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Sources of the highly expressed wheat bread making (*wbm*) gene in CIMMYT spring wheat germplasm and its effect on processing and bread-making quality

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Abstract Bread-making quality is a core trait for wheat breeding programs. Recently, the expression of a novel gene named wheat bread making (*wbm*) gene has been associated with good bread-making quality. In this study, 54 historical and modern bread wheat genotypes from CIMMYT were screened by PCR marker for the presence of the allele associated with high *wbm* expression. Eight of the 54 wheat genotypes tested positive for the *wbm* allele and the genotype Waxwing was identified as the most likely donor of the allele in part of CIMMYT germplasm. The *wbm* allele had a significant effect on overall gluten quality, gluten strength, gluten extensibility and bread-making quality, although its effect was smaller than the effects of other quality related genes as *Glu-D1, Glu-B1* or the negative effect of the 1BL.1RS translocation. The *wbm* allele was associated with higher values of the traits mentioned but not with higher protein content. The identification of this new *wbm* gene/protein is a step forward in understanding wheat quality genetic control. Implementation of marker assisted selection in breeding programs to detect the *wbm* allele is highly recommended.

Keywords Wheat quality · Bread-making · *wbm* gene · Glutenins

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Wheat is one of the most important crops in the world, occupying 219 million hectares and resulting in global production of about 715 million tonnes a year (FAOSTAT 2013). High adaptability to diverse environments is important but probably the key characteristic for understanding the success of this crop are the unique properties of the dough formed from wheat flours, which is processed into a range of products including cakes, biscuits, pasta, noodles, and other processed foods of which probably bread is the most important worldwide (Shewry 2009).

Bread-making quality is a core trait for wheat breeding programs. The International Maize and Wheat Improvement Center (CIMMYT) wheat breeding program aims to develop new wheat genotypes for release as varieties in developing countries that not

only produce high yields for farmers but also satisfy subsequent actors in the value chain, including food manufacturers and consumers. Integrating bread-making quality in a breeding program is not simple, as quality analyses are expensive, time-consuming and require certain amounts of seed that are usually not available until late generations of the breeding process. Molecular markers can be useful for determining various wheat quality components and enhancing selection for bread-making quality (Kuchel et al. 2007).

The viscoelastic properties of wheat dough occur due to the seed storage proteins, which form a complex responsible of the elasticity and extensibility of the dough called gluten when the flour is mixed with water. Gluten quality is important to understand and ensure good bread-making quality. Intensive research has been carried out to identify its components and understand their underlying genetics (see Wrigley et al. 2006 for a complete review). Glutenins (high and low molecular weight subunits, HMW and LMW, respectively) and gliadins have been identified as the main components of storage proteins. HMW glutenins have been the most studied, probably for their relatively simple pattern by SDS-PAGE electrophoresis and for their great impact in rheological and bread-making properties, in spite of the fact that they only represent 10 % of the total storage protein in the grain. The genes coding for these proteins (*Glu-A1, Glu-B1* and *Glu-D1*, Payne and Lawrence 1983) and their alleles are well known, and molecular markers are available to detect them (ÓOvidio and Anderson 1994; Liu et al. 2008). The presence of 1BL.1RS translocation has also been associated to low quality (Liu et al. 2005; Peña et al. 1990), due to the loss of proteins from the *Glu-B3* and *Gli-B1* and incorporation of secalins from rye. However, observed polymorphisms of these and other grain proteins associated with additional quality traits (hardness, starch properties, etc.) still do not explain the genetic variation found in bread-making quality.

Recently Furtado et al. (2015) identified a new gene that is expressed in developing seeds, called the wheat bread making (*wbm*) gene. This gene codifies for a small sulphur-rich protein not previously associated with wheat quality. The *wbm* gene has shown highly differential expression in genotypes varying in bread-making quality: genotypes with high *wbm* expression all had good bread-making quality. Furtado et al. (2015) also identified the sequence variant in promoter region of the gene (GWseqVar3) associated with high expression of the gene, which presence can be determined by a simple molecular marker using PCR. Therefore, the objective of the current study was to screen CIMMYT germplasm for the presence of GWseqVar3 sequence variant associated to high *wbm* expression and to analyse the effect of this gene on processing and bread-making quality traits.

For this purpose 54 CIMMYT bread wheat lines (ESM 1), including historical and modern varieties and advanced lines, released from 1960 to 2014, were sown in an alpha lattice design with three replicates in 2013 and 2014 crop seasons in Ciudad Obregon in the state of Sonora, Mexico, under six different field management conditions, including drought and heat stress. Grain from two field replicates was analysed for seven quality traits, including grain protein content (%) (GPC), SDS-sedimentation volume (ml) (SDSS), mixograph optimum dough development time (min) (DDT) and torque (%) (TQ), alveograph gluten strength (ALVW) and tenacity/extensibility ratio (ALVP/L), and bread loaf volume (LV), using the methodologies of the American Association of Cereal Chemist (AACC 2000), with some modifications (Guzman et al. 2015). In addition, the HMW glutenins composition and the presence of the 1BL.1RS translocation were determined by SDS-PAGE in polyacrylamide gels, according to the methodology of Peña et al. (2004). To detect the presence of the *wbm* allele associated to high expression, PCR screening was carried out using genomic DNA extracted from young leaves, with primers NWPFor and NWPRev and PCR conditions described by Furtado et al. (2015). Two more bread wheat lines, Kiritati and Waxwing, were tested for the *wbm* allele.

Eight of the 54 wheat genotypes tested positive for the *wbm* allele associated to high expression of the gene, including some popular varieties released in different countries, such as Seher 06 (Pakistan) and Baj #1 (India). Two of the advanced lines that were positive for the *wbm* allele had the line Waxwing in their pedigree as well as Baj #1, Grackle #1 and Munal #1. Therefore it seemed that Waxwing was probably the source of the *wbm* allele in part of CIMMYT germplasm, which was confirmed with the PCR marker. Kiritati, which is the other parental of Munal #1, tested also positive for the *wbm* allele. The origin of the *wbm* allele in two other genotypes (Kanchan and Babax/Lr42//Babax*2/4/Sni/Trap#1/3/Kauz*2/Trap//Kauz) that tested positive for the marker is not clear

and further screenings in more lines will be required to elucidate it.

The effect of the presence of the *wbm* allele on the seven measured quality traits was analysed by a combined variance analysis across the two years and six field managements, together with the composition of HMW glutenins (*Glu-A1, Glu-B1* and *Glu-D1*) and 1BL.1RS translocation data (data not shown) (Table 1). The analysis revealed that *wbm* allele had a significant effect on all of the quality traits analysed, including those related to overall gluten quality (SDSS), gluten strength (TQ and ALVW), gluten extensibility (ALVP/L) and bread-making quality (LV). The same results were revealed for the additional genes included in the study. For most of the traits, the locus *Glu-D1* showed the greatest effect, followed by the *Glu-B1* locus and the 1B/1R translocation. The *Glu-A1* effect was lower, but also more significant than the effect of the *wbm* allele for TQ and ALVW. Overall, the effect of *wbm* was less significant than that of the *Glu-1* genes in traits related to gluten strength, but in traits related to gluten extensibility (ALVP/L and to a minor extent SDSS) its effect was more prominent and comparable to that of the other genes but far from *Glu-D1*. On bread-making, the *wbm* effect was small, particularly if compared with the effect of *Glu-D1*. The two major alleles at the *Glu-D1* locus, 5 + 10 and 2 + 12, have repeatedly shown a contrasting effect on quality traits (Gupta et al. 1994; He et al. 2005), so it was not surprising to find a strong *Glu-D1* effect in this study. The interaction of the *wbm* gene and other genes, except *Glu-D1,* with the environment was not significant.

The results shown in Table 2 reveal that the presence of the *wbm* allele was associated with higher quality for all the traits. This higher quality is not associated with higher protein content in the genotypes carrying the *wbm* allele, which agrees with Furtado et al. (2015). The association of the *wbm* allele with higher gluten strength and extensibility (lower ALVP/L value) is what probably leads to higher loaf volumes values. The deduced protein sequence of the *wbm* gene showed the presence of four cysteine residues which could participate in intra- or intermolecular disulphide bonds with other gluten proteins enhancing the visco-elastic properties of the dough (Furtado et al. 2015). However, based on current results, we cannot state that the presence of the allele associated to high expression of the gene automatically leads to good bread-making quality. In the current study, lines with low loaf volume values carrying the *wbm* allele were found, and vice versa (data not shown). Furtado et al. (2015) made the same discovery in the cv. Bobwhite, which carries the 1BL.1RS translocation.

In summary, the identification of the *wbm* allele in several CIMMYT genotypes is important, as CIMMYT develops spring wheat lines for all major wheat growing

Table 1 Effects of environment, different loci and their interactions with environment on quality traits: mean squares from ANOVA analysis

Effect	DF	SDSS Mean square	DDT Mean square	TQ Mean square	ALVW Mean square	ALVP/L Mean square	LV Mean square
Environment (E)	11	38.4***	1.1*	2580.5***	70534.7***	0.45*	15262.3***
Glu-A1	1	159.7***	NS	9959.6***	586612.1***	1.40*	42694.6**
Glu-B1	4	293.6***	15.8***	30510.8***	29169.1***	5.23***	72120.2***
Glu-D1	1	399.2***	261.6***	335778.7***	2182667.4***	8.98***	628273.7***
1BL.1RS	1	1333.3***	55.6***	115411.9***	1267197.7***	1.75*	86109***
wbm	1	265.4***	3.1*	6370.6*	91368.1**	1.69*	40969.1**
E × *Glu-A1*	11	NS	NS	NS	NS	NS	NS
E × *Glu-B1*	44	NS	NS	NS	NS	NS	NS
E × *Glu-D1*	11	18.6***	1.17***	1759.1*	20808.8**	2.20***	NS
E × *1B/1R*	11	NS	NS	NS	NS	NS	NS
E × *wbm*	11	NS	NS	NS	NS	NS	NS

NS not significant, *SDSS* SDS sedimentation, *DDT* mixograph optimum dough development time, *TQ* mixograph torque, *ALVW* alveograph strength, *ALVP/L* alveograph tenacity/extensibility ratio, *LV* bread loaf volume

Significant at the probability level of * $p < 0.01$, ** $p < 0.001$, and *** $p < 0.0001$

Table 2 Adjusted least square means analysis of genotypes positive (+) or negative (−) for the *wbm* allele

	GPC (%)	SDSS (ml)	DDT (min)	TQ (%)	ALVW (J × 10^{-4})	ALVP/L	LV (ml)
wbm −	13.8*	13.7	2.45	98	261	0.97	838
wbm +	13.8*	15.3	2.62	106	291	0.84	852

GPC grain protein content, *SDSS* SDS sedimentation, *DDT* mixograph optimum dough development time, *TQ* mixograph torque, *ALVW* alveograph strength, *ALVP/L* alveograph tenacity/extensibility ratio, *LV* bread loaf volume

Means not significantly different at * $p < 0.05$ level

areas in the developing world (Rajaram and van Ginkel 2001). Advanced breeding lines are distributed annually to around 250 partners in more than 60 countries, where they are released directly as varieties or are used as parents for crossing (Singh et al. 2007). Therefore CIMMYT lines have served as donors of the highly expressed *wbm* allele in many other breeding programs, helping to enhance bread-making quality worldwide. The frequency of the *wbm* gene (14 %) is still relatively low in CIMMYT germplasm and can be increased. Dough systems are a complex mixture of diverse proteins, carbohydrates and lipids, each of them contributing to the dough and products characteristics. The identification of this new *wbm* gene/protein is a step forward in understanding wheat quality genetic control. Further studies are necessary to analyse in detail the functional role of this protein in combination with other grain factors. Implementation of marker assisted selection in breeding programs to detect the *wbm* allele is highly recommended.

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