Influence of the Year and HMW Glutenin Subunits on End-use Quality Predictors of Bread Wheat Waxy Lines

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The effects of environment and the high molecular weight glutenins on some quality properties (sedimentation volume, % protein content, and starch pasting viscosity) of bread wheat mutant waxy lines were evaluated. Thirty eight 100% amylose-free F_2 derived F_6 and F_7 lines were used. The results indicated that the environment did not influence sedimentation volume, mixograph parameters and starch viscosity parameters of waxy flour. Variation in the % protein content was determined mainly by the environment. The sedimentation volume and the mixograph peak development time were influenced by the variation at over expression of Bx7 and the mixograph peak development time was influenced by the *Glu-D1* locus. One starch viscosity parameter, time to peak viscosity, was influenced by variation at the *Glu-A1* locus. This parameter is significantly lower in the waxy lines than the parent line, which shows the influence of the waxy loci. No significant correlation was observed for sedimentation volume, mixograph parameters, protein content and viscosity parameters of waxy lines.

Keywords: *Triticum aestivum;* bread wheat quality; starch viscosity

Introduction

In the majority of bread wheat varieties (*Triticum aestivum* L. genome AABBDD), the endosperm starch is composed of the polysaccharides amylose and amylopectin in the approximate ratio of 25:75% (Preiss 1991). Amylose has a linear structure while amylopectin is branched. Amylose is synthesized mainly through the action of the enzyme "granule-bound starch synthase"(GBSS) (Echt and Schwartz 1981) which is coded by the *Waxy* loci (*Wx*). These loci are located on the short arms of chromosomes 7A and 7D and on the long arm of chromosome 4A, as a result of translocation from chromosome 7B (Naranjo et al. 1987). Their products are three waxy isoenzymes which may be separated by electrophoresis (Nakamura et al. 1993).

Some wheat varieties have been found to have low amylose content and it has been observed from electrophoretic gels that one or more of these GBSS isoenzymes are not expressed. These varieties are referred to as "partial waxy mutants". It has also been observed that there is a relationship between the physico-chemical properties of starch from bread wheat and their content of amylose. In the case of wheats whose amylose content is close to 0% (is named waxy mutant), it has been observed that their starch viscosity properties differ from those of normal wheat as do those of the different "partially waxy mutants" (Hayakawa et al. 1997). Kiribuchi-Otobe et al. (1997) and Kim et al. (2003) found that, in waxy wheat lines, the peak viscosity of starch measured by the Rapid Visco Analyser (RVA) occurred at earlier times than non-waxy wheats and at a temperature lower by between 10 and 15 °C than normal or partially waxy wheats. Kiribuchi-Otobe et al. (1997) and Kim et al. (2003) found that waxy lines had a peak viscosity higher than non-waxy, while the peak viscosity of waxy lines analyzed by Hayakawa et al. (1997), Yasui et al. (1999) and Sasaki et al. (2000) had lower values than non-waxy. Bettge et al. (2000) reported that starch granules of waxy wheat were more susceptible to physical damage than those that contain amylose. Yoo and Jane (2002) and Kim et al. (2003) found that the crystallinity of waxy bread wheats was significantly greater than that of normal lines. This characteristic has also been observed in waxy lines of other cereal species such as barley (Song and Jane 2000) and potato (McPherson and Jane 1999). Also the lack of amylose produces a reduction of staling in bread (Nakamura et al. 1995).

In bread wheat, in addition to the type of starch that a variety has protein quality characteristics such as the baking strength and protein content are also of great interest to the flour and baking industry. The baking strength or gluten strength depends to a large extent on the genetic composition of the variety and the specific composition of the endosperm protein, particularly the insoluble fraction of glutenin of high molecular weight (Payne et al. 1979). These proteins, in bread wheat, are controlled by complex loci located on the long arms of group 1 chromosomes 1A, 1B and 1D (Payne et al. 1982; Shewry et al. 1992). The endosperm protein content, on the other hand, depends to a greater extent on the environment and agricultural practices.

The objective of the present work was to study the waxy mutant lines of bread wheat and the influence that the environment and the genotype (composition of HMW glutenin subunits) has on some of their quality parameters (sedimentation volume and mixograph parameters) and starch paste viscosity.

Materials and Methods

Plant materials

The experimental material included 38.0% amylose (i.e., waxy) F_6 and F_7 lines of bread wheat (*Triticum aestivum* L.) derived from crosses between the cultivar 'Califa' and the 'Waxy Line' triple mutant (100% amylopectin). This parental 'Waxy Line' was obtained by crossing the cultivar 'Bai Huo', which lacks the waxy protein Wx-D1 (alleles *Wx-A1a, Wx-D1b, Wx-B1e)* with a Chinese bread wheat variety, which lacks the proteins Wx-A1 and Wx-B1 (alleles *Wx-A1b, Wx-D1a, Wx-B1b*). The waxy line mutants and the parentals were grown in the field in an experimental design with two random complete blocks each, in E.T.S.I. Agrónomos Agricultural Experimental Station, Madrid, Spain, in 2003 and 2004.

Electrophoretic analysis

Glutenins were extracted from crushed grain (Singh et al. 1991) and the extracts fractionated by SDS-PAGE electrophoresis (Payne et al. 1980). The 1B/1R translocation was detected by A-PAGE electrophoresis (Lafiandra and Kasarda 1985). Waxy proteins were extracted from the flour, and electrophoresis performed as described by Rodríguez-Quijano et al. (1998).

DNA extraction was performed according to Saghai-Maroof et al. (1984). PCR to determine the over-expression of Bx7 (7^{OE}) was performed as described in Ragupathy et al. (2008).

Evaluation of quality traits

Sedimentation volume (SDSS) was measured according to Dick and Quick (1983). Mixograph peak mixing development time [MDT] and resistance to breakdown [BDR] were determined using 10 g of flour and a National Manufacturing Co. Mixograph (Lincoln, NE), as described by Finney and Shogren (1972). All measurements were replicated twice.

Starch properties

Starch viscosity was analysed using a Rapid Visco Analyser (RVA-3D, Newport Scientific, Pty. Ltd.). Flour (3.5 g) was mixed with 24±0.1ml of distilled water and 1ml of $AgNO_3$ (10%) (Batey et al. 1997), and the mixture processed using profile standard 1 (Method ICC 162). Peak starch viscosity (PV), trough viscosity (TV), final viscosity (FV) and were then derived in rapid visco units (RVU), and the time to peak viscosity (TP), in min. from the rapid visco analysis curve. From these values were calculated breakdown (BKD, peak minus trough viscosity) and setback (SB, final minus trough viscosity). All measurements were replicated twice.

Statistical analysis

Statistical analysis was performed using statistical software (Version SAS Institute, 8.2). Analysis of variance (General Linear Model Procedure, F-test on type III sum of squares) was conducted to assess whether siguificant variation existed among genotype protein alleles and environments. The differences between the pairs of alleles were tested using the *t*-test. Linear relationships among all parameters were examined by generating Pearson correlation coefficients.

Results

The HMW glutenin subunit compositions of the parental 'Califa' and 'Waxy line' were 1, 7^{OE}+8, 5+10 and Null, 7*+9, 2+12, for *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. 'Califa' showed a 1B/1R translocation but in this study the lines analysed did not have the translocation. Figure 1 shows the over-expression of Bx7 (7^{OE}) glutenin subunit present in 'Califa'. Figure 2 shows the RVA curves for 'Califa' and 'Waxy Line' (parents) and two waxy lines (W16 and W149). The HMW glutenin subunits of *Glu-B1* locus of 'Califa' (7+8* subunits) have been called *al* alelle because until now the over-expression has been only described on this allele (Butow et al. 2004).

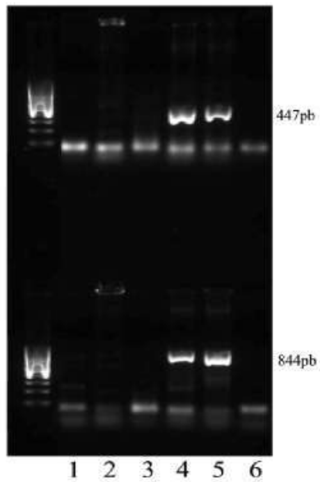

Figure 1. Composition in Bx7^{OE} encoded by *Glu-B1* locus: two waxy lines without Bx7^{OE} (slots 1-2), and their parents ('Waxy Line' and 'Califa', slots 3 and 4, respectively). The varieties controls 'Glenlea' (slot 5) and 'Chinese Spring' (slot 6). Molecular marker is included on the left

Averages and ranges for the parameters measured

Table I shows data for the sedimentation volume (SDSS), mixograph parameters (MDT and BDR) and viscosity parameters (PV, BKD, SB and TP) for the waxy lines F_6 and F_7, the parent lines and the control varieties. Results of the tests (sedimentation volume, mixograph and starch viscosity) for the waxy lines had indicated that, for the majority of the tests, there is a wide range of values, as seen in Table 1. For sedimentation volume, mixograph values and value of starch peak viscosity (PV) and breakdown (BKD) the

Table 1. Means (± standard deviation) and range for the sedimentation volume (SDSS) and mixograph (MDT and BDR) and viscosity variables (PV, BKD, SB and TP) of waxy lines, 'Califa' and the 'Waxy Line' parents in two years (2003 and 2004) analysed

Quality Test	Waxy Lines		'Califa'	'Waxy Line'
	Mean±Sd	Range	Mean±Sd	Mean±Sd
SDSS (mm)	98.6±10.5	70.0-117.5	101.0±1.8	87.5±2.7
MDT (s)	162±5.0	53-290	256±1.0	82±2.0
BDR (%)	25.6±6.7	13.6-46.7	16±1.2	33±1.4
PV (RVU)	203.7±34.5	131.9-271.5	137.0±0.8	195.4±22.6
BKD (RVU)	131.1±24.3	79.9-178.9	47.5±0.1	131.8±2.0
SB (RVU)	32.4±6.1	17.5-42.2	80.8±0.1	43.9±3.3
TP (min)	3.6±0.1	3.5-3.8	5.7±0.1	3.7±0.1

ranges indicate that some of the waxy lines have values higher than the parents (Table 1) and that the average peak viscosity of the waxy lines are higher than their parents.

Influence of environment and genotype on the different parameters

Analysis of variance (Table 2) shows that the sedimentation volume in these waxy lines is not influenced by environment but is influenced by the over expression of HMW glutenin subunit Bx7 ($Bx7^{OE}$) controlled by locus *Glu-B1*. The interactions and field replications are not including in the Table 2 of results because they are not significant. The model only explains 27% of the variation due to this effect. The sedimentation volume of the waxy lines that carry the $Bx7^{OE}$ is significantly greater than those that do not (Table 3).

Table 2. Mean squares from analysis of variance for sedimentation volume (SDSS), viscosity RVA (PV, BKD, SB and TP) and mixograph parameters (MDT and BDR) of waxy lines F6 and F7

Source	**df**	**SDSS**	**PV**	**BKD**	**SB**	**TP**
Year	1	238.0	2732.2	1170.7	10.1	0.004
Glu-A1	1	147.5	1040.9	720.4	18.2	0.041**
Bx7OE	1	545.6*	101.9	10.2	19.0	0.007
Glu-D1	1	46.6	165.6	16.6	8.4	0.000
Error	71	84.8	1201.8	633.0	49.43	0.000

Source	**df**	**MDT**	**BDR**
Year	1	9106.5**	260.2**
Glu-A1	1	2590.8	5.5
Bx7 OE	1	25414.2**	42.1
Glu-D1	1	8150.5**	320.8**
Error	65	697.1	25.5

** and * significant at $P < 0.01$ and $P < 0.05$, respectively.

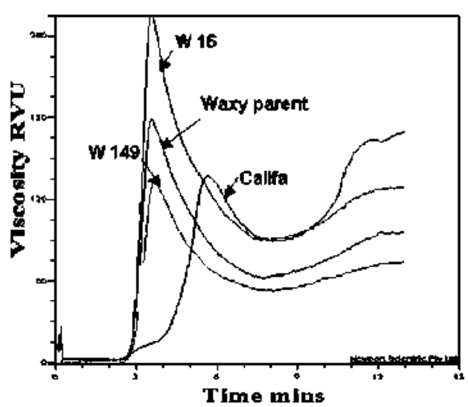

Figure 2. RVA viscosity curves of parents ('Califa' and 'Waxy Line') and two waxy lines, W16 and W149

Analysis of variance for the starch paste viscosity measurements by RVA indicated that, in the waxy lines, none are influenced by environment (Table 2). Of the parameters that were analyzed, only the TP was influenced by genotype, this being due to variation at *Glu-A1*. The waxy lines with HMW glutenin subunit 1 at the *Glu-A1* locus showed peak viscosity at an earlier time than those with the null allele (Table 3), even though the model explains only 22% of the variation.

Table 3. T tests returning significant results for sedimentation volume (SDSS), mixograph parameters (MDT and BDR) and time to peak viscosity (TP) in the waxy lines analysed

Parameters	Allelic composition compared			Means		df	*T-test*
	X		Y	X	Y		
SDSS	$Bx7^{OE}$ +	*vs*	$Bx7^{OE}$ -	104.8±6.9	94.5±10.5	74	4.7**
MDT	$Bx7^{OE}$ +	*vs*	$Bx7^{OE}$ -	209±34	128±28	68	11.1**
TP	*Glu-A1* Null	*vs*	Glu-A1 1	3.62±0.1	3.56±0.1	74	3.6**
MDT	*Glu-D1* 5+10	*vs*	Glu-D1 2+12	187±45	118±22	68	7.4**
BDR	*Glu-D1* 5+10	*vs*	Glu-D1 2+12	22.6±5.7	30.9±4.8	68	–6.3**

** Significant at P < 0.01.

The mixograph parameters (analysed for 35, instead of 38, waxy lines) have an environmental influence (Table 2). For variation of the parameters MDT and BDR, there is an influence of genotype at locus *Glu-D1* and for the variation of MDT, variation at *Glu-B1* also has an influence. The model explains 74% and 46% of variability (for MDT and BDR respectively) due to environmental and genotypic variation. The waxy lines that carry the $Bx7^{OE}$ or the pair 5+10 HMW glutenin subunits have higher MDT and lower BDR than those that do not have them (Table 3).

Correlations between the quality parameters

The correlations between parameters are shown in Table 4.

Table 4. Pearson correlation coefficients for the variables analysed in the waxy lines

	SDSS	MDT	BDR	PV	BKD	SB
MDT	0.44**	–	–	–	–	–
BDR	–0.35**	–0.45**	–	–	–	–
PV	n.s.	n.s.	n.s.	–	–	–
BKD	n.s.	n.s.	n.s.	0.99**	–	–
SB	n.s.	n.s.	n.s.	0.82**	0.80**	–
TP	n.s.	n.s.	n.s.	–0.49**	–0.53**	–0.44**

** Significant at P < 0.01; ns, not significant

The sedimentation volume correlates positively with MDT and negatively with BDR, both mixograph parameters. There is no significant correlation between sedimentation volume and the different parameters for starch paste viscosity. Among the parameters of starch viscosity, there is positive correlation between all of them, except for the time to reach peak (TP) which has a negative correlation with the rest of the parameters for starch viscosity.

Discussion

The results of this work indicate that the sedimentation volume, the mixograph parameters and the starch paste viscosity have a wide variability in the waxy lines of bread wheat. This variability is due mainly to influence of genotype for sedimentation volume, mixograph time to peak development (MDT) and time to reach peak starch paste viscosity (TP). It was also observed that the various waxy lines had higher values than their parent lines in the case of sedimentation volume, mixograph MDT and some of the starch paste viscosity parameters (PV and TV), which indicates that the waxy lines are transgressive for these parameters. The wide variability indicates that for the majority of the parameters that were analyzed, the behaviour does not differ from that expected for non-waxy lines, except that the time to peak paste viscosity were always less than those for the non-waxy parent line, 'Califa'. The behaviour for TP agrees with that described by other workers who have studied the viscosity for waxy lines of bread wheat (Hayakawa et al. 1997; Fujita et al. 1998; Kiribuchi-Otobe et al. 1997, 1998; Yasui et al. 1997, 1999; Graybosch et al. 2003; Kim et al. 2003).

In non-waxy bread wheat, various authors (Butow et al. 2003, 2004; Radovanovic et al. 2002; Juhász et al. 2003) have described the effect of the presence of over expression of HMW-GS Bx7 ($Bx7^{OE}$) at *Glu-B1* locus in increasing the sedimentation volume and mixograph parameters. This effect had not been described for waxy lines of bread wheat. The results indicated that the $Bx7^{Oe}$ subunit of the parent line 'Califa' agreed with that reported recently for non-waxy lines of bread wheat. The presence of this glutenin subunit exerts a positive effect on sedimentation volume and mixograph peak development time. In this way, it has a greater effect than that of the HMW glutenin subunits pair 5+10, which is considered to have the greatest effect on sedimentation volume and mixograph development time (Payne et al. 1980). On the other hand, mixograph breakdown which estimates the dough resistance to over mixing is not influenced by the $Bx7^{OE}$ subunit but influenced by HMW glutenins subunits 5+10.

These differences that have been observed in the waxy lines in our study due to the genotypic effect of loci *Glu-B1* and *Glu-D1* do not agree with the results of Graybosch et al. (2003) when they also analysed sedimentation volume and mixographs for waxy lines obtained from different origins and evaluated in different environments. In their case, the waxy lines had lower values of sedimentation volume and mixograph parameters than the non-waxy controls. These authors questioned whether this behaviour was due to the inherent nature of the waxy lines or if these lines, in particular, had a genotype with lower values for baking quality parameters.

Our results have indicated that the year does not have an effect on any of the parameters analyzed by the RVA for the waxy lines which is in agreement with the results of Graybosch et al. (2003) who analyzed waxy lines at three locations. Viscosity parameters for waxy lines were spread over a wide range as reported by Graybosch et al. (2003) and Han et al. (2004), who also found a wide variation in the viscosity of waxy lines of bread wheat and rice respectively. It is interesting to note the wide range found for the peak starch pasting viscosity. This parameter is considered to be of great interest since it is one of the indicators for starch viscosity relevant to end use quality of wheat varieties. When the variety is non-waxy, a greater or lesser peak viscosity has been associated with the ratio of amylose/amylopectin of the endosperm starch (Zeng et al. 1997) and consequently with variation of genes at the *Wx* locus that control amylose synthesis. Thus, the fact that in this study the waxy lines which lack amylose, also showed variation in the peak viscosity, indicates that amylopectin and its genetic control could play an important role in determining the paste viscosity of these lines. In view of this, future work would be needed to measure the variability for those loci involved in the synthesis of amylopectin (loci *Sbe*) for waxy lines of bread wheat. The correlations detected between different parameters that have been analyzed in waxy lines show that there is no correlation between the parameters that measure baking strength (sedimentation volume and mixograph parameters) and starch paste viscosity.

It can be concluded that, for the waxy lines that were analyzed, the genotype of the variety is involved in quality parameters of the waxy flour. The independence of baking strength and starch viscosity and the effect of genotype on both, could have much application for improvement to obtain lines with different characteristics, permitting selection for different end uses, based on physico-chemical properties

Acknowledgement

This work was supported by Grant No. AGL 2006-8025 from Comisión Interministerial de Ciencia y Tecnología (CICYT) of Spain.

References

Batey, I.L., Curtin, B.M., Moore, S.A. 1997. Optimization of Rapid-Visco-Analyser test conditions for predicting Asian noodle quality. Cereal Chem. 74:497–501.

Bettge, A.D., Giroux, M.J., Morris, C.F. 2000. Susceptibility of waxy starch granules to mechanical damage. Cereal Chem. 77:750–753.

Butow, B.J., Ma, W., Gale, K.R., Cornish, G.G., Rampling, L., Larroque, O., Morell, M.K., Békés, F. 2003. Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. Theor. Appl. Genet. **107**:1524–1532.

Butow, B.J., Gale, K.R., Ikea, J., Juhász, A., Bed, Z., Tamás, L., Gianibelli, M.C. 2004. Dissemination of the highly expressed Bx7 glutenins subunit (Gli-B1 allele) in wheat as revealed by novel PCR markers and RP-HPLC. Theor. Appl. Genet. **109**:1525–1535.

Dick, J.A., Quick, J.S. 1983. A modified screening test for rapid estimation of gluten strength in early generation durum wheat breeding lines. Cereal Chem. **60**:315–318.

Echt, C. S., Schwartz, D. 1981. Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. Genetics **99**:275–284.

Finney, K.F., Shogren, M.D. 1972. A ten-gram mixograph for determining and predicting functional properties of wheats flours. Baker's Digest **46**:32–35, 38–42, 77.

Fujita, S., Yamamoto, H., Sugimoto, Y., Morita. N., Yam, M. 1998. Thermal and crystalline properties of waxy wheat (*Triticum aestivum* L.) starch. J. Cereal Sci. **27**:1–5.

Graybosch, R.A., Souza, E., Berzonsky, W., Baenziger, P.S., Chung, O. 2003. Functional properties of waxy flours: Genotypic and environmental effects. J. Cereal Sci. **38**:69–76.

Han, Y., Xu, M., Liu, X., Yan, Ch., Korban, S.S., Chen, X., Gu, M. 2004. Genes coding for starches branching enzymes are major contributors to starch viscosity characteristics in waxy rice (*Oryza sativa* L.). Plant Sci. **166**:357–364.

Hayakawa, K., Tanaka, K., Nakamura, T., Endo, S., Hoshino, T. 1997. Quality characteristics of waxy hexaploid wheat (*Triticum aestivum* L.): Properties of starch gelatinization and retrogradation. Cereal Chem. **74**:576–580.

Juhász, A., Larroque, O.R., Tamás, L., Hsam, S.L.K., Zeller, F.J., Békés, F., Bedő, Z. 2003. Bánkuti 1201 – an old Hungarian wheat variety with special storage protein composition. Theor. Appl. Genet. **107**:697–704.

Kim, W., Johnson, J.W., Graybosch, R.A., Gaines, C.S. 2003. Physicochemical properties and end-use quality of wheat starch as a function of waxy protein alleles. J. Cereal Sci. **37**:195–204.

Kiribuchi-Otobe, C., Nagamine, T., Yanagisawa, T., Ohnishi, M., Yamaguchi, I. 1997. Production of hexaploid wheats with waxy endosperm character. Cereal Chem. **74**:72–74.

Kiribuchi-Otobe, C., Yanagisawa, T., Yamaguchi, I., Yoshida, H. 1998. Wheat mutant with waxy starch showing stable hot paste viscosity. Cereal Chem. **75**:71–672.

Lafiandra, C., Kasarda, D.D. 1985. One and two-dimensional (two-pH) polyacrylamide gel electrophoresis in a single gel: separation of wheat proteins. Cereal Chem. **62**:314–319.

McPherson, A.E., Jane, J. 1999. Comparison of waxy potato with other root and tuber starches. Carbohydrate Polymers **40**:57–70.

Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S. 199??????. Identification of three Wx proteins in wheat (*Triticum aestivum* L.). Biochem. Genet. **31**:75–86.

Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S., Nagamine, T. 1995. Production of waxy (amylose-free) wheats. Mol. Gen. Genet. **248**:253–259.

Naranjo, T., Roca, A., Goicoecha, P.G., Giraldez, R. 1987. Arm homoeology of wheat and rye chromosomes. Genome **29:**873–882.

Payne, P.I., Law, C.N., Mudd, E.E. 1980. Control of homoeologous group 1 chromosomes of the high-molecular-weight subunits, a major protein of wheat endosperm. Theor. Appl. Genet. **58**:113–120.

Payne, P.I., Corfield, K.G., Blackman, A.J. 1979. Identification of a high-molecular-weight subunit of glutenin whose presence correlates with breadmaking quality in wheats of related pedigree. Theor. Appl. Genet. **55**:153–159.

Payne, P.I., Holt, L.M., Worland, A.J., Law, C.N. 1982. Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. Part 3: telocentric mapping of the subunit genes on the long arm of the homoeologous group 1 chromosomes .Theor. Appl. Genet. **63**:129–138.

Preiss, J. 1991. Biology and molecular biology of starch synthesis and its regulation. Plant Mol. Cell. Biol. 7:59–114.

Radovanovic, N., Cloutier, S., Brown, D., Humphreys, D.G., Lukow, O.M. 2002. Genetic variance for gluten strength contributed by high molecular weight glutenins proteins. Cereal Chem. **79**:843–849.

Ragupathy, R., Naeem, H.A., Reimer, E., Lukow, O.M., Sapirstein, H.D., Cloutier, S. 2008. Evolutionary origin of the segmental duplication encompassing the wheat GLU-B1 locus encoding the overexpressed Bx7 ($Bx7^{OE}$) high molecular weight glutenin subunit. Theor. Appl. Genet. **116**:283–296.

Rodriguez-Quijano, M., Nieto-Taladriz, M.T., Carrillo, J.M. 1998. Polymorphism of waxy proteins in Iberian hexaploid wheats. Plant Breed. **117**:341–344.

Sasaki, T., Yasui, T., Matsuki, J. 2000. Effect of amylose content on gelatinization, retrogradation and pasting properties of starches from waxy and nonwaxy wheat and their F_1 seeds. Cereal Chem. 77:58–63.

Saghai-Maroof, M., Soliman, K.M., Jorgensen, R.A., Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Nat. Academy of Sci. USA **81:**8014–8018.

Shewry, P.R., Halford, N.G., Tatham, A.S. 1992. High-molecular-weight subunits of wheat glutenin. Plant Mol. Cell. Biology **15**:105–120.

Singh, N.K., Shepherd, K.W., Cornish, G.B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin J. Cereal Sci. **14**:203–208.

Song, Y., Jane, J. 2000. Characterization of barley starches of waxy, normal and high amylose varieties. Carbohydrate Polymers **41**:365–377.

Yasui, T., Sasaki, T., Matsuki, J. 1999. Milling and flour pasting properties of waxy endosperm mutant lines of bread wheat (*Triticum aestivum* L.). J. Sci.Food Agr. **79**:687–692.
Yasui, T., Sasaki, T., Matsuki, J., Yamamori, M. 1997. Waxy endosperm mutants of bread wheat (*Triticum aestivum* L.) and their starch properties. 1997. Breeding Science **47**:161–163.
Yoo, S.G., Jane, J. 2002. Structural and physical characteristics of waxy and other wheat starches. Carbohydrate Polymers **49**:297–305.
Zen, M., Morris, C.F., Batey, I.L., Wrigley C.W. 1997. Sources of variation for starch gelatinization, pasting, and gelation properties in wheat. Cereal Chem. **74**:3–71.