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# Durum wheat for pasta and bread-making. Comparison of methods used in breeding to determine gluten quality-related parameters

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**SUMMARY –** Gluten strength and gluten extensibility influence both pasta-making and bread-making qualities of durum wheat. This study examined the relationship among various gluten quality-related screening parameters. It also determined (using stepwise regression analysis) which screening parameters were more suitable to predict gluten strength (alveographic parameters ALVW and ALVPL) and bread loaf volume (LV). The experimental material used was practically all (152 of 156 samples) of the LMW-2 glutenin type and it included conventional and exotic durum wheat germplasm from CIMMYT. In addition, the relationship between various *Glu-B1* – controlled HMW-glutenin subunits and gluten quality-related parameters was determined. Gluten Index (GLUIND), grain SDS-sedimentation (GRNSED), and Mixograph curve width (MWP3), were highly significantly correlated with ALVW and bread LV. Prediction equations for W and LV included protein + GLUIND + GRNSED + MWP3 and protein + GLUIND + GRNSED + Mixograph peak height (MPH), respectively. The prediction equation for ALVPL included only GRNSED and MPH. GRNSED and the Mixographic parameters MWP3 and MPH showed the highest value as screening parameters to select for gluten quality. The *Glu-B1* subunit 6+8 was associated with slightly stronger gluten type than 7+8 and 13+16, while subunit 20 was associated with weak gluten properties. To breed for medium to strong gluten types, breeders should avoid, in addition to LMW-1 type glutenins, HMW-glutenin subunit 20.

**Key words:** Durum wheat, breeding for quality, gluten strength, glutenin proteins, pasta-making quality, bread-making quality.

***RESUME –*** *"Blé dur pour pâtes alimentaires et boulangerie. Comparaison de méthodes utilisées en amélioration pour déterminer les paramètres du gluten liés à la qualité". La force et l'extensibilité du gluten influent sur les qualités pastières et boulangères du blé dur. Cette étude examine la relation entre différents paramètres d'évaluation de la qualité du gluten et détermine (avec analyse de régression par échelon) quels sont les paramètres les plus adaptés pour prédire la force du gluten (paramètres alvéographiques ALWM et ALVPL) et le volume du pain (LV). Le matériel expérimental était presque quasi exclusivement (152 des 156 échantillons testés) constitué de matériel à gluténine de faible poids moléculaire LMW-2, et incluait du germoplasme conventionnel et exotique du CIMMYT. De plus, la relation entre plusieurs sous-unités gluténine* Glu-B1 *de haut poids moléculaire (HMW) et les paramètres de qualité du gluten ont été déterminés. L'indice de gluten (GLUIND), la sédimentation-SDS du grain (GRNSED) et l'épaisseur de la courbe du Mixographe (MWP3) étaient hautement et significativement corrélés avec ALVM et LV. Les équations de prévision pour W et LV contenaient la protéine + GLUIND + GRNSED + MWP3 et la protéine + GLUIND + GRNSED + la hauteur du pic de la courbe du Mixographe (MPH), respectivement. L'équation de prévision pour ALVPL était constituée seulement de GRNSED et MPH. GRNSED, MPH et MWP3 sont les paramètres les plus adaptés pour prédire la force du gluten. La sous-unité 6+8* Glu-B1 *était associée à un gluten de type légèrement plus fort que 7+8 et 13+16, alors que la sous-unité 20 était associée à du gluten aux propriétés médiocres. Afin de sélectionner vis-à-vis du gluten de type fort ou moyen, les sélectionneurs devraient éviter, en plus des gluténines de type LMW-1, la sous-unité 20 de gluténine HMW.*

***Mots-clés :*** *Blé dur, amélioration de la qualité, force du gluten, protéines de gluténine, qualité pastière, qualité boulangère.*

## Introduction

Durum wheat is the main material used to manufacture pasta products, cous-cous, bulghur, and, in some countries (mostly in the Mediterranean region), bread. High protein content and high protein (gluten) quality are necessary because both traits directly affect the processing and culinary properties of pasta and the crumb and keeping properties of bread (Liu *et al.,* 1996, for a review; Marchylo *et al.,* 1998).

In the pasta- and bread-making industries, wheat quality is commonly determined by evaluating its performance in food processing and by determining the cooking/baking quality characteristics of the finished product. This practice uses large amounts of raw material (semolina and flour) and is time-consuming. Alternatively, the industry has adopted dough rheological methods as less time-consuming means of predicting pasta-cooking and bread-making qualities. Dough rheological methods (using the Mixograph, the Farinograph, and the Alveograph, among others) used to measure the viscoelastic properties (strength and extensibility) of the gluten protein correlate well with the firmness and springiness of cooked pasta and with the loaf volume and crumb structure of bread (Landi and Guarneri, 1992; Marchylo *et al.,* 1998).

Most rheological and sensory tests used in industry to assess durum wheat quality are not suitable to screen hundreds of experimental breeding lines at the segregating and the early-advanced stages, due to the limited amount of testing sample and short testing time. Several small-scale parameters used to screen germplasm at early breeding stages are strongly associated with rheological quality and with pasta-cooking quality attributes. These include protein content, gluten content, Gluten Index (GLUIND), manual gluten quality score, sodium dodecyl sulfate (SDS)-sedimentation, and Mixograph mixing parameters (Matsuo *et al.,* 1982; Autran *et al.,* 1986; D'Egidio *et al.,* 1990; Cubadda *et al.,* 1992; Kovacs *et al.,* 1997).

Although screening parameters are particularly useful to discriminate between weak and strong gluten types, results of different studies comparing the screening value of the same or similar parameters may not agree (Matsuo *et al.,* 1982; D'Egidio *et al.,* 1990; Kovacs *et al.,* 1997). This disparity is partly due to: (i) different testing conditions used to determine a given parameter; (ii) the high degree of complexity of the grain factors determining pasta-cooking and bread-making quality; and (iii) differences in the type of germplasm used in the different studies. For example, Dexter *et al.* (1998) observed that despite their lower protein content and lower sedimentation volume, Italian varieties showed longer mixing time and larger Alveographic strength values than North American varieties. The more distinctive difference between the two groups of genotypes compared was dough extensibility.

The International Maize and Wheat Improvement Center (CIMMYT) conducts durum wheat breeding using a large, genetically wide gene pool to develop germplasm which is widely distributed among breeding programs of durum-producing countries. Until now, protein and SDS-sedimentation have been the only parameters used to screen for gluten strength. At present CIMMYT advanced durum wheat germplasm predominantly (>90%) possesses the LMW-glutenin block known as LMW-2 (mainly 2 but also $2^-$, 2*) and shows sedimentation values corresponding to medium to strong gluten types. The gluten strength of CIMMYT durum wheat germplasm must be further improved to increase its value in other breeding programs. To achieve this, in addition to the sedimentation test, other gluten quality screening parameters need to be implemented. Therefore, the objectives of this study were to determine in the durum wheat germplasm of CIMMYT: (i) the relationship between quality screening parameters and gluten strength and bread-making quality; (ii) the set of screening parameters that would better predict gluten strength and gluten extensibility (Alveograph W and P/L values, respectively) and bread making quality (bread loaf volume); and (iii) the relationship between HMW-glutenin (*Glu-1)* composition and gluten quality-related parameters.

## Materials and methods

Five sets of experimental lines and varieties of durum wheat (n = 192) developed by CIMMYT were used. These included breeders' lines (n = 37), lines used in nitrogen (n = 8) and phosphorous (n = 16) fertilization trials, lines with exotic (Kharkov 5 and tetraprelude) backgrounds (n = 44), and durum wheat 1DL (1AL) translocation lines carrying the *Glu-D1* HMW-glutenin subunit 5+10 (n = 55). Breeders' lines were grown under irrigation in central Mexico during summer, 1999; all other materials were grown under irrigation in northwestern Mexico during crop cycle Y, 1998-99.

Whole meal and refined flour samples were obtained with a UDY Cyclone mill (0.5 mm sieve) and with a Brabender Jr. mill (9xx sieve), respectively. Protein (PRT) and SDS-sedimentation (SED) were determined in both whole meal flour (GRN) and refined flour (FLR) using NIR analysis and the 1.0-g sample method described by Peña *et al.* (1990), respectively. Wet and dry gluten (WETGLU, DRYGLU, respectively) and GLUIND were determined using the AACC method 38-12 (AACC, 1995). The gluten mass removed from the washing equipment was further washed by hand between the palms for 10 seconds under a light stream of tap water before it was centrifuged.

Mixographic parameters, peak time (MPT), peak height (MPH), curve width at peak time (MWP), and curve width 3 minutes after peak time (MWP3) were determined in a 10-g Mixograph according to the

AACC method 54-40A (AACC, 1995), but using a constant water absorption (60%) regime. The Alveographic assay was conducted in 60-g flour samples, using variable water absorption (60-70%) as determined by filling the consistency of the dough after the first 4-5 minutes of mixing. Alveographic mixing time was also variable (8-12 min) and was judged optimum when the dough achieved a continuous, cohesive, and smooth condition. The gluten strength value W (ALVW) and the tenacity/extensibility ratio P/L (ALVPL) were recorded. Loaf volume (LV) was determined in breads baked from 100-g flour samples using the AACC straight dough method 10-09 (AACC, 1995). Total grain protein extracts and glutenin protein extracts were fractionated by SDS-PAGE (12.5% acrylamide gel), following the methods described by Payne *et al.* (1980) and by Singh *et al.* (1991), respectively. High- (HMW-Glu) and Low- (LMW-Glu) molecular weight glutenins were numbered according to Payne and Lawrence (1983) and Payne *et al.* (1984), respectively. Pearson correlation coefficients and stepwise regression analysis were carried out using the SAS statistical computer software (SAS, 1996).

## Results and discussion

### Relationship between screening parameters

#### *Correlation among quantitative parameters*

Correlation coefficients among grain protein (GRNPRT), flour protein (FLRPRT), and dry and wet gluten (DRYGLU and WETGLU, respectively) were very high (0.84-0.99, $p \leq 0.0001$). The very high correlation between DRYGLU and WETGLU ($r = 0.99$) indicates that gluten hydration capacity is rather a measure of gluten quantity than of gluten quality.

#### *Relationship between quantitative and qualitative parameters*

None of the correlation coefficients between quantitative screening parameters and SED were significant (Table 1). Neither GRNPRT nor FLRPRT were correlated with MPT, while FLRPRT, DRYGLU, and WETGLU were not correlated with the width of the MWP3. Protein and gluten content showed medium-low negative correlation with GLUIND. Cubadda *et al.* (1992) also found a negative relationship between GLUIND and DRYGLU. This negative correlation suggest that gluten extensibility, as affected by protein content, plays a role in the definition of GLUIND and could be influenced by the fact that for a given wheat cultivar, as the protein content increases, gluten extensibility also increases. An explanation for this relationship is that increased extensibility may increase the passing of gluten through the sieve during the centrifugation stage in the GLUIND determination. This view is supported by the negative relationship observed between quantitative parameters and ALVPL in this study (see below; see also Table 2). An increase in gluten extensibility with an increase in protein content is commonly seen in bread wheat.

Table 1. Correlation coefficients between quantitative and qualitative gluten strength screening parameters (n = 192)

| Qualitative parameters | Code | Quantitative parameters† | | | |
|---|---|---|---|---|---|
| | | GRNPRT (%) | FLRPRT (%) | DRYGLU (%) | WETGLU (%) |
| Grain SDS-sedimentation (ml) | GRNSED | NS | NS | NS | NS |
| Flour SDS-sedimentation (ml) | FLRSED | NS | NS | NS | NS |
| Gluten Index (%) | GLUIND | -0.25** | -0.27** | -0.45** | -0.41** |
| Mixograph | | | | | |
| Peak time (min) | MPT | NS | NS | -0.28** | -0.27** |
| Peak height (cm) | MPH | 0.59** | 0.55** | 0.60** | 0.64** |
| Width at peak time (cm) | MWP | 0.32** | 0.27** | 0.22** | 0.26** |
| Width 3 min after peak (cm) | MWP3 | 0.14* | NS | NS | NS |

†GRNPRT = grain protein; FLRPRT = flour protein; DRYGLU = dry gluten; WETGLU = wet gluten.
**Significant at P < 0.01, *significant at P < 0.05, NS = not significant.

Table 2. Correlation coefficients between quality screening parameters and variables related to pasta- and bread-making quality†

| Screening parameter | Code | Alveograph | | Bread loaf volume (ml) |
|---|---|---|---|---|
| | | W x $10^{-4}$ J | P/L | |
| Quantitative | | | | |
| Grain protein (%) | GRNPRT | 0.34** | -0.29** | 0.32** |
| Flour protein (%) | FLRPRT | 0.28** | -0.30** | 0.32** |
| Dry gluten (%) | DRYGLU | 0.15* | -0.31** | 0.24** |
| Wet gluten (%) | WETGLU | 0.17* | -0.32** | 0.24** |
| Qualitative | | | | |
| Grain SDS-sedimentation (ml) | GRNSED | 0.50** | 0.25** | 0.61** |
| Flour SDS-sedimentation (ml) | FLRSED | 0.51** | NS | 0.45** |
| Gluten Index (%) | GLUIND | 0.51** | 0.19* | 0.43** |
| Mixograph | | | | |
| Peak time (min) | MPT | 0.54** | 0.15* | 0.33** |
| Peak height (cm) | MPH | 0.44** | -0.38** | 0.34** |
| Width at peak time (cm) | MWP | 0.50** | -0.24** | 0.26** |
| Width 3 min after peak (cm) | MWP3 | 0.64** | NS | 0.42** |

†Number of samples used in correlation analysis was 190 for W and P/L and 149 for loaf volume.
**Significant at P < 0.01, *significant at P < 0.05, NS = not significant.

The low but significant correlation between all the quantitative parameters and the MWP suggests that MWP is significantly influenced by protein content. The largest significant correlation occurred between all quantitative parameters, especially WETGLU, and MPH, indicating that MPH is mainly a measure of flour absorption capacity, as affected chiefly by protein content.

## Relationship between screening parameters and variables related to pasta- and bread-making quality

### *Correlation between screening parameters and variables predicting pasta- and bread making quality*

According to Landi (1995), the leading pasta making industry of Italy has relied since the early 1980s on an equation considering DRYGLU and both Alveograph gluten strength value W (ALVW) and the tenacity/extensibility ratio, P/L (ALVPL), as good predictors of cooking quality. Gluten strength and extensibility are also widely recognized to be associated with bread-making quality. Thus Alveographic parameters are quite relevant variables for predicting pasta- and bread-making quality.

ALVW and LV generally had higher correlation coefficients with qualitative screening parameters than with quantitative ones. In contrast, with the exception of MPH, the tenacity/extensibility ratio, P/L (ALVPL), had higher correlation coefficients with quantitative than with qualitative parameters (Table 2). ALVW had a similar correlation coefficient with SED and GLUIND, indicating that these two parameters have similar gluten strength screening efficiency. This suggestion is also supported by the results obtained by Cubadda *et al.* (1992), which compared these three parameters. The screening parameters showing the highest correlation with ALVW, ALVPL, and LV were MPW3, MPH, and GRNSED, respectively.

Bread LV had its largest correlation with ALVW (0.63, p < 0.001), followed closely by GRNSED. The strong relationship between these parameters is in agreement with previous reports by Boggini and Pogna (1989), Peña *et al.* (1994) and Dexter *et al.* (1998), who used Italian, CIMMYT, and North American and Italian durum wheat, respectively.

*Best screening parameters to predict variables related to pasta- and bread-making quality*

The best combinations of screening parameters to predict variables related to pasta- and bread-making quality, determined by stepwise regression analysis, are presented in Table 3. GRNSED appeared in the three predicting equations; GLUIND and MPH were present in two; and MWP3 and FLRPRT appeared in the equation for ALVW and in that for LV, respectively. The great value of the SDS-sedimentation test to screen for gluten viscoelastic properties was clearly manifested. The presence of Mixographic parameters in all three predicting equations indicates that this instrument is also of great value in screening for gluten strength-related parameters. These results agree with those of Kovacs *et al.* (1997), who evaluated several small-scale tests and found that GRNESED, MPH, and Mixograph total energy (a parameter most likely associated with the MPW3 examined in the present study) were the best small-scale tests to predict pasta chewiness and firmness.

Table 3. Stepwise regression analysis to predict variables related to pasta- and bread-making quality†

| Screening parameter | Alveograph | | | | Bread loaf volume (ml) | |
|---|---|---|---|---|---|---|
| | W x $10^{-4}$ J | | P/L | | | |
| | Parameter estimate | Model** $R^2$ | Parameter estimate | Model $R^2$ | Parameter estimate | Model $R^2$ |
| Intercept | -326.67 | – | – | – | – | – |
| MWP3 | 60.36 | 0.407 | – | – | – | – |
| GRNPRT | 20.10 | 0.468 | – | – | – | – |
| GRNSED | 14.31 | 0.545 | – | – | – | – |
| GLUIND | 145.57 | 0.569 | – | – | – | – |
| Intercept | – | – | 5.64 | – | – | – |
| MPH | – | – | -1.34 | 0.146 | – | – |
| GRNSED | – | – | 0.48 | 0.240 | – | – |
| Intercept | – | – | – | – | -447.59 | – |
| GRNSED | – | – | – | – | 40.69 | 0.370 |
| FLRPRT | – | – | – | – | 31.67 | 0.530 |
| GLUIND | – | – | – | – | 316.14 | 0.607 |
| MPH | – | – | – | – | 36.42 | 0.631 |

†Number of samples used in correlation analysis was 190 for W and P/L and 149 for loaf volume.
**($p < 0.01$).

In this study GLUIND had less weight in the prediction equations than GRNSED. Actually protein content had greater weight than GLUIND in the prediction equations. Nonetheless, and in agreement with Cubadda *et al.* (1992), GLUIND is an acceptable single alternative to screen for gluten strength and bread LV in durum wheat, better than PRT, DRYGLU, and WETGLU parameters, and similar to SED, when used individually.

## Relationship between *Glu-B1*-controlled HMW-glutenin subunits and parameters related to pasta- and bread-making quality

It has been demonstrated that LMW glutenins, especially those controlled by genes at the *Glu-B3* locus, play a major role in determining gluten strength in durum wheat. The LMW-2 type glutenins confer stronger gluten character and better pasta quality attributes than the LMW-1 type glutenins (du Cros, 1987; Pogna *et al.,* 1988; Feillet *et al.,* 1989; Vazquez *et al.,* 1996). The HMW-glutenin subunits also confer, although to a lesser extent than the LMW glutenins, differential quality effects in durum wheat (Boggini and Pogna, 1989; Carrillo *et al.,* 1990; Kovacs *et al.,* 1993; Peña *et al.,* 1994). However, the influence of the various *Glu-B1*-controlled HMW-glutenin subunits on gluten strength is still controversial.

The relationship between *Glu-B1*-controlled HMW glutenin subunits and quality parameters was examined in durum wheats uniform for *Glu-A1* (null allele), and for *Glu-B3* (LMW-2 type glutenins) allelic composition. *Glu-B1* HMW-glutenin subunits encountered were 20, 6+8, 7+8, and 13+16. Quality characteristics of durum wheat lines associated with each subunit are shown in Table 4.

Table 4. Quality characteristics of durum wheat sets with differing *Glu-B1* glutenin composition (all the lines possess the LMW-2 type glutenin composition)

| *Glu-B1* subunit | Quality parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | FLRSED[†] (ml) | GLUIND (%) | MPT (min) | MWP3 (cm) | ALVW ($x10^{-4}$ J) | ALVPL | LV (ml) |
| 20 (n = 8)[††] | 7.3 ± 1.0 | 43 ± 9 | 1.8 ± 0.2 | 1.3 ± 0.2 | 133 ± 46 | 4.1 ± 3.8 | 435 ± 68 |
| 13 + 16 (n = 9) | 10.5 ± 3.4 | 64 ± 16 | 2.5 ± 0.6 | 1.7 ± 0.4 | 249 ± 65 | 3.3 ± 1.8 | 680 ± 141 |
| 7 + 8 (n = 64) | 9.9 ± 2.8 | 63 ± 16 | 3.3 ± 1.4 | 1.9 ± 0.6 | 235 ± 81 | 3.7 ± 1.7 | 637 ± 112 |
| 6 + 8 (n = 12) | 10.7 ± 2.4 | 67 ± 12 | 3.0 ± 1.0 | 2.0 ± 0.6 | 337 ± 116 | 3.6 ± 2.4 | 718 ± 88 |

[†]Mean and standard deviation.

[††]Number of samples used to obtain LV values was 4, 3, 39, and 9, for 20, 13+16, 7+8, and 6+8, respectively.

No difference in gluten extensibility (ALVPL) was observed among the four HMW-glutenin groups. All presented tenacious gluten character. The group of lines possessing subunit 20 showed the lowest values for all parameters related to gluten strength and for loaf volume. The other three glutenin groups showed similar values for all the parameters, although the 6+8 glutenin group showed consistently higher values than the other glutenin groups. This was particularly so for ALVW and for LV. These results are in agreement with those of Carrillo *et al.* (1990), Kovacs *et al.* (1993) and Peña *et al.* (1994), in that subunit 20 is associated with weak gluten strength and subunits 6+8 and 7+8 are associated with medium to strong gluten character. The present results disagree, however, with those of Boggini and Pogna (1989), who found that subunit 20 had a bread-making quality effect similar to that of 6+8, and with those of Boggini and Pogna (1989) and Peña *et al.* (1994), who found that 7+8 imparts better breadmaking quality than 6+8. Differences in genotypic backgrounds of the durum wheat cultivars used in the different studies could partially explain the conflicting results obtained.

The overall results of this study indicate that genotypes having subunit 6+8 generally show superior gluten strength, which is expected to produce high pasta- and bread-making quality. On the contrary, and in spite of the presence of LMW-2 type glutenins, genotypes having the HMW glutenin 20 possess weak gluten and consequently poor pasta- and bread-making quality. Genotypes possessing either 7+8 or 13+16 showed medium to strong gluten characters. Lines with superior gluten strength and bread-making quality were found among the germplasm possessing any of these two HMW-glutenin subunits (Table 4).

## Conclusions

The CIMMYT-derived durum wheat germplasm used in this study was characterized as having mainly medium to strong gluten types, possessing tenacious gluten character, and possessing the LMW-2 glutenin block in a very high frequency (>95%). In addition, a large proportion of the lines showed superior bread-making quality, which was strongly associated with gluten strength. These characteristics of CIMMYT-derived germplasm have resulted from early generation screening using the SDS-sedimentation test as the main screening criterion.

The SDS-sedimentation test remained the best single small-scale test to screen for gluten strength and consequently for pasta-cooking and bread-making quality in durum wheat. Although the Gluten Index is as good a predictor of gluten strength as GRNSED, the latter is more practical and allows a much larger number of samples to be screened in a given time. GRNSED and GLUIND, by themselves and in conjunction with GRNPRT, do not make it possible to select only germplasm with strong gluten type; medium-to-weak gluten types are still retained. Therefore, Mixographic parameters more closely related to gluten strength than the previous ones should be used in a second screening phase to ensure that only medium-strong to strong gluten types are retained in the breeding process.

As noted, the population used in this study was practically all of the LMW-2 type. Even so, the population possessed a wide range of gluten strength types. The weak types were mainly associated with the presence of the *Glu-B1*-controlled subunit 20, and the stronger ones with subunit 6+8. Subunits 7+8 and 13+16 were present in cultivars possessing, on average, intermediate-to-strong gluten types. These findings indicate that to attain superior gluten strength it is not enough to incorporate the LMW-2 type glutenin block. In breeding durum wheat for quality characteristics, more importance should be given to specific allelic variations at *Glu-3* and at *Glu-1*. This study provides further evidence that glutenin subunit 20 should be avoided in breeding programs.

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