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GLUTENIN DIVERSITY OF FLOUR WHEAT (*Triticum aestivum* L.) FOR COOKIE QUALITY IN MEXICO

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ABSTRACT

Glutenins are high- and low-molecular-weight proteins (HMW-G and LMW-G). They play a determining role in genetic improvement to define industrial quality in flour wheat, so understanding their variability is necessary for directing selection toward a specific quality. The objective of this research was to determine the diversity of HMW-G and LMW-G alleles of genotypes from the flour wheat breeding program of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) useful for cookie quality. Twenty-five genotypes were used, including six Mexican cookie varieties, as well as 21 genotypes introduced from an international collection of accessions from the International Maize and Wheat Improvement Center (CIMMYT). HMW-G and LMW-G were identified based on their separation on polyacrylamide gels in the presence of sodium dodecyl sulfate. For HMW-G, the 2* variant in *Glu-A1* was the most frequent variant for the trial and introduction nursery, with more than 80 %. At the *Glu-B1* locus, the 7+9 and 7 variants were the most frequent, with 60 and 38.1 % in trial and nursery, respectively. For *Glu-D1*, the 2+12 variant was the most frequent in the trial with 64 %, and 5+10 with 85.7 % in nursery. The latter allele favors baking quality. For the *Glu-A1* and *Glu-D1* loci, it is necessary to introduce genotypes with null alleles or with the $2+12_1$ variant in *Glu-D1* associated with cookie quality. For the highest-frequency variants of LMW-G, c in *Glu-A3*, h in *Glu-B3*, and b in *Glu-D3* are associated with baking quality. With the aforementioned, it is concluded that there is a low frequency and little variation in useful alleles of HMW-G and LMW-G for cookie quality in the introduced accessions analyzed, implying that new genetic sources that favor it should be explored.

Keywords: proteins, high and low molecular weight glutenins, breeding.

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INTRODUCTION

Within the objectives of flour wheat (*Triticum aestivum* L.) breeding, it is necessary to combine variety traits with higher yield potential, disease tolerance, and qualities for a specific industrial use (Kiszonas and Morris, 2018). Within the flour wheat breeding program, there is selection for a better cracker factor, which combines larger diameter and smaller cracker thickness, which is a way of indicating its quality (Ma *et al*., 2020;

Zhang *et al*., 2020). This variable is commercially expressed as producing more cookies per unit of flour used in their production.

One of the main components that define the cookie factor are flour proteins called glutenins. This protein, along with gliadins, forms gluten, which is an insoluble polymer in the presence of water that gives the dough the characteristics of strength, tenacity, extensibility, and viscosity, which partially defines the industrial use of flour wheat genotypes as suitable for cookie or bakery use (Zhang *et al*., 2023).

Glutenins are classified as high- and low-molecular-weight glutenins (HMW-G and LMW-G) based on their separation on polyacrylamide gels in the presence of sodium dodecyl sulfate as a reducing agent. The *Glu-A1*, *Glu-B1*, and *Glu-D1* loci code for HMW-G subunits (Payne and Lawrence, 1983), and LMW-G is encoded by the *Glu-A3*, *Glu*-B3, and *Glu-D3* loci (Singh and Shepherd, 1988). Through genetic recombination, combinations of HMW-G and LMW-G alleles can be generated for bakery and cookie making. These qualities differ because bread requires strong doughs to increase its volume, whereas cookies require weak doughs that favor cookie diameter (Sarkar *et al*., 2015).

Research has identified the alleles and their combinations that favor cookie quality. In this context, it has been reported that the HMW-G combinations associated with higher cookie factor are: the absence of subunit (null allele) in *Glu-A1*, the presence of the 7+8 allele in *Glu-B1*, as well as the 2+12 or null allele in *Glu-D1* (Zhang *et al*., 2018; Ma *et al*., 2019). A new allelic variant in *Glu-D1* called $2+12_1$ was also identified and associated with the biscuit expansion (Ma *et al*., 2020). In addition, Sharma *et al*. (2021), Zhang *et al*. (2018), and Ma *et al*. (2020) proposed the *Glu-A1*, *Glu-B1*, and *Glu-D1* combinations of HMW-G: 2*, 7, 2+12; 0, 7+8, 0, and 1, 7+8, $2+12_1$, associated with larger cookie diameter.

In Mexico, there are several studies in flour wheat that have identified HMW-G and LMW-G alleles and their best combinations of with the aim of favoring baking quality (Hernández-Espinosa *et al.*, 2013; Martínez-Cruz *et al*., 2014; Muñoz-Calixto *et al*., 2022). However, there is a lack of assessment to identify variants and their combinations that benefit cookie quality. An important condition for selection in a breeding program is to know the existing genetic variability in order to derive progenies with the desired characteristics. The National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) wheat breeding program consists of genotypes with HMW-G and LMW-G alleles associated with excellent cookie quality. The present research aimed to know the diversity of these alleles in the genotypes of the program and to identify those useful for cookie quality.

MATERIALS AND METHODS

Plant material and field evaluation

Two groups of genotypes from the INIFAP breeding program were used. The group called the Fifth National Soft Wheat Trial (5th ENSUVES) consisted of 25 genotypes, of which seven were commercial varieties (Cortázar S94, Bárcenas S2002, Maya S2007, Urbina S2007, Torocahui S2008, Borlaug 100 F2014, and Faisán S2016) and 18 were advanced F_6 lines generated by the breeding programs of INIFAP and the International Maize and Wheat Improvement Center (CIMMYT). Varieties with a letter "S" in their name were released by INIFAP for use in the cookie industry in Mexico, indicating their soft dough is suitable for cookie production, while the number refers to the year of release. The Borlaug 100 F2014 variety was used as a reference control because of its high yield in irrigated areas, which is representative of strong dough varieties for baking. A second group consisted of 21 F_6 lines generated in 2019 for CIMMYT international evaluation trials and introduced by INIFAP with the aim of identifying new genetic sources to increase the variability of alleles associated with higher cookie quality.

Planting took place in the 2019–2020 autumn–winter cycle at INIFAP's Bajío Experimental Field (CEBAJ) in Celaya, Guanajuato, Mexico. The experimental design was a randomized complete block design with two replications. The experimental unit consisted of four 3 m-long furrows with a spacing of 30 cm and a sowing density of 120 kg ha^{-1}. Sowing took place in the first week of December under irrigated conditions. The fertilizer rate of 240-60-00 was used; half of the N and all of the P_2O_5 were applied at sowing, and the rest of the N was applied with the first relief irrigation. Fertilizer sources were urea with 46 % N [CO $(NH_2)_2$] and triple calcium superphosphate with 46 % P_2O_5 [Ca $(H_2PO_4)_2$]. Narrow-leaved weeds were controlled with an application of Topik® 240 EC (clodinafop-propargyl + cloquintocet-mexyl) 30 days after sowing irrigation, and broad-leaved weeds with Esteron® 47 EC (2-4-dichlorophenoxyacetic acid) 35 days after emergence. An application of 2.5 % cypermethrin® ($C_{22}H_{19}C_{l2}NO_3$) was made for aphid control at the grain formation and filling stages.

Laboratory analysis and information

One ear from each plot was collected and threshed for the extraction of HMW-G and LMW-G. The grains from each ear were milled in a UDY-type mill, and the electrophoretic analysis of glutenins was performed using the method of Guzmán *et al*. (2022) at the Cereal Chemistry and Quality Laboratory of CIMMYT. Separation of the protein subunits was obtained from a 40 mg sample of wholemeal flour using 14 % acrylamide gels at pH 8.5, applying 9 mA per gel for 17 h. The HMW-Gs (loci *Glu-A1*, *Glu-B1*, and *Glu-D1*) were identified based on the nomenclature proposed by Payne and Lawrence (1983), and the LMW-Gs (loci *Glu-A3* and *Glu-B3*) according to Singh *et al*. (1991), Jackson *et al*. (1996), and Branlard *et al*. (2003). For the *Glu-D3* locus, the nomenclature proposed by Branlard *et al*. (2003) was used.

For each group of genotypes, allele frequencies were calculated for the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci coding for the HMW-Gs, as well as for the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci of the LMW-Gs. All HMW-G and LMW-G allele combinations were identified for each of the genotypes.

RESULTS AND DISCUSSION

The frequency of allelic variants coding for HMW-Gs for both groups of lines analyzed (Figure 1) shows that, for the *Glu-A1* locus, allelic variants 2*, 0, and 1 and the heterologous allele 2*/1 were identified in the trial, while for the lines introduced from the nursery, alleles 2* and 1 were identified. Variant 2* was the most frequent variant in the trial and introduction nursery, with more than 80 %, so new lines should be evaluated to favor the presence of the 0 allele, which is associated with higher cookie quality (Zhang *et al*., 2012).

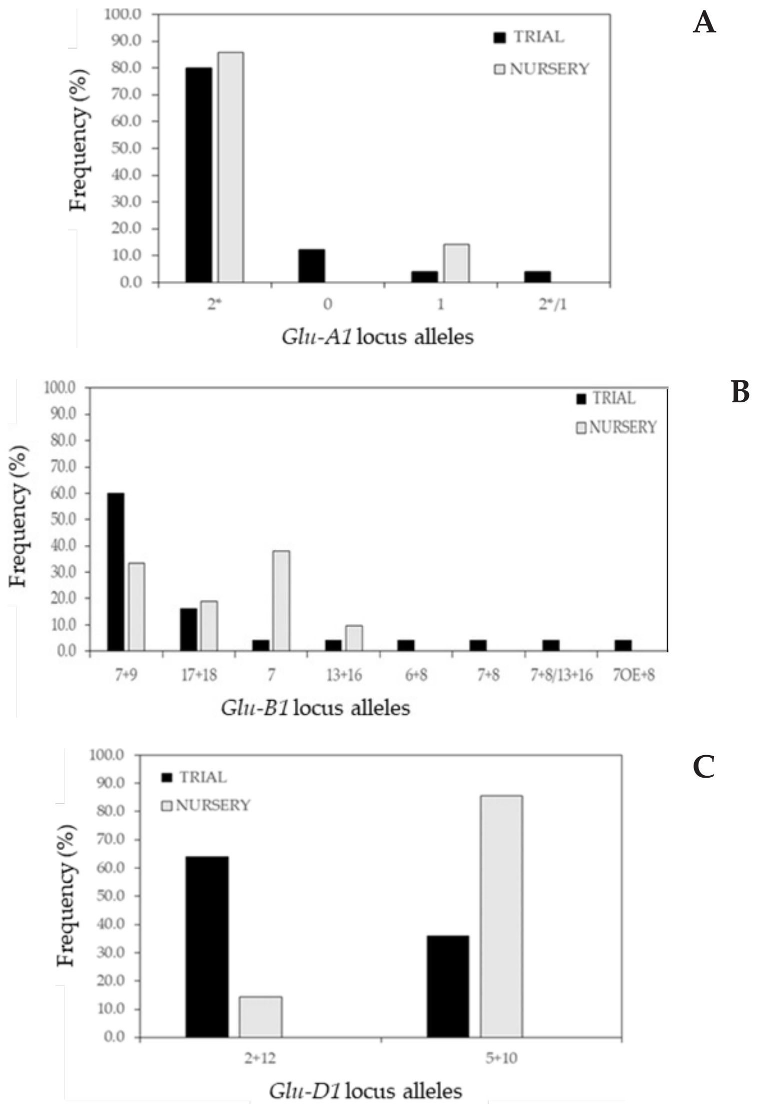

Figure 1. Allelic variants of the loci coding for HMW-Gs of flour wheat (*Triticum aestivum* L.) genotypes from the breeding program of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP). A: *Glu-A1*; B: *Glu-B1*; C: *Glu-D1*.

At the *Glu-B1* locus, seven allelic variants (7+9, 17+18, 7, 13+16, 6+8, 7+8, and 7OE+8) and one heterologous allele (7+8/13+16) were found in the trial, and 7+9, 17+18, 7, and 13+16 alleles were identified in the nursery. For this locus, the highest frequency variants in the trial were 7+9 and 17+18, with 60 and 16 %, respectively. For the introduction nursery, they were 7, 7+9, and 17+18, with values of 38.1, 33.3, and 19 %, respectively. According to Moiraghi *et al*. (2013), allele 7 is suitable for cookie fitness, so these genotypes should be selected.

For the *Glu-D1* locus, the 2+12 and 5+10 alleles were identified. The 2+12 variant was the most frequent in the trial (64 %), while 5+10 was the most frequent for the introduction nursery (85.7 %). This is partly due to the fact that the national trial included the varieties Cortázar S94, Bárcenas S2002, Maya S2007, Urbina S2007, Torocahui S2008, and Faisán S2016, which had the 2+12 allele and were released for use in the cookie industry. Zhang *et al*. (2018) and Ma *et al*. (2019) mentioned that zero or null alleles in *Glu-A1*, 7 and 7+ 8 in *Glu-B1*, and 2+12 in *Glu-D1* favor cookie quality, so it is necessary to maintain genotypes containing these alleles and continue exploring genotypes to introduce a greater number of lines with these variants. Likewise, according to Ma *et al.* (2020), it is imperative to introduce materials with the 0 and $2+12_1$ variants in *Glu-D1*, which favor the cookie factor, to reduce the presence of the 5+10 allele, which is associated with higher baking quality (Guzmán *et al*., 2022), as is the case of the control variety Borlaug 100 F2014.

Regarding the frequency of alleles coding for the LMW-G (Figure 2), for the *Glu-A3* locus, variants c, d, and b were common between the trial and the introduction nursery, while f occurred in the trial and e in the nursery. Variants c and d were the most frequent variants (40 %) in the trial, while c (52 %), b (19 %), and e (19 %) were the most frequent in the introduced lines. At the *Glu-B3* locus, 11 allelic variants were identified, which corresponds to the highest number of all loci analyzed. The highest frequency variants were h (56 %), d (12 %), and i (12 %), respectively, for the test lines. In the case of the h allele, it was again found most frequently (47.7 %) in the nursery lines; the second most frequent was the b’ variant (33.3 %).

Six alleles in *Glu-D3* and one heterologous allele (b/c) were identified. The most frequent alleles for the trial and nursery were b, a, and c, with b accounting for more than 60 % in both groups of lines. The most frequent alleles were c in *Glu-A3*, d and h in *Glu-B3*, as well as b in *Glu-D3* in both groups of lines. According to Zhang *et al.* (2012) and Wang *et al.* (2016), these alleles were associated with higher sedimentation volume and resistance in the extensograph, which favors baking quality. However, in the introduction nursery, the e and h variants in *Glu-A3* and *Glu-B3*, which decrease dough strength (Zhang *et al*., 2012; Bonafede *et al*., 2015; Franaszek and Salmanowicz, 2021) and may favor cookie quality, were identified in low frequency. Therefore, it is recommended to increase their frequency by introducing new genetic material.

According to Moiraghi *et al.* (2013) and Zhang *et al*. (2018), the combinations of HMW-Gs in *Glu-A1*, *Glu-B1*, and *Glu-D1* that favored the cookie factor were: 0, 7+8, and 2+12; 1, 7+8, and 0; and 0, 7+8, and 0, respectively. Within the group of lines of the national

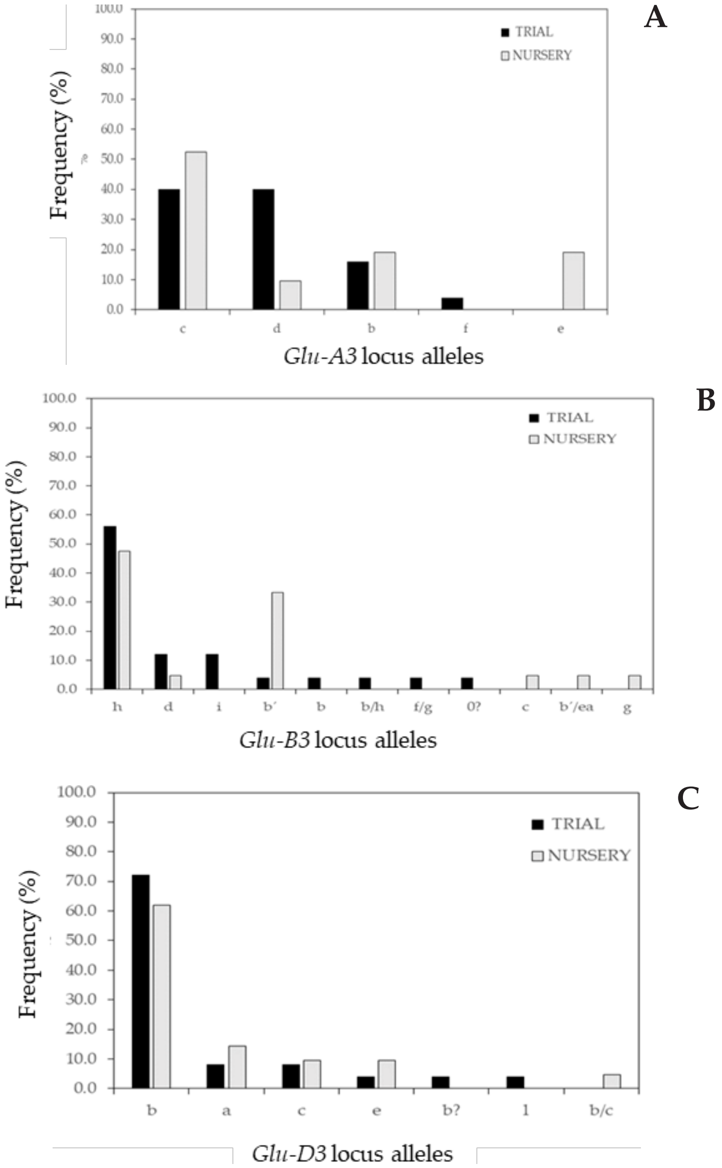

Figure 2. Allelic variants of the loci coding for LMW-Gs of flour wheat (*Triticum aestivum* L.) genotypes from the breeding program of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP). A: *Glu-A3*; B: *Glu-B3*; C: *Glu-D3*.

trial, the genotype Maya S2007 and lines 1, 11, and 12 (Table 1) were identified as genetic sources for the genetic improvement of the cookie quality as they present at least two desirable alleles: 0 in *Glu-A1*, 7+8 in *Glu-B1*, and 2+12 in *Glu-D1*. On the other hand, lines 16, 19, 20, and 21 from the introduction nursery showed allele 7 in *Glu-B3* and alleles e and c of the *Glu-A3* and *Glu-B3* loci in LMW-G, respectively, which are

Table 1. Combinations of high and low molecular weight glutenins (HMW-G and LMW-G) of flour wheat (*Triticum aestivum* L.) genotypes from the National Soft Wheat Trial of the breeding program at the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP).

	HMW-G			LMW-G		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Maya S2007	0	6+8	2+12	f	null	B
Line 1	0	7+9	2+12	d	i	B
Line 11	0	7+9	2+12	d	i	B
Urbina S2007	1	13+16	2+12	b	d	B
Torocahui S2008	2*	7+9	2+12	c	h	E
Cortazar S94	2*	7+9	2+12	d	h	B
Bárcenas S2002	2*	7+9	2+12	c	H	b?
Line 4	2*	7+9	2+12	b	H	L
Line 6	2*	17+18	2+12	c	f, g	B
Line 7	2*	17+18	2+12	d	h	B
Line 8	2*	7+9	2+12	c	h	B
Line 12	2*	7+8	2+12	d	h	B
Line 13	2*	7+9	2+12	d	h	B
Line 15	2*	7+9	2+12	d	i	B
Line 16	2*	7+9	2+12	b	h	C
Faisán S2016	2*/1	7+8/13+16	2+12	d	b/h	B
Borlaug 100 F2014	2*	7	5+10	c	h	C
Line 2	2*	7OE+8	5+10	c	h	B
Line 3	2*	7+9	5+10	c	h	A
Line 5	2*	7+9	5+10	c	h	B
Line 9	2*	17+18	5+10	d	b`	B
Line 10	2*	7+9	5+10	c	h	A
Line 14	2*	17+18	5+10	d	b	B
Line 17	2*	7+9	5+10	b	d	B
Line 18	2*	7+9	5+10	c	d	B

associated with lower dough strength and may favor cookie quality, so these lines should be selected (Table 2).

It was not possible to identify the desirable combination for higher cookie quality of HMW-G (0, 7+18, 2+12) or LMW-G (e and c) within the genotypes analyzed, with the Maya S2007 variety being the genotype that presented the highest number of desirable alleles for HMW-G. For LMW-G, the desirable alleles e for *Glu-A3* and c for *Glu-D3* were not matched by any genotype.

According to Bonafede *et al*. (2015), it is important to note the difficulty in the individual identification of LMW-Gs on rheological quality and cookie or baking quality due to the linkage between the *Glu-3* and *Gli-1* loci. However, Ibba *et al*. (2017) indicate that there are different relationships between alleles of the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on cookie diameter. With some lines identified in the trial and introduction nursery, it will be possible to create combinations of HMW-G and LMW-G that favor cookie

Table 2. Combinations of high and low molecular weight glutenins (HMW-G and LMW-G) of flour wheat (*Triticum aestivum* L.) genotypes from the International Maize and Wheat Improvement Center (CIMMYT) introduction nursery.

	HMW-G			*LMW-G*		
	Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*
Line 17	2*	13+16	2+12	b	b	E
Line 12	2*	17+18	2+12	c	h	B
Line 10	2*	7+9	2+12	c	h	C
Line 5	2*	7	5+10	d	b`	B
Line 6	2*	7	5+10	c	b`/ea	E
Line 8	2*	7	5+10	b	b	B
Line 15	2*	7	5+10	c	b`	B
Line 16	2*	7	5+10	e	b`	B
Line 19	2*	7	5+10	e	h	A
Line 20	2*	7	5+10	e	h	B
Line 21	2*	7	5+10	e	h	A
Line 18	1	13+16	5+10	c	h	B
Line 11	2*	17+18	5+10	c	h	B
Line 13	2*	17+18	5+10	c	b`	B
Line 14	2*	17+18	5+10	c	b`	B
Line 3	1	7+9	5+10	c	g	B
Line 4	1	7+9	5+10	d	c	B
Line 1	2*	7+9	5+10	b	d	B
Line 2	2*	7+9	5+10	b	h	b/c
Line 7	2*	7+9	5+10	c	h	A
Line 9	2*	7+9	5+10	c	h	C

quality. Through genetic recombination of the variety Maya S2007 and lines 16, 19, 20, 21, and 4, glutenin combinations associated with higher cookie quality can be generated.

CONCLUSIONS

The flour wheat germplasm in Mexico contains high-molecular-weight glutenin alleles 0 in *Glu-A1*, 7+8 in *Glu-B1*, and 2+12 in *Glu-D1*, as well as low-molecular-weight glutenin alleles e in *Glu-A3* and c in *Glu-B3*, in lines introduced from the International Maize and Wheat Improvement Center, which can favor cookie quality; however, their frequency is very low and dispersed. There is a need to introduce genetic sources that increase their frequency and diversity. It is recommended to introduce the 0 and $2+12_1$ variants of the *Glu-D1* locus, which are associated with a larger cookie diameter. The use of genotypes with these variants and their genetic recombination will make it possible to obtain a greater accumulation of alleles to obtain lines with greater cookie aptitude.

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