohexaploid wheat with genome size $\sim 17\text{Gbp}$ (Brenchley et al. 2012; McIntosh et al. 2008) comprising of three complex genomes A, B and D derived from two different *Aegilops* and a *Triticum* species. Due to its polyploidy character and wide adaptability to diverse climatic conditions, it has become a crop of immense financial and nutritional importance (Dubcovsky and Dvorak 2007). The inheritance of glutenin subunits follows Mendelian genetics with multiple alleles at each locus. Glutenin subunits are classified into high molecular weight glutenin subunits (HMWGS) and low molecular weight glutenin subunits (LMWGS) (Payne et al. 1981a). The nomenclature of a single HMWGS is based on its coding genome (A, B, D), the type (x, y) and the mobility in SDS-PAGE. Genes controlling HMW glutenin are present on three loci on long arms of 1A, 1B and 1D chromosomes and are designated as *Glu-1A*, *Glu-1B* and *Glu-1D*, respectively (Payne 1987). At each locus there are two tightly linked genes coding for HMWGS x-subunit with slow mobility and y-subunit with higher mobility. Both x- and y-genes are tightly linked and are highly polymorphic without environmental effect (Payne et al. 1981b). The LMWGS comprise one-third of the total seed endosperm protein and 60 % of total gluten (Bietz and Wall 1973). These LMWGS are controlled by genes at *Glu-3* loci present on the short arm of the chromosomes 1A, 1B and 1D and designated as *Glu-3A*, *Glu-3B* and *Glu-3D* (McIntosh et al. 2008) respectively. However, there is another locus coding for LMW glutenins designated as *Glu-B2* which is present on the short arm of 1B (Shewry 2003). Each wheat variety possesses at least 3–4 different HMWGS and each subunit has additive role in the bread making quality. This vast allelic variation of glutenin subunits from high molecular weight to low molecular weight can be easily distinguished by SDS-PAGE (Payne et al. 1981a). By understanding the genetics of the wheat storage proteins, this variation is mainly used by plant breeders to develop wheat lines for

desired dough elasticity and extensibility. The diversity of grain flour characteristics (Kaur et al. 2013), quality traits (Katyal et al. 2016) and rheological properties (Kaur et al. 2016) of several Indian wheat varieties have been extensively evaluated and utilized in food technology. The related wild species are rich reservoirs of genes for wheat improvement including disease resistance as well as quality (Zhang et al. 2010). Several progenitor and non-progenitor species of wheat have been used for development of wheat-alien addition, substitution and translocation lines for the precise transfer of useful alien traits into wheat cultivars (Friebe et al. 2000). Wheat-*Ae. biuncialis* addition lines have been used for the transfer of HMW glutenin subunits from *Ae. biuncialis* to wheat through induced homoeologous pairing (Zhou et al. 2014) while wheat-*Ae. kotschy* addition lines were used for enhancement of grain protein, Fe and Zn content (Verma et al. 2016a). The use of irradiated seed is an alternative approach for precise gene transfer from related species of wheat and mapping (Verma et al. 2016b). In wheat, radiation hybrids have been used for transfer of genes controlling high grain iron and zinc (Verma et al. 2016a, b). It is a very useful approach for precise transfer of genes from non-progenitor genomes to wheat cultivars. Some of the *Aegilops* species possesses higher molecular weight x and y glutenin subunits than any of the wheat genotypes (Tiwari et al. 2010). These subunits could be transferred into wheat cultivars through radiation hybrid strategy. A wheat-*Ae. kotschy* substitution line (49-1-73-10) developed for high grain micronutrients had 1U chromosome of *Ae. kotschy* with higher MWGS than that of the recipient wheat cultivar. However, the role of higher molecular weight subunits from non-progenitor genome in modulating dough characteristics and their precise transfer without linkage drag has not been reported. Pollen radiation induced precise transfer of quality traits from non-progenitor species into bread wheat cultivar is an efficient and recombination independent approach. This article deals with the pollen radiation induced transfer of 1U specific higher MWGS into wheat cultivar and their interaction with the wheat HMWG subunits for improving bread making quality. To the best of our knowledge, this is the first report on radiation hybridization mediated transfer of HMWGS from substitution lines of a non-progenitor *Aegilops* species.

Experimental

Plant material and development of radiation hybrids

The experimental material used in the present study comprising wheat-*Ae. kotschy* derivative (49-1-73-10) and the pollen radiation hybrid derivatives of PBW343/wheat-*Ae. kotschy* (49-1-73-10) were obtained from the wheat

germplasm collection, Eternal University, Baru Sahib. Initially, *Ae. kotschy* acc. 396 was crossed with *T. aestivum* cv. Chinese Spring (Ph^1) for induced homoeologous pairing with wheat. The F_1 was then crossed with the wheat cultivar PBW343. The BC_1 plants were again crossed with the wheat cv. UP2425. The BC_2F_1 plants were then allowed to self up to BC_2F_6 . One of the wheat-*Aegilops* BC_2F_6 derivative namely 49-1-73-10 $\otimes$ (CS(Ph^1)/*Ae. kotschy* acc. 396//PBW343-3//UP2425(49)-1-73-10 $\otimes$) with 1U and 2S chromosomes of *Ae. kotschy* substituted for their wheat homeologues was used for induced introgression of 1U high molecular weight glutenin subunits (HMWGS) through pollen radiation hybridization. Spikes of 49-1-73-10 $\otimes$ gamma irradiated at a dose of 20 Gy, were used as male parent to pollinate *T. aestivum* cv. PBW343 with *Lr24* and *GPCB1* genes. Spikes of 49-1-73-10 $\otimes$ were detached (ready to dehisce the next day), and exposed to gamma radiation at 20 Gy in the evening and kept their peduncles immersed in water. The PBW343 recipient plants were emasculated 2 days before the dehiscence of pollen so that on the day of pollination, ovaries were fully receptive. Seed set was checked at regular intervals. The fully matured crossed seeds were harvested, dried and stored at 4 °C temperature till the next sowing season. The Pollen Radiation Hybrids (PRH₁) and their subsequent generations were then grown for generation advancement in the experimental fields of the Indian Institute of technology Roorkee, Roorkee (IITR), Punjab Agricultural University, Ludhiana and Eternal University, Baru Sahib, India during 2011–2012, 2012–2013 and 2013–2014, respectively. The pollen irradiated derived hybrid progenies PRH₂, PRH₃ and PRH₄ were used in the present study.

Cytological studies

The spikes at appropriate meiotic stages were collected early in the morning and were fixed in Carnoy's solution (6 ethanol : 3 chloroform : 1 acetic acid) for 24 h and then stored in 70 % ethanol at 4 °C. The anthers were squashed in 2 % acetocarmine and the pollen mother cells (PMC) at various meiotic stages were analyzed for chromosome constitution and pairing under microscope (Magnus, New Delhi, India). Genomic in situ Hybridization (GISH) was done at GBPUA&T, Pant Nagar to analyze and visualize the alien chromosome introgression in the pollen radiation hybrids using the method described by elsewhere (Verma et al. 2016a; Dou et al. 2006). Actively growing root tips were collected and treated with chilled water for 24 h and then fixed in Clarke's solution. Root tips were squashed in 45 % glacial acetic acid and further stained with 1 % acetocarmine solution. The sheared genomic DNA of *Ae. umbellulata* (UU) was labeled with tetramethyl rhodamine-5-dUTP (red) (Roche Applied Science, Indianapolis, IN)

and used as genomic probes for GISH analysis. QIAquick Nucleotide Removal Kit (Qiagen Valencia, CA) was used for purification of labeled DNA. Unlabelled sheared genomic DNA of *T. aestivum* cv. Chinese Spring was utilized as blocking agent (1 ng labelled probe:120 ng blocking DNA). The 4', 6-diamidino-2-phenylindole (DAPI) dye was used to counter stain the chromosomes. All the slide preparations were analyzed by Epifluorescent microscope (Labexchange - Die Laborgerateborse GmbH, Germany).

HMW glutenin SDS-PAGE

HMW glutenin protein subunits were extracted from single seeds as well as from bulk seed material using the method described by Smith and Payne (1984) with some modifications. 30 mg of each seed sample was crushed in the 400 μ l extraction buffer and incubated in the water bath (80 °C) for 18 min. Extraction buffer (pH-6.8) comprised 0.75 g of Tris (hydroxymethyl) aminomethane buffer (Merck), 2gm of Sodium Dodecyl Sulfate (Himedia), 5 ml of β - mercaptoethanol (Sigma) and dissolved in double-distilled water (total volume—100 ml). After centrifuging the solution for 10 min at 4000 rpm, supernatant was collected for further analysis. HMW glutenin subunits present in the protein solution were separated by SDS-PAGE (Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis). The resolving and stacking gels were composed of 10 and 4.5 % acrylamide (Merck), respectively. Gels were stained with Commassie brilliant blue (CBB-R250) (Himedia) and images were captured by Gel documentation system (Gel DocTM EZ, Bio-Rad). Five seeds for bulk analysis and at least 6 individual seeds from each radiation hybrid line were analyzed.

Micro SDS sedimentation test

The selected wheat-*Aegilops* radiation hybrids with 1Ux and 1Uy HMWGS were studied for bread making quality using micro SDS sedimentation test (MST). Due to less availability of seeds, micro SDS sedimentation test was carried out for only seven selected lines in addition to the cultivars and wheat-*Aegilops* derivative. Micro sedimentation test was performed according to the method of Dick and Quick (1983). About 80 grains were used to get 3 g of wheat flour. The grains were milled into very fine powder using Ball Mill (Retsch MM 400) with vibration frequency of 30/s. The wheat flour (1 g) of each line was transferred to three test tubes as different replications. 4 ml of distilled water was added to each test tube and the contents were mixed thoroughly by using vortex mixer and allowed to stand for 5 min. Then the samples were again mixed and allowed to stand for 5 min. Then 12 ml of working solution

(1 ml 85 % lactic acid and 48 ml of 2 % SDS) was added to each tube and inverted them upright-down position 10 times. The samples were then allowed to settle for 10 min. The mixture separated into two phases, the upper liquid phase and the lower solid phase. The raised dough height of the solid phase was measured in millimeters (mm) by a measuring scale. The average of three MST replications was taken for each line.

Protein content estimation

Percentage protein content was determined using Pelican Kjeloplus-Kjelodist CAS, VA according to (AACC 1983) method (46-11A).

Results and discussion

SDS-PAGE HMWGS profile of selected pollen radiation hybrids (PRH)

In the wheat-*Aegilops kotschy* acc. 396 derivative 49-1-73-10, two chromosomes of *Ae. kotschy* viz., 1U and 2S were substituted for their wheat homologues (Rawat et al. 2011). The SDS-PAGE of the derivative having 1Ux and 1Uy HMWGS confirmed the presence of 1U substitution (Fig. 1). The 1Ux HMWGS had the slowest SDS-PAGE mobility whereas 1Uy HMWGS had mobility corresponding to *Glu-1Bx7* of PBW343. The addition of 1U of *Ae. kotschy* had high molecular weight glutenin subunits with higher molecular weight than those of wheat (Rawat et al. 2011). Non-progenitor *Aegilops* species possesses higher molecular weight x and y glutenin subunits than any of the wheat genotypes (Tiwari et al. 2010). The SDS-PAGE of *Ae. umbellulata*(UU) and *Ae. longissima*(S¹S¹) also confirmed the higher molecular weight of their *Glu-*

1Ux and *Glu-1Sx* HMWGS equivalent to that of 1Ux of the derivative. HMWG y subunit of *Glu-1Sy* of *Ae. longissima* and *Ae. sharonensis* also corresponded to the *Glu1Bx7* of the Chinese Spring (Jiang et al. 2012). Ma et al. (2013) characterized five novel HMWGS from different accessions of *Ae. kotschy* designated as *AKx1**, *AKx3**, *AKx2.3**, *AKy20** and *AKy8** on the basis of their electrophoretic mobility.

A number of fertile pollen irradiation hybridization derivatives (PRH) of PBW343/49-1-73-10 were developed which had 1U specific 1Ux and 1Uy HMWGS in subsequent generations indicating the effectiveness of radiation hybridization in their possible precise transfer of useful variability from non-progenitor alien substitution lines (Fig. 1). Only a few pollen radiation hybrids were used to identify the introgressed 1U specific HMWGS (Supplementary Table 1). Seven stable radiation hybrid lines PRH₄ B-14-1-2, PRH₄ B-14-2-1, PRH₄ B-16-1-3, PRH₄ B-16-2-1, PRH₅ B-56-1-4-2, PRH₅ 56-1-4-3 and PRH₄ B-58-3-1 (Fig. 1; Table 1) which did not segregate for 1U HMWGS among the individual seeds were selected for further analysis. All these PRH lines showed an additional HMWGS of 1Ux and 1Uy of *Aegilops kotschy* (Fig. 1). The SDS-PAGE electrophoretic pattern of five PRH lines B-14-1-2, B-16-1-3, B-16-2-1, B-56-1-4-2 and B-56-1-4-3 (Table 1) was the same as that of wheat cultivar PBW343 *Glu-Ax1*, *Glu-Bx7* and *Glu-Dx5* + *Glu-Dy10* indicating with either the addition of 1U chromosome or non-compensated transfer of its HMWGS to wheat genome. One PRH₃ B-14-2-1 plant, however, showed *Glu-1A1* null, *Glu-1B7*, *Glu-1D 2 + 12* (Fig. 1). All the B-58-3 plants were found null for *Glu-1D* (Fig. 2a) indicating that these might have either compensating transfer of 1U HMWGS for *Glu-1D* HMWGS or substitution of 1U for 1D but B-16-2 plants had *Glu-1Ux* + 1Uy in addition to *Glu-1D* (Fig. 2b). SDS-PAGE of PRH derivatives showed that some had 1U

Fig. 1 HMWGS profile of selected radiation hybrid lines from single seeds Lane 1: B-16-1-3; Lane 2: *Aegilops kotschy* K396; Lane 3: Chinese Spring; Lane 4: 49-1-73; Lane 5: PBW343; Lane 6–7: B-56-1-4-2, B-56-1-4-3; Lane 8: B-14-1-2; Lane 9: B-14-2-1; Lane 10: B-16-2-1

Table 1 Allelic variations of HMWGS in selected radiation hybrids

Wheat lines	1Ux, 1Uy	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>
Chinese spring	–	Null	7 + 8	2 + 12
PBW343	–	1	7	5 + 10
UP2425	–	1	17 + 18	5 + 10
WL711	–	2*	17 + 18	2 + 12
49-1-73-10	1Ux, 1Uy	1	7	5 + 10
B-14-1-2	1Ux, 1Uy	1	7	5 + 10
B-14-2-1	1Ux, 1Uy	Null	7	2 + 12
B-16-1-3	1Ux, 1Uy	1	7	5 + 10
B-16-2-1	1Ux, 1Uy	1	7	5 + 10
B-56-1-4-2	1Ux, 1Uy	1	7	5 + 10
B-56-1-4-3	1Ux, 1Uy	1	7	5 + 10
B-58-3-1	1Ux, 1Uy	1	7	Null

Fig. 2 SDS-PAGE of single seed. **a** Lane 1: *Aegilops kotschy*; Lane 2: derivative 49-1-73-8-5⊗; Lane 3: WL711; Lane 4: PBW343; Lane 5–10: single seed of B-58-3(1Ux, 1, 1Dx null, 7 + 1Uy, 1Dy null). **b** Lane 1: WL711; Lane 2–6: seed protein of six individual seeds of B-16-2

HMWGS addition while others had substitution of 1U for 1A or 1D. Out of the seven selected derivative lines, the PRH₃ B-14-2 had *Glu1Dx2* + *Glu1Dy12* along with 1Ux and 1Uy substituting for *Glu 1Ax*. In only one derivative PRH₃ B-58-3 ($2n = 42$; 21II), *Glu-1U* HMWGS were substituted for *Glu-1Dx* + 1Dy indicating the possible compensating transfer of *Glu1U* for *Glu 1D*. Enhanced bread making quality was observed in the substitution line CS-1S¹(1B) which showed the substitution of alien HMWGS for *Glu-1Bx7* + *Glu-1By8* in presence of 1Dx2 + 1Dy12 (Wang et al. 2013).

Cytological studies

Four radiation hybrid lines were observed through cytology for stable chromosome number ($2n = 6x = 42$). Cytological analysis of PRH₃ B-14-1-6, PRH₃ B-14-2-1, PRH₃ B-16-1-2 and PRH₄ B-56-1-4-3, with $2n = 42$ chromosomes showed 21 bivalents whereas PRH₃ B-16-2-2

showed mostly 19 II + 1 IV (Supplementary Fig. S1; Supplementary Table 2).

GISH of PRH₃ derivative B-14-2 showed a small homozygous 1U specific transfer in a pair of chromosome as well as two dots in the nucleus confirming the precise compensating transfer of 1U as it had normal seed set and seed size without any linkage drag (Supplementary Fig. S2). The other line B-16-2, however, had two complete 1U chromosomes suggesting that the irradiation might not have been effective in all the pollen grains.

Micro SDS sedimentation and total protein content

The MST value of PBW343 and PBW343 (GPCB1 + *Lr24*) were 24.3 mm and 33 mm respectively. All the selected derivatives with addition of 1Ux and 1Uy along with either 1Dx2 + 1Dy12 or 1Dx5 + 1Dy10 had significantly higher MST values which were almost more than double than the recipient cultivar PBW343 (Table 2). The B-58-3 line with both 1Ux and 1Uy but null for *Glu-*

Table 2 Micro sedimentation test mean values and total protein content of lines non-segregating for HMWGS

Sr. no.	Wheat lines	MST value ^a Mean (mm)	Protein content (%)
1	PBW343	24.3	10.2
2	PBW343 (GPCB1 + <i>Lr24</i>)	33.0	8.39
3	49-1-73 (<i>T. aestivum</i> + 1U, 2S)	60.6	10.84
4	B-14-1(<i>T. aestivum</i> + 1U)	65.6	13.38
5	B-14-2(<i>T. aestivum</i> + 1U)	62.6	15.74
6	B-16-1(<i>T. aestivum</i> + 1U)	48.0	16.55
7	B-16-2 (<i>T. aestivum</i> + 1U)	43.3	13.58
8	56-1-4-2(<i>T. aestivum</i> + 1U)	63.3	10.61
9	56-1-4-3(<i>T. aestivum</i> + 1U)	67.0	12.24
10	B-58-3 (<i>T. aestivum</i> + 1U)	10.3	10.31

^a MST values are based on triplicate determinations

1D, exhibited much lower MST value and hence the gluten strength indicating clearly that the 1U specific HMWGS enhance MST value and hence bread making quality only in the presence of *Glu*-1D HMWGS. The presence of *Glu*-1D 5 + 10 have been found to give better bread making quality as compared to the wheat cultivars with *Glu*-1D 2 + 12 (Payne 1987). Addition of alien HMWGS in wheat-*Ae. biuncialis* addition line also enhanced the SDS Sedimentation value (Zhou et al. 2014). The micro-sedimentation test of the PRH derivatives indicated that the addition of 1U specific HMWG subunits increased the MST values only in the presence of *Glu*-1D as their absence in PRH B-58-3 derivative gave extremely low MST values. The favorable role of *Glu*-1D HMWG subunits in bread wheat over *T. durum* without D genome has been demonstrated in several studies (Kerber and Tipples 1969; Ceoloni et al. 1996; Radaelli et al. 1997; Lafandra et al. 2000). However the molecular and biochemical basis for 1U and 1D interaction, which led to high MST values, are not well known and need to be further investigated.

The addition of 1Ux and 1Uy HMW glutenin subunits of the derivatives significantly increased the protein content over the wheat cultivars (Table 2). The protein content of the selected derivatives B-14-1, B-14-2, B-16-1, B-16-2 B-56-1-4-2 and B-56-1-4-3 ranging from 10.61 to 16.55 % was higher than the recipient cultivar PBW343 with *Lr24* and *GPCB1* except B-58-3 (null *Glu*-1D) with lower protein content. All the selected derivatives had their protein content more than the recipient PBW343 (GPCB1 + *Lr24*) suggesting that the transfer of 1U also increased the protein content in the derivatives. Wang et al. 2013 reported that with the alien HMWGS introgression, the protein content also increased probably due to higher expression of 1U specific genes for storage proteins. The addition of HMW glutenin subunits from A genome donors in the derivatives may also increase the protein content

(Waines and Payne 1987). The addition of 1U/1S had higher MWGS than those of wheat and the addition of 2S in most of the derivatives enhanced grain protein content by over 20 % (Rawat et al., 2011).

The substantial increase in micro SDS sedimentation value with better protein content and hence bread making quality of wheat with the addition and transfer of HMWGS from related non-progenitor species suggests that the work on the precise transfer of genes from group 1 chromosome addition and substitution lines in the presence of *Glu*-1D in *Triticeae* should be continued.

Conclusion

High molecular weight glutenin subunits (HMWGS) of *Aegilops kotschy* were transferred into bread wheat by radiation hybridization. The presence of *Glu*-1A and/or *Glu*-1B HMWGS did not affect the micro sedimentation values. Transfer of *Glu*-1Ux + 1Uy for *Glu*-1D showed very low MST values indicating that *Glu*-1Ux + 1Uy enhance MST values only in the presence of *Glu*1D HMWGS. The addition of alien HMWGS or their transfer/substitution for *Glu*-1A and or *Glu*-1B loci only can lead to improved bread making quality of wheat.

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