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IDENTIFICATION OF TECHNOLOGICALLY IMPORTANT GENES AND THEIR PRODUCTS IN THE COLLECTION OF BREAD WHEAT GENOTYPES

Milan Chňapek, Renata Peroutková, Martin Vivodík, Zdenka Gálová*

Address(es): Ing., PhD. Milan Chňapek,
Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, phone number: +421376414277

*Corresponding author: chnapek.m@gmail.com

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ABSTRACT

Wheat is the second most cultivated crop on the world and is very important plant for feed not only mankind but also animals. Because of this is necessary to develop new varieties with better properties. Bread making quality of wheat grain is one of the most important paramaters for quality evaluation. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of wheat storage proteins and allelic specific polymerase chain reaction (AS-PCR) are analysis suitable for identification, differentiation and characterization of bread wheat (*Triticum aestivum* L.). There were analysed 16 genotypes of new varieties of bread wheat in our work by SDS-PAGE and obtained results were verified by AS-PCR. Analysed genotypes of bread wheat genotypes were homogenous and single line with very good bread making quality. Our results confirmed hypothesis, that cultivated bread wheat genotypes are uniformed with high production and quality but there is a risk of sensitivity to environmental conditions. SDS-PAGE analyses of wheat grain proteins are fast and not very expensive technique, which provide us information of bread making quality of grains. However, there is possibility of environmental influence on protein synthesis and because of this is necessary to couple these analysis with analysis of DNA.

Keywords: AS-PCR, Bread wheat, HMW-GS, SDS-PAGE, technological quality

INTRODUCTION

Current annual global production of bread wheat (*Triticum aestivum* L.) is over 860 million tonnes providing approximately one-fifth of the total calorific input of the world population which means, that bread wheat is one of the most important food crops. Important bread wheat quality determinants are storage proteins, because they are responsible for dough elasticity and extensibility, and thus for determining the processing qualities in the production of a range end-products (**Ma *et al.*, 2009)**. Selection efficiency of breeding materials with desirable genes has been dramatically improved by development and utilization of functional markers or gene-specific markers for high molecular weight glutenin subunit (HMW-GS) (**Liu *et al.*, 2012)**. HMW-GS represent about 12% of the total seed storage protein corresponding for around 1,0–1,7% of the flour dry weight. Genes coding HMW-GS are located at *Glu-1* loci on the long arms of homoeologous group 1 chromosomes are named as *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively (**Payne *et al.*, 1980)**. There are two subunits of different size, called x-type and y-type subunits (e.g., 1Ax and 1Ay), with higher and lower molecular weights produces by each locus. 1Bx, 1Dx and 1Dy subunits are expressed in all bread wheat cultivars, while some cultivars express 1By and 1Ax subunits as well. The gene encoding the 1Ay subunits usually remains silent in bread and durum wheat because of wheat domestication syndrome (**McIntosh *et al.*, 2013)**. The complete coding sequences of 10 HMW-GS alleles including Ax1, Ax2*, the silent Ay subunit, Bx7, Bx14, Bx17, By9, Dx2, Dx5, Dy10, and Dy12 are known (**Forde *et al.*, 1985; Halford *et al.*, 1987, 1992**). This is very important point for breeding program to predict and to design the primers to sequence the other alleles used in breeding programs. Widely used method for HMW-GS diagnosis is sodium dodecyl sufate poly-acrylamide gel electrophoresis (SDS-PAGE). The main limitations of SDS-PAGE are overestimation of molecular mass and low resolution, but it is still the cheapest and simplest technique suitable for high-throughput and large-scale screening of HMW-GS for wheat genotypes in breeding programs (**Gao *et al.*, 2010)**. Utilization of specific PCR markers has enabled protein-based limitations of HMW glutenin allele resolution to overcome. These markers development is based on DNA polymorphisms among the glutenin subunit genes and once available they can be considered as perfect or functional markers for HMW-GS alleles. High-throughput capability for assessing different alleles in breeding materials and possibility of genotyping during the vegetative growth stages is the major advantages. Allele-specific PCR (AS-PCR) markers provide us fast and very efficient tool for screening desirable HMW-GS for maintenance and improvement of quality parameters in breeding programs (**Liu *et al.*, 2008)**. The objective of our study was utilization of AS-PCR markers for detection of genes and SDS-PAGE for detection of proteins with desirable properties influenced technological quality of wheat.

MATERIAL AND METHODS

Material

We have analyzed 16 genotypes of bread wheat (*Triticum aestivum* L.): IS Ezopus, Faustina, Gallus, IS Median, Arnold, Impulsiv, Midas, Helmut, IS Apage, IS Jarisa, Fulvio, Cornelius, Blasius, Pannonikus, IS Questor, Lukullus.

Methods

Analysis of DNA

Sample preparation
DNA was isolated from 14 days old young leaves by Genomic DNA Purification Kit (**Thermo Scientific**). Sufficiency of DNA isolation was confirmed by horizontal agarose gel electrophoresis in Tris-borate buffer and by BioSpec-nano Micro-volume UV-Vis Spectrophotometer (Shimadzu).

PCR analysis
Polymerase chain reaction was performed in C1000 Touch™ Thermal Cycler (**Bio-Rad**) according to several protocols. Multi Ax2*, Bx7-17 and Dx5 genes were analysed according to **Ma *et al.*, 2003**. Ax1-2* and Dx5-Dy10 genes were detected by **D´Ovidio *et al*. (1994)** protocol.
Amplified products were visualised by 1,2% horizontal agarose gel electrophoresis in Tris-borate buffer.

Analysis of proteins

Sample preparation
Proteins were extracted from individual grains according to standard ISTA method (**Wrigley *et al.*, 1992**). Seed storage proteins were isolated from whole, dry and mature grains. There were analysed 20 individual grains from each genotype.

Electrophoretical separation of proteins in SDS-PAGE

Seed storage proteins separation was realized in vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe. SDS polyacrylamide gel was used as a separation medium according to standard ISTA method (**Wrigley *et al.*, 1992**). Volume 5µl of each sample was loaded into gel. Seed storage proteins separation was running for 20 hours with constant current 10 mA.

Gel staining and image analysis

Protein fraction of separated seed storage proteins were stained in solution of Commasie Brilliant Blue R 250 in ethanol and 10% TCA. Image analysis of SDS-PAGE gels was carried out using DocIt-LS software (Ultraviolet Products) using an automated process supplemented with occasional manual adjustments. Each lane was first defined where the average intensity across the width of these lanes was depicted as a function of the distance in pixels from the top of the image. The background was subtracted from each profile (parameters adjusted on a case-by-case basis), after which the bands were identified. The DocIt-LS software was also used for statistical interpretation of the eletrophoreographs.

RESULTS AND DISCUSSION

High molecular weight glutenin subunits (HMW-GS) were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (**Wrigley, *et al.*, 1992).** Agarose electrophoresis were used for detection of genes coded HMW-GS. There were analysed 16 new genotypes of bread wheat (*Triticum aestivum* L.). Electrophoretical profile of wheat genotypes Marquis and Chinese Spring were used as standards for identification of electrophoretic spectra. All analysed genotypes were homogenous and single lines (Figure 1), (Table 1).

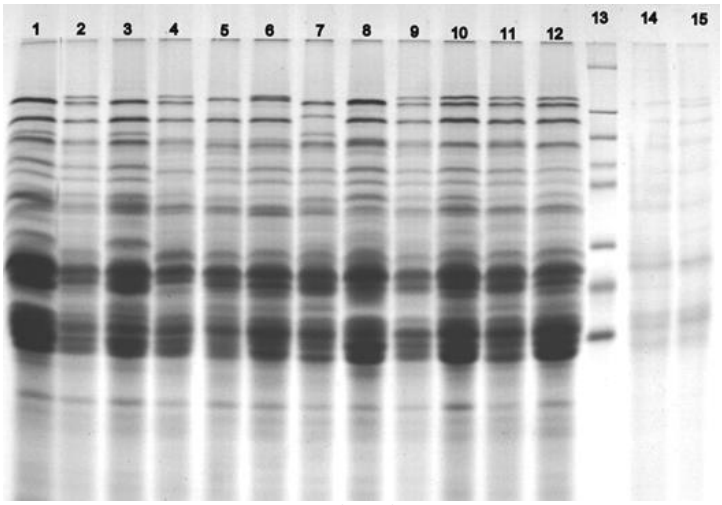

Figure 1 Protein electrophoretic profile of bread wheat genotypes (*Triticum aestivum* L.): 1 = IS Apage; 2 = IS Ezopus; 3 = IS Median; 4 = IS Questor; 5 = Faustina; 6 = Blasius; 7 = Helmut; 8 = Arnold; 9 = Cornelius; 10 = Fulvio; 11 = Gallus; 12 = Impulsiv; 13 = Molecular Marker (SigmaMarker™ wide range, mol wt 6,500-200,000 Da) ; 14 = Wheat standard – Marquis; 15 = Wheat standard – Chinese Spring;

Table 1 HMW-GS composition, allele presence and Glu-score calculation of individual bread wheat genotype

Name	Proteins			Genes					Glu score
	HMW-GS			Glu-A1 loci		Glu-B1 loci		Glu-D1 loci	
	Glu-A1	Glu-B1	Glu-D1	Ax1/null	Ax2*	Bx7H7	Bx7-17	Dx5	
IS EZOPUS	1	7+9	5+10	+	-	+	+	+	9
FAUSTINA	0	7+9	2+12	+	-	+	+	-	5
GALLUS	1	7+9	5+10	+	-	+	+	+	9
IS MEDIAN	0	7+8	5+10	+	-	+	+	+	8
ARNOLD	2*	7+9	5+10	-	+	+	+	+	9
IMPULSIV	1	7+9	5+10	+	-	+	+	+	9
MIDAS	1	7+9	5+10	+	-	+	+	+	9
HELMUT	0	6+8	5+10	+	-	-	-	+	6
IS APAGE	2*	7+8	5+10	-	+	+	+	+	10
IS JARISA	1	7+9	5+10	+	-	+	+	+	9
FULVIO	1	7+9	5+10	+	-	+	+	+	9
CORNELIUS	1	7+9	5+10	+	-	+	+	+	9
BLASIUS	1	7+9	2+12	+	-	+	+	-	7
PANNONIKUS	0	7+9	5+10	+	-	+	+	+	7
IS QUESTOR	1	7+9	5+10	+	-	+	+	+	9
LUKULLUS	1	7+9	5+10	+	-	+	+	+	9

There were observed 8 electrophoretic profiles. The most frequent HMW-GS composition were 1; 7+9; 5+10 (Figure 2) detected in 9 genotypes (56%). Allelic polymorphism on Glu-A1 loci is main factor of wheat flour qualitative parameters. Alleles 1 and 2* located on Glu-A1 loci influenced technological quality more positively than null allele. Glu-B1 loci may contain HMW-GS allele with good technological quality (7+8, 7+9) or allele with negative effect (6+8 or 6+7). The highest impact on wheat technological quality was investigated within the alleles coded by Glu-D1 loci and 5+10 allele is the most valuable (**Dumur, *et al.*, 2010).**

Electrophoretic analysis of individual genotypes of bread wheat confirmed (Figure 3) presence of 3 alleles null, 1 and 2* coded by Glu-A1 loci. The most frequent was allele 1 which was observed in 67% of genotypes. Glu-B1 loci showed presence of 4 alleles (6+8, 7+8 and 7+9) with dominancy of allele 7+9 which was detected in 78% of samples. Analysed genotypes of bread wheat showed presence of 2 combination of allele (5+10, 2+12) and allele 5+10 was the dominating one (83%).

Protein spectrums of individual genotypes evaluated by SDS-PAGE were confirmed by AS-PCR analysis of bread wheat collection. There were used 5 pairs of AS-PCR primers (Figure 4-8). Two sets of primers were used for detection of gene coded HMW-GS 7 localized on Glu-B1 loci. HMW-GS null and 1 were monitored by one pair of primer. Glu-A1 loci coded not only null and 1 allele, but also allele 2* which were evaluated by one pair of primer. Gene of HMW-GS 5 is localized on Glu-D1 loci were detected by one pair of primer. PCR detection of genes coded specific HMW-GS confirmed presence of HMW-GS determined by protein analysis.

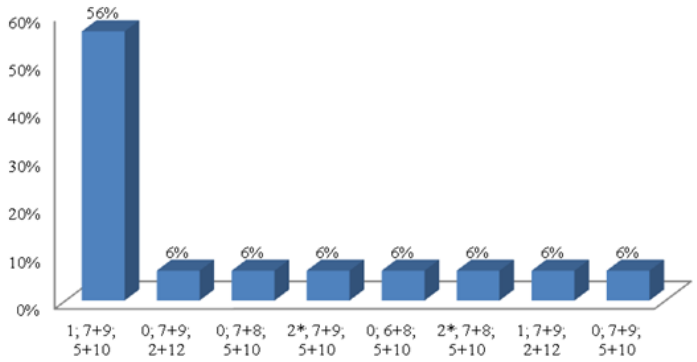

Figure 2 Frequency of HMW-GS composition of individual bread wheat

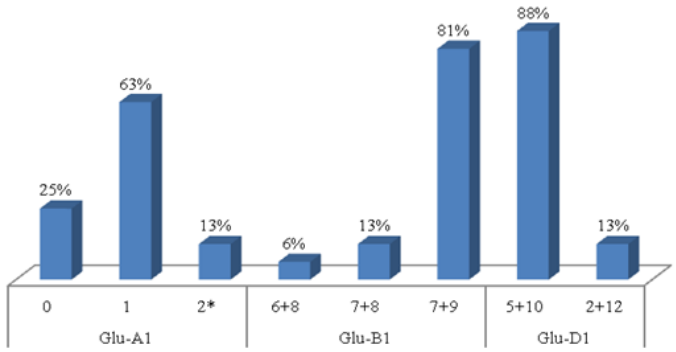

Figure 3 Allelic compositions of individual Glu loci

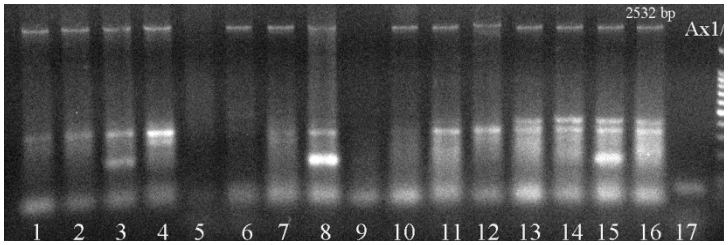

Figure 4 AS-PCR detection of gene coding Ax1 and null alleles: 1= IS Ezopus; 2=Faustina; 3=Gallus; 4=IS Median; 5=Arnold; 6=Impulsiv; 7=Midas; 8=Helmut; 9=IS Apage; 10=IS Jarisa; 11=Fulvio; 12=Cornelius; 13=Blasius; 14=Pannonikus; 15=IS Questor; 16=Lukullus; 17=negative control; 18=molecular marker

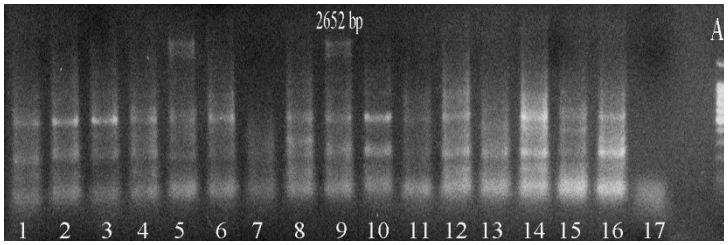

Figure 5 AS-PCR detection of gene coding Ax2* allele: 1= IS Ezopus; 2=Faustina; 3=Gallus; 4=IS Median; 5=Arnold; 6=Impulsiv; 7=Midas; 8=Helmut; 9=IS Apage; 10=IS Jarisa; 11=Fulvio; 12=Cornelius; 13=Blasius; 14=Pannonikus; 15=IS Questor; 16=Lukullus; 17=negative control; 18=molecular marker

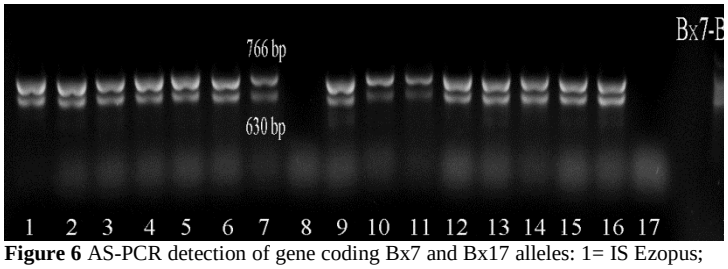

Figure 6 AS-PCR detection of gene coding Bx7 and Bx17 alleles: 1= IS Ezopus; 2=Faustina; 3=Gallus; 4=IS Median; 5=Arnold; 6=Impulsiv; 7=Midas; 8=Helmut; 9=IS Apage; 10=IS Jarisa; 11=Fulvio; 12=Cornelius; 13=Blasius; 14=Pannonikus; 15=IS Questor; 16=Lukullus; 17=negative control; 18=molecular marker

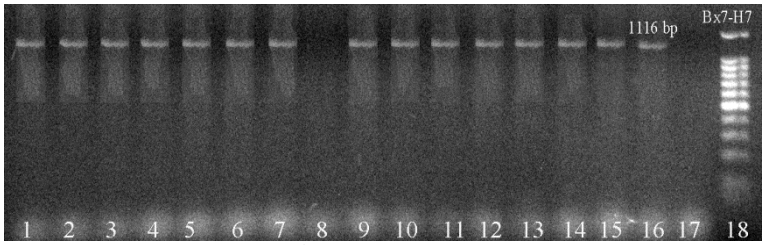

Figure 7 AS-PCR detection of gene coding Bx7 allele: 1= IS Ezopus; 2=Faustina; 3=Gallus; 4=IS Median; 5=Arnold; 6=Impulsiv; 7=Midas; 8=Helmut; 9=IS Apage; 10=IS Jarisa; 11=Fulvio; 12=Cornelius; 13=Blasius; 14=Pannonikus; 15=IS Questor; 16=Lukullus; 17=negative control; 18=molecular marker

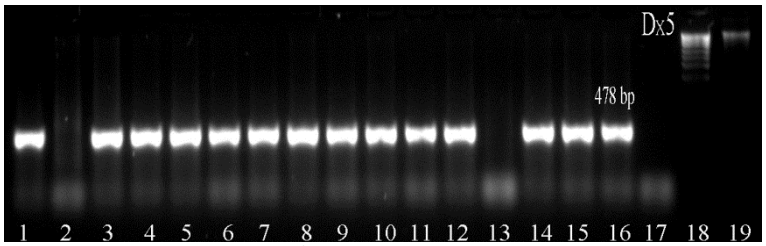

Figure 8 AS-PCR detection of gene coding Dx5 allele: 1= IS Ezopus; 2=Faustina; 3=Gallus; 4=IS Median; 5=Arnold; 6=Impulsiv; 7=Midas; 8=Helmut; 9=IS Apage; 10=IS Jarisa; 11=Fulvio; 12=Cornelius; 13=Blasius; 14=Pannonikus; 15=IS Questor; 16=Lukullus; 17=negative control; 18=molecular marker; 19=positive control

Wheat technological quality is influenced by presence or absence of specific HMW-GS. Bread making quality of wheat depends on Glu-score of individual wheat genotypes. Glu-score is calculated according to composition of individual alleles coded by A1, B1 and D1 loci of analysed bread wheat genotypes. **Payne, *et al.*, (1987)** analysed collection of bread wheat genotypes and evaluated individual alleles by points depends on their contribution to bread making properties of selected genotype. Wheat genotypes with Glu-score more than 7 have good technological quality. Electrophoretic separation of HMW-GS and analysis of genes by PCR provide identification of allele on individual loci and calculation of Glu-score (Table 1). The best technological quality on the basis of Glu-score calculation showed genotype IS Apage. Genotype Faustina reached the worst Glu-score value (5) and together with genotype Helmut (Glu-score 6) is not recommended for bread making. Genotypes IS Questor, Arnold, Cornelius, Fulvio, Gallus, Impulsiv, Lukullus and Midas with Glu-score 9 have very good technological quality.

CONCLUSION

Utilization of SDS-PAGE analysis of bread wheat protein as well as AS-PCR analysis is useful tools for identification, differentiation and characterization of some technological important properties. Our results showed that bread wheat genotypes are homogenous and single lines and they have very good bread making quality, in generally. SDS-PAGE analyses of wheat grain proteins are fast and not very expensive technique, which provide us information of bread making quality of grains. However, there is possibility of environmental influence on protein synthesis and because of this is necessary to couple these analysis with analysis of DNA.

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REFERENCES

D' OVIDIO, R., ANDERSON, O.D. 1994. PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread making quality. *Theor. Appl. Genet.*, 88, 759-763. http://dx.doi.org/10.1007/bf01253982

DUMUR, J., JAHIER, J., DARDEVET, M. 2010. Effects of the replacement of Glu-A1 by Glu-D1 locus on agronomic performance and bread-making quality of the hexaploid wheat cv. Courtot. *Journal of Cereal Science*, 51, 175-181. http://dx.doi.org/10.1016/j.jcs.2009.11.009

FORDE, J., MALPICA, J.M., HALFORD, N.G., SHEWRY, P.R., ANDERSON, O.D., GREENE, F.C.,MIFLIN, B.J. 1985. The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1A of wheat (*Triticum aestivum* L.). *Nucleic Acids Res*., 13, 6817-6832. http://dx.doi.org/10.1093/nar/13.19.6817

HALFORD, N.G., FORDE, J., ANDERSON, O.D., GREENE, F.C., SHEWRY, P.R. 1987. The nucleotide and deduced amino acid sequence of an HMW glutenin subunit gene from chromosome 1B of bread wheat (*Triticum aestivum* L.) and comparison with those of genes from chromosomes 1A and 1D. *Theor. Appl. Genet*., 75, 117-126. http://dx.doi.org/10.1007/bf00249151

HALFORD, N.G., FIELD, J.M., BLAIR, H., URWIN, P., MOORE, K., ROBERT, L., THOMPSON, R., FLAVELL, R.B., TATHAM, A.S., SHEWRY, P.R. 1992. Analysis of HMW glutenin subunits encoded by chromosome 1A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality. *Theor. Appl. Genet*., 83, 373-378. http://dx.doi.org/10.1007/bf00224285

MA, W., ZHANG, W., GALE, K.R. 2003. Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. *Euphytica* 134, 51-60.

MA, W., ANDERSON, O., KUCHEL, H., BONNARDEAUX, Y., COLLINS, H., MORELL, M.K., LANGRIDGE, P., APPELS, R. 2009. Genomics of quality traits. Feuillet, C., Muehlbauer, G.J. (Eds.), Genetics and Genomics of the Triticeae. Springer, New York, 611-652. http://dx.doi.org/10.1007/978-0-387-77489-3_21

McINTOSH, R., DUBCOVSKY, J., ROGERS, J., MORRIS, C., APPELS, R., XIA, X.C. 2013. Catalogue of gene symbols for wheat. 12th *International Wheat Genetics Symposium*, Yokohama, Japan.

LIU, S.X., CHAO, S.M., ANDERSON, J.A. 2008. New DNA markers for high molecular weight glutenin subunits in wheat. *Theor. Appl. Genet*., 118, 177-183. http://dx.doi.org/10.1007/s00122-008-0886-0

LIU, Y., HE, Z.H., APPELS, R., XIA, X.C. 2012. Functional markers in wheat: current status and future prospects. Theor. Appl. Genet. 125, 1e10. http://dx.doi.org/10.1007/s00122-012-1829-3

PAYNE, P. I. - LAWRENCE, G. J. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, Glu-D1, which code for high molecular weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun*., 1983, 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. http://dx.doi.org/10.1146/annurev.pp.38.060187.001041

WRIGLEY, D. 1996. Giant proteins with flour power. *Nature*, 381, 738-739. http://dx.doi.org/10.1038/381738a0