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Original Research Article

ASSESSMENT OF HIGH MOLECULAR WEIGHT PROTEINS IN SELECTED INDIGENOUS BREAD WHEAT VARIETIES OF PAKISTAN

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Abstract: *Higher molecular weight gluten subunits (HMW-GS) articulate variation in allelic frequency, reflecting a strong correlation with bread quality. In order to determine this quality trait, 23 different Pakistani indigenous wheat varieties were screened that may be used as selection criteria in a breeding program. For detecting variations in HMW-GS, sodium dodecyl sulfate acrylamide gel electrophoresis was used. In all, 77 Glu-1 were identified including 25 at Glu-A1, 26 at Glu-B1 and 26 at Glu-D1. Alleles at Glu-1 were 2* with a frequency of 53.3% and N with 60% frequency at Glu-A1, 6+8 with 60%, 17+18 with 45 % at Glu-B1 and 5+10 with 60% and 72% at Glu-D1. The highest frequency of subunits 20 and 5+10 were found at Glu-B1-e and Glu-D1 (a), followed by a frequency of 2* and Null at Glu-A1 (b, c), which is then followed by 2+12 at GluD-1 (a). The lowest frequency of 13+16, 17+18 and 6+8 were observed at Glu-B1 (f, i, d), respectively. Glu-A1, Glu-B1, and Glu-D1 alleles showed genetic index (i.e. 0.587, 0.54), (0.587. 0.065 and 0.48, 0.39, respectively) with an average of 0.55 and 0.37. The glu-D1 locus has a lower genetic index comparatively. The composition N, 20, 5+10 was found in maximum number (4) in Fakhr-e-Sarhad, Pasban, Pirsabak-2004 and Tatara genotypes, followed by the composition of 2*, 20, 2+12 in one check (Marvi-2000) and two varieties i.e. Chakwal-97 and Bakhtawar-92. The results describing the allelic frequency and composition of bread wheat would benefit further breeding plans for wheat to select good quality parameters.*

Keywords: HMW-GS, Allelic variation, Bread wheat, Protein, Storage protein.

Introduction

Wheat is one of human civilisation's most important food crops, providing about 20% of the total dietary calories and proteins worldwide (FAO, 2023). Wheat has a long history of domestication and cultivation, dating back to 10,000 years ago in the Fertile Crescent. Wheat has many advantages over other crops, such as requiring less water, being adaptable to different climates and soils, and being suitable for processing various food products, such as bread, pasta, noodles, cakes, biscuits and more. Wheat also contains many essential nutrients for human health, such as carbohydrates, fibre, vitamins (especially B vitamins), minerals (such as calcium and iron), and phytochemicals. Wheat fibre helps regulate digestion, lower cholesterol levels, control blood sugar levels, and prevent inflammation and cancer. Wheat is, therefore, a vital crop for global food security and human well-being. Bread wheat (*Triticum et al.*) endosperm comprises 70% starch and 10-15% protein content (Dai *et al*., 2023). Generally, wheat gluten is characterised by two main subunits: glutenin and gliadin. Glutenin is divided into high *Glu-1* and low molecular weight *Glu-3* subunits (Rathan *et al*., 2020). Both glutenin and gliadin (*Gli-1*) locus determine the dough strength of bread wheat (Velu *et al*., 2020). Wheat quality is indicated by the high molecular weight of glutenin subunits (Gupta *et al.*, 1994).

Higher molecular weight gluten subunits (HMW-GS) show variation in allelic frequency, which are supposed to be strongly correlated with differences in bread-making quality (Guzmán *et al.*, 2019). Genetic diversity in wheat can be explored using HMW-GS because they also serve as reliable genetic markers. In order to improve this quality, HMW-GS are subjected to analysis, which acts as a criterion in breeding for quality (Shewry *et al.*, 1995). On the long arm of chromosomes, *1A, 1B*, and 1D genes are present, which encode high molecular weight glutenin and are identified as *Glu-A1, Glu-B1* and *Glu-D1* (Payne *et al.*, 1980).

High molecular weight glutenin subunits are present in the endosperm of wheat and are seed storage proteins (MacRitchie, 1992). These comprise 5-10% of flour protein (Shewry *et al.*, 1992) and about 12% of the total protein content in wheat grains (Payne, 1987; Shewry *et al.*, 1995 & 2002). Electrophoresis was used to check the mobility and size-based separation of high molecular weight glutenin subunits in both bread wheat (Payne et al., 1980) and pasta wheat (Branlard et al., 1989), which exposed substantial polymorphism.

The high molecular weight glutenin subunits may act as carbon and nitrogen sources for the germinating seedlings since they possess a high proportion of nitrogenous amino acids in their primary structures. End-use properties of wheat grains are mainly determined based on these subunits, and extensive studies have been conducted on their role (Payne *et al.*, 1981; Shewry *et al.*, 1995). Gluten strength and end-use quality of grains in tetraploid and hexaploid wheat are affected by the composition of high molecular

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weight glutenin subunits (Payne, 1987; Shewry *et al.*, 1995 & 2002). Genetically, high molecular weight glutenin subunits are encoded by the x and y genes, which are present in the *Glu-1* locus. Owing to the presence of three *Glu-1* loci (*Glu-A1, Glu-B1 and Glu-D1*) and gene silencing, there are usually three to five subunits expressed in individual hexaploid wheat varieties (Payne & Lawrence, 1983; Payne, 1987).

Due to the multidimensional aspect of HMW-GS, the present study was designed to evaluate the traditional wheat varieties that may be used in wheat breeding programmes to enhance the quality and quantity of wheat yield.

Methodology

This study was performed at the Wheat Wide Crosses National Agricultural Research Center from 2022 to 2023. In the experiment, 26 parents were analysed, four of which were used as standards (Dirk et al.).

SDS-PAGE: A single spike was harvested separately from each genotype for SDS-PAGE analysis. A single grain from the spiked sample was crushed to make powder and 10 mg of each was weighed and taken in a microtube. 1 ml of protein extraction buffer (0.05M Tris + 0.2% SDS + 5 M Urea, adjusted to pH 8.0 with HCl) was added to the microtube to extract protein from flour. After a few minutes, 10 µl of mercaptoethanol was added into the microtube and mixed well with the help of a Vortex mixer. The HMW-SG was analysed through slab-type SDS-PAGE using 8% and 12% of polyacrylamide gels without urea. Electrophoresis was run at 200 V until the blue line marker passed through the bottom of the gel plates. Gels were removed from plates and stained with 0.2% (w/v) Coomassie brilliant blue for 20-30 min over a shaker. A 5% methanol solution was prepared using 7.5% acetic acid for de-staining.

Allele Identification: The allelic classification at *Glu-A1* and *Glu-B1* loci and the numbering of HMW-GS were made after Payne & Lawrence (1983). The alleles at the *Glu-D1* locus were identified according to Pena *et al*. (1995) and William *et al*. (1993). Name sources of all the alleles were made by using MacGenes (McIntosh *et al.*, 2008, 2010).

Statistical Analysis: The genetic diversity at each locus was calculated by using Nei's index (Nei, 1973) as follows:

$H = 1 - \Sigma Pi^2$

Where H and Pi denote the genetic variation index and the frequency of the number of alleles at the locus, respectively, allelic frequencies were determined by summing the allelic frequencies in the individual accessions, irrespective of the HMW-GS composition, either homogeneous or heterogeneous and then dividing this total by the number of accessions.

Results & Discussion

Allelic frequency (%) for *Glu1* loci of 23 different Pakistani wheat genotypes is shown in Table 1 and Table 2. In all, 77 *Glu-1* were identified including 25 at *Glu-A1*, 26 at *Glu-B1* and 26 at *Glu-D1*. At *Glu-A1*, frequencies of occurrence of Null, 2* and 1 were 33.3, 53.3 and 13.3% (Table 1) and 0.1, 0.3 and 0.6%, respectively (Table 2). At *Glu-B1*, subunits 7+8, 13+16, 17+18, 20 and 6+8 were found at frequencies of (20, 0.272) (6.66, 0.181) (6.66, 0454) (60, 0.090) and (6.66, 0) respectively (Table 1, 2). At *Glu-D1*, subunits 2+12 and 5+10 frequencies were found to be 40 and 60 (Table 1) and 0.272 and 0.727%, respectively (Table 2). The most prevalent allele was 5+10 with a frequency of 72 and 60% at *Glu-D1*, 2* with a frequency of 53.3% at *Glu-A1*, 6+8 with 60% at *Glu-B1* and 5+10 with 60 % at *Glu-D1* and 45% frequency of 17+18 at *Glu-B1* (Tables 1, 2) (Alhabbar *et al*., 2018a). The highest frequencies of 20 and 5+10 were found at Glu-B1 (e) and Glu-D1 (a), followed by a frequency of 2* at *Glu-A1* (b) and 2+12 at *GluD-1* (a). The lowest frequency of 13+16, 17+18 and 6+8 were observed at *Glu-B1* (f, i, d), respectively (Battenfield *et al*., 2016). The scarcity of the 6+8 subunit is advantageous because it has been associated with poor bread-making quality (Payne *et al.*, 1987; Dong *et al.*, 19991). The frequency of the 2+12 allele at *Glu-D1* is comparable with that already reported in several studies (Nakamura *et al.*, 2000; Branlard *et al.*, 2003).

The genetic index of the three loci was H=0.55 and 0.37 (Table 1 & 2). The genetic index for *Glu-A1*, *Glu-B1* and *Glu-D1* were (0.587, 0.54), (0.587, 0.0657) and (0.48, 0.3976), respectively. In the case of the *Glu-D1* locus, the same was lowered compared to other loci. Subunits 5+10 were identified at this locus in more than 60% of cultivars. The allelic subunit composition of varieties differs (Table 3). The composition N, 20, 5+10 was found in a maximum number (5) of genotypes such as Fakhr-e-Sarhad, Pasban, Pirsabak-2004, Tatara and Bahawalpur-97, followed by the allelic composition 2*, 17+18 and 5+10 in three wheat varieties (Manthar-2003 et al.). Similar number of varieties (i.e. Marvi-2000, Chakwal-97 and Bakhtawar-92) showed the composition of 2*, 20, 5+10

Table 1. Allelic frequency (%) for *Glu1* loci of 15 different Pakistani wheat genotypes

Locus	Alleles	Subunit composition	Frequency	Relative frequency pi= Frequency/Total frequency	$(pi)^2$	Genetic Variation/Diversity $H=1-\sum (pi)^2$	Genetic variation mean (Average of H)
Glu-A1	A	1	2	0.133333	0.017778	0.5866	0.5511
	B	2*	8	0.533333	0.284444		
	C	N	5	0.333333	0.111111		
Glu-B1	B	7+8	3	0.2	0.04	0.5866	
	F	13+16	1	0.066667	0.004444		
	I	17+18	1	0.066667	0.004444		
	E	20	9	0.6	0.36		
	D	6+8	1	0.066667	0.004444		
Glu-D1	A	2+12	6	0.4	0.16	0.48	
	D	5+10	9	0.6	0.36		

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Table 2. Allelic frequency (%) for *Glu1* loci of 11 different Pakistani wheat genotypes

Locus	Alleles	Subunit composition	Frequency	Relative frequency pi= Frequency/Total frequency	$(pi)^2$	Genetic Variation/Diversity $H=1-\sum (pi)^2$	Genetic variation mean (Average of H)
Glu-A1	A	1	1	0.1	0.01	0.54	
	B	2*	3	0.3	0.09		
	C	N	6	0.6	0.36		
Glu-B1	B	7+8	3	0.272	0.0739	0.0657	0.327
	F	13+16	2	0.181	0.327		
	I	17+18	5	0.454	0.206		
	E	20	1	0.090	0.09		
	D	6+8	0	0	0		
Glu-D1	A	2+12	3	0.272	0.0739	0.3976	
	D	5+10	8	0.727	0.5285		

Table 3. Subunit composition for 23 Pakistani wheat varieties along with standards (E-1-52).

Allelic composition			Variety Name
Glu-A1	**Glu-B1**	**Glu-D1**	
N	6+8	2+12	E-1-52 (Standard)
N	20	5+10	Fakhr-e- Sarhad, Pasban, Pirsabak-2004, Tatara, Bahawalpur-97
N	7+8	2+12	Chinese Spring
N	7+8	5+10	Fareed -2006
N	13+16	5+10	Inqilab-91
N	13+16	2+12	Sehr-2006
N	17+18	5+10	Miraj-2008
2*	7+8	5+10	Shafaq, Marvi-2000
2*	13+16	5+10	Dirk (Check)
2*	7+8	2+12	Zam-2009
2*	20	5+10	Marvi-2000 (Check), Chakwal-97, Bakhtawar-92
2*	20	2+12	Khyber-87, Daman-98
2*	17+18	5+10	Manthar-2003, Dirk and Punjnad-2001
1	7+8	2+12	NARC-2009
1	17+18	2+12	Chanab-2000 (Check)

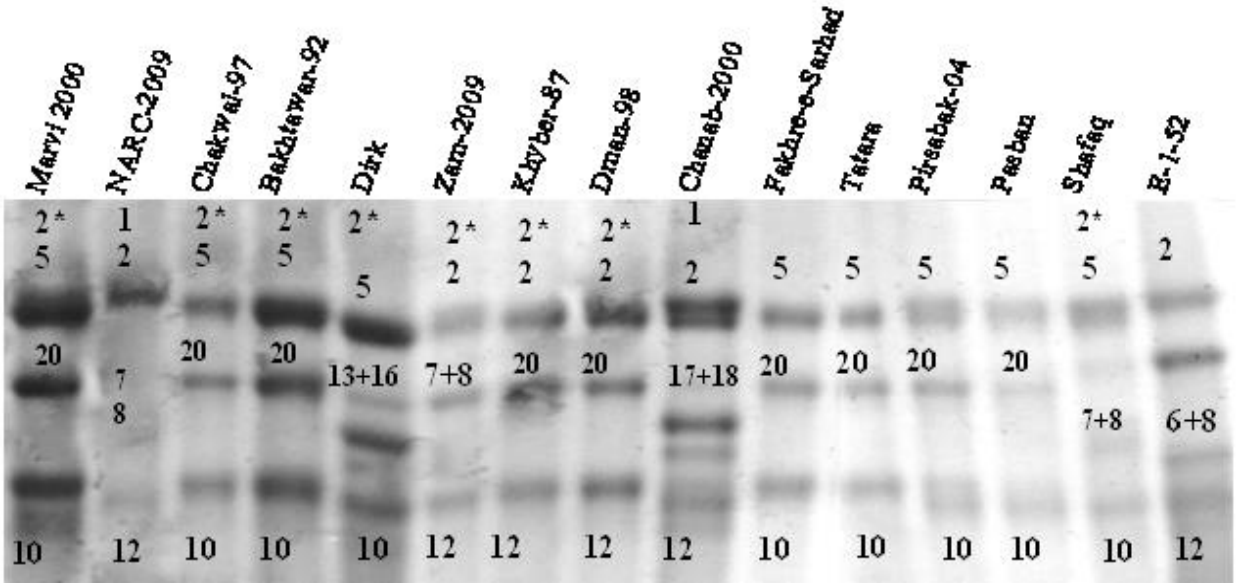

Fig.1. HMW-GS profile. (From left) 1=Marvi-2000 (Check), 2= NARC-2009, 3= Chakwal-97, 4= Bakhtawar-92, 5= Dirk (Check), 6= Zam-2009, 7= Khyber-87, 8= Daman-98, 9= Channab-2000 (Check), 10= Fakhre-e-Sarhad, 11= Tatara, 12= Pirsabak-2004, 13= Pasban, 14= Shafaq and 15= E-1-52 (Check)

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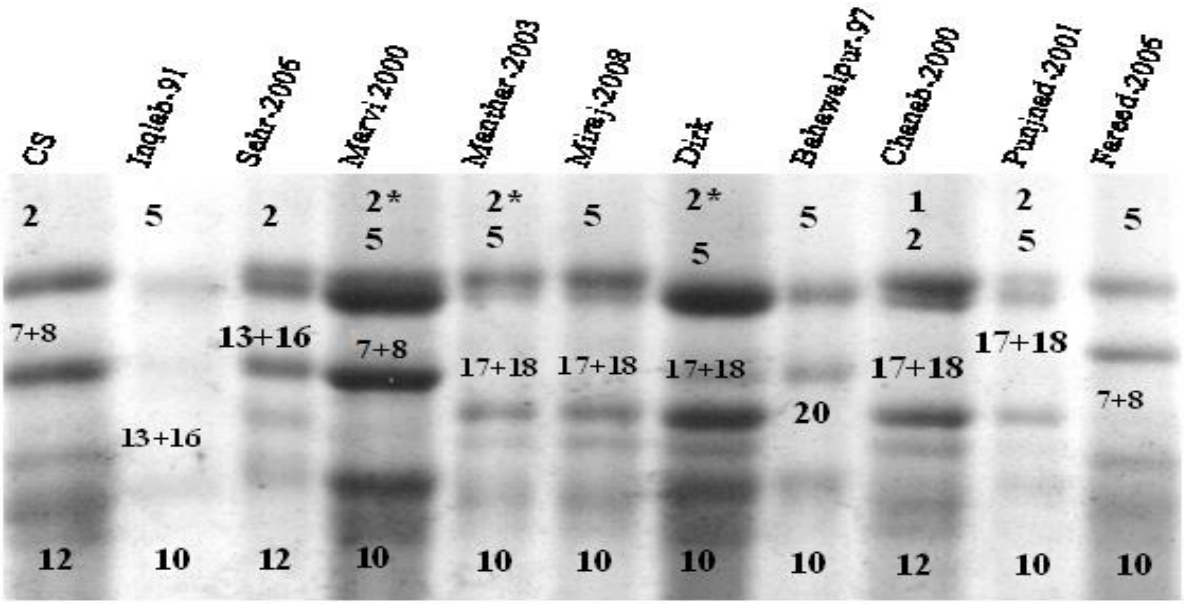

Fig.2. HMW-GS profile. (From left) 1= Chinese Spring, 2= Inqlab-91, 3= Sehr-2006, 4= Marvi-2000, 5= Manthar 2003), 6= Miraj-2008, 7= Dirk (Check), 8= Bahawalpur-97, 9= Channab-2000 (Check), 10= Punjnad-2001, 11= Fareed-2006.

Other allelic composition was depicted by different varieties e.g. E-1-52 (N, 6+18, 2+12), Shafaq and Marvi -2000 (2*, 7+8, 5+10), Dirk (2*, 13+16, 5+10), Zam-2009 (2*, 7+8, 2+12), NARC-2009 (1, 7+8, 2+12) and Chanab-2000 (1, 17+18, 2+12). Locus, linked to some genes having agronomic importance, is greatly influenced by environmental adaptation, which might be the reason for the relatively low variability found at the *Glu-B1 (*McIntosh *et al.,* 1998). It has been shown that specific alleles, such as 5+10, have a positive influence, whereas others, such as Null, hurt dough strength and bread-making quality (Payne *et al.,* 1987). The alleles 7+8 at *Glu-B1* were present in Shafaq, Zam-2009, and NARC-2009, and subunits 17+18 and 18+16 were present in control varieties (i.e. Chanab-2000 and Dirk), respectively that are associated with good bread-making quality and are responsible for the medium-quality scores. The subunits 2+12 and 5+10 are highly correlated with weak and robust glutens, respectively (Johansson *et al.,* 1994), and various genotypes through environment interactions (Blumenthal *et al.*, 1995).

Since the advent of human civilisation, food quality has been an immense desire and need of humans, with the main focus on wheat. Moreover, since then, efforts have been made to improve wheat's processing and bread-making quality through the exploitation of genetic variability. With the importance of *Glu-D1* encoded proteins, the breeding becomes more precise for quality improvement in wheat (Lagudah *et al.*, 1987; William *et al.*, 1993). In our study, we have identified good quality enhancing HMW-GS having different genetic backgrounds with superior combinations from indigenous wheat varieties, which can be used as promising breeding material for crop improvement.

Conclusion

Evaluating these varieties effectively determines the quality of the alleles and allelic combinations. This will be helpful and useful when selecting local varieties with better and improved quality. It could also be used as a genetic source to breed new, improved-quality lines.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department Concerned.
Consent for publication
Approved
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Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

References

Alhabbar, Z., Yang, R., Juhasz, A., Xin, H., She, M., Anwar, M., et al. (2018a). NAM gene allelic composition and its relation to grain-filling duration and nitrogen utilisation efficiency of Australian wheat. *PLoS ONE* 13:e0205448. Doi: 10.1371/journal.pone.0205448

Battenfield, S. D., Guzmán, C., Gaynor, R. C., Singh, R. P., Peña, R. J., Dreisigacker, S., et al. (2016). Genomic selection for processing and end-use quality traits in the CIMMYT spring bread wheat breeding program. Plant Genome 9, 1–2. doi: 10.3835/plantgenome2016.01.0005

Branlard, G., J. C. Autran , P. Monneveux. 1989. High molecular weight glutenin subunits in durum wheat *(Triticum durum*). *Theor. Appl. Genet.*, 78: 353-358.

Branlard, G., M. Dardevet, N. Amiour and G. Igrejas. 2003. Allelic diversity of HMW and LMW glutenin subunits and omega-gliadins in French bread wheat (Triticum et al.). Genet. Resour. & Crop Evol., 50: 669-679.

Blumenthal, C., P.W. Grass, F. Bekes, E.W.R. Barlow, C.W. Wrigley. 1995. A possible role for the Glu-D1 locus concerning tolerance to dough-quality change after heat stress. *Cereal Chem.*, 72:135-136.

Dai Y, Li J, Shi J, Gao Y, Ma H, Wang Y, Ma H. Molecular Characterization and Marker Development of the *HMW-GS* Gene from *Thinopyrum elongatum* for Improving Wheat Quality. Int J Mol Sci. 2023 Jul 4;24(13):11072.

Dong, H., T.S. Cox, R.S. Sears, G.L. Lockhart. 1991. High molecular weight glutenin genes: effect on quality in wheat. *Crop Sci.*, 31:974-979.

FAO. 2023. Agribusiness handbook: Wheat flour. Rome. (Available at http:// www.fao.org/docrep/012/al376e/al376e.pdf).

Gupta, R.B., J.G. Paul, G.B. Cornish, G.A. Palmer, F, Bekes and A.J. Rathjen. 1994. Allelic variation at glutenin subunit and gliadin loci, Glu-1, Glu-3 and Gli-1 of common wheat. *J. Cereal. Sci*., 19: 9-17.

Guzmán, C., Ammar, K., Velu, G., and Singh, R. P. (2019). Genetic improvement of wheat grain quality at CIMMYT. *Front. Agr. Sci. Eng.* 6:260. doi: 10.15302/J-FASE-2019260.

He, Z. H., R. J. Pena., S. Rajaram. 1992. High Molecular weight glutenin subunit composition of Chinese bread wheat. *Euphytica*, 64: 11-20.

Johansson, E. G. Svensson, W.K. Heneen. 1994. Quality evaluation of the high molecular weight glutenin subunits in Swedish wheat material. In: *Proceedings of 5th international workshop gluten proteins*, Association of Cereal Research, Dermold, Germany. 568-575.

Lagudah, E. S., F. MacRitchie, G. M. Halloran. 1987. The influence of high-molecular-weight glutenin from *Triticum tauschii* on synthetic hexaploid wheat flour quality. *J. Cereal Sci.*, 5:129-138.

MacRitchie, F. 1994. Role of Polymeric Proteins in Flour Functionality. In: *Wheat kernel proteins: molecular and functional aspects*. Bitervo, Italy, Universita degli studi Della Tuscia. 145-150.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, D. Somers, R. Appels and K. Devos. 2008. Catalogue of Gene Symbols for Wheat. In: *11th International Wheat Genetics Symposium*. Brisbane Qld Australia. Pp.?

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, F. Morris, D.J. Somers, R. Appels and K.M. Devos. 2010. *Catalogue of gene symbols for wheat*. MacGene.http://www.shigen.nig.ac.jp/wheat/komugi /genes/download.jsp.

Nei, M. 1973. Analysis of gene diversity in a subdivided population. Proceedings of the National Academy of Sciences of the USA. 70: 3321-3323.

Nakamura, H. 2000. The association between high-molecular-weight glutenin subunit composition and Chinese and Japanese hexaploid wheat bread-making quality. *Austr. J. Agricul. Res.*, 51: 371-375.

Payne, P. I., C. N. Law and E. E. Mudd. 1980. Control by homoeologous Group 1 chromosome of the high-molecular-weight glutenin subunits, a significant wheat endosperm protein. *Theor. Appl. Genet.*, 58: 113-120.

Payne, P. I., K.G. Corfield, L. M. Holt and J. A. Blackman. 1981. Correlation between the inheritance of specific high-molecular-weight glutenin subunits and bread-making quality in progenies of six crosses of bread wheat. *J. Sci. Food. Agric.*, 32: 51-60.

Payne, P. I. and G. J. Lawrence, 1983. Catalogue of alleles for the complex gene loci GluA1, GluB1, and GluD1, which code for high-molecular-weight glutenin subunits in hexaploid wheat. *J. Cereal Res. Communications*, 11: 29-35.

Payne, P.I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annu. Rev. Plant Physiol.*, 38:141-153.

Pena, R.J., J. Zarco-Hernandez and A. Mujeeb-Kazi (1995) Glutenin subunit compositions and bread making quality characteristics of synthetic hexaploid wheat derived from Triticum turgidum × Triticum tauschii (Coss.) Schmal Crosses. *J. Cereal Sci.*, 21: 15–23.

Rathan ND, Mahendru-Singh A, Govindan V and Ibba MI. 2020. Impact of High and Low-Molecular-Weight Glutenins on the Processing Quality of a Set of Biofortified Common Wheat (*Triticum aestivum* L.) Lines. *Front. Sustain—food Syst.* 4:583367. doi: 10.3389/fsufs.2020.583367.

Shewry, P. R., A. S. Tatham, P. Barro and P. Lazzeri. 1995. Biotechnology of breadmaking, unravelling and manipulating the multi-protein gluten complex. *Biotechnology*, 13:1185-1190.

Shewry, P. R., N. G. Halford, P. S. Belton and A. S. Tatham. 2002. The structure and properties of gluten, an elastic protein from wheat grain. *Phil. Trans. R. Soc. Lond. B.*, 357:133-142.

Shewry, P. R., N. G. Halford and A. S. Tatham. 1992. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.*, 15: 105-120.William, M.D.H.M., R.J. Pena, A. Mujeeb-Kazi. 1993. Seed protein and isozyme variations in *Triticum tauschii* (*Aegilops squarossa*). *Theor. Appl. Genet*., 87: 257-263.

Velu, G., Crespo-Herrera, L., Guzmán, C., Huerta, J., Payne, T., and Singh, R. P. (2019). We assess genetic diversity to breed competitive biofortified wheat with enhanced grain Zn and Fe concentrations. *Front. Plant Sci.* 9:1971. doi: 10.3389/fpls.2018.01971.

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