Wheat quality improvement at CIMMYT and the use of genomic selection on it

Carlos Guzman a, *, Roberto Javier Peña a, Ravi Singh a, Enrique Autrique a, Susanne Dreisigacker a, Jose Crossa a, Jessica Rutkoski a, Jesse Poland b, Sarah Battenfield c

a Global Wheat Program, International Maize and Wheat Improvement Center, Texcoco, Mexico
b Wheat Genetics Resource Center, Department of Plant Pathology, Kansas State University, 4011 Throckmorton Plant Science Center, Manhattan, KS 66506, USA
c AgriPro Wheat, Syngenta, 11783 Archer Rd., Junction City, KS 66441, USA

A B S T R A C T

The International Center for Maize and Wheat Improvement (CIMMYT) leads the Global Wheat Program, whose main objective is to increase the productivity of wheat cropping systems to reduce poverty in developing countries. The priorities of the program are high grain yield, disease resistance, tolerance to abiotic stresses (drought and heat), and desirable quality. The Wheat Chemistry and Quality Laboratory has been continuously evolving to be able to analyze the largest number of samples possible, in the shortest time, at lowest cost, in order to deliver data on diverse quality traits on time to the breeders for making selections for advancement in the breeding pipeline. The participation of wheat quality analysis/selection is carried out in two stages of the breeding process: evaluation of the parental lines for new crosses and advanced lines in preliminary and elite yield trials. Thousands of lines are analyzed which requires a big investment in resources. Genomic selection has been proposed to assist in selecting for quality and other traits in breeding programs. Genomic selection can predict quantitative traits and is applicable to multiple quantitative traits in a breeding pipeline by attaining historical phenotypes and adding high-density genotypic information. Due to advances in sequencing technology, genome-wide single nucleotide polymorphism markers are available through genotyping-by-sequencing at a cost conducive to application for genomic selection. At CIMMYT, genomic selection has been applied to predict all of the processing and end-use quality traits regularly tested in the spring wheat breeding program. These traits have variable levels of prediction accuracy, however, they demonstrated that most expensive traits, dough rheology and baking final product, can be predicted with a high degree of confidence. Currently it is being explored how to combine both phenotypic and genomic selection to make more efficient the genetic improvement for quality traits at CIMMYT spring wheat breeding program.

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1. Understanding wheat quality - quality and what it means to different people

Wheat quality is a very wide subject that will be defined differently by the different stakeholders of the wheat chain, which makes it an extremely complex and variable concept. For farmers in some countries wheat quality is considered what allows them to allocate their harvested grain at the grain market and get the highest price for it. This is usually different among countries, where each one has different regulations that may prime farmers for producing better grain quality or not. Good morphological characteristics (grain size and density through test weight) and absence of grain damage are some of the most common traits considered at grain markets to determine the grade and

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sometimes the prize of the produced wheat. In other countries such as Australia or Canada there are more complex grading systems, in which protein content is usually an important trait (Blakeney et al., 2009). In other countries subsistence farmers will mill and process the wheat to feed their families and in these cases farmers consider wheat quality what allows them to produce a good product with desirable organoleptic properties.

For millers, wheat quality is the ability of a wheat variety to produce high levels of flour or semolina during the extraction process. In this process the level of contamination of the flour/semolina with bran fractions is also important and is linked in most cases to undesirable characteristics for the end-use quality of the product. For milling quality the traits probably most important are the grain morphology, grain density (test weight) (Matsuo and Dexter, 1980) and grain hardness (Edwards et al., 2010). Millers prefer large grain with plump shape which is well filled and not shriveled. These characteristics are also targets for breeders to increase grain yield in the field. On the other hand, food manufactures are more focused on processing quality, the ability of a wheat variety to be processed with minimum cost to give a uniform product, and end-use quality, the ability of a wheat variety to produce a specific product according to the consumers’ preferences. For both types of quality, grain hardness and gluten quality and quantity are critical. Nutritional quality, the ability of a food to supply nutrients for a complete physical and mental development and a healthy life, is becoming also a big priority for food manufacturers due to the interest of consumers in that issue.

Last, but not least, consumers could have very different ideas of what wheat quality means. Some of them will think about the end-use quality of the product, while others could think about the processing conditions (artisanal or handmade vs. mechanized or industrial) or the nutritional quality of food products. End-use consumers vary in terms of quality demands, although there are several traits well identified among consumers as desired for specific products (soft crumb for bread, yellow color in pasta, shelf life of products, etc.). Having all this in mind breeding for quality to satisfy the demands of all the mentioned stakeholders is no simple task.

2. Bread wheat quality improvement at CIMMYT

The International Center for Maize and Wheat Improvement (CIMMYT) leads the Global Wheat Program of the Consultative Group on International Agriculture Research (CGIAR), whose main objective is to increase the productivity of wheat cropping systems to reduce poverty in developing countries. For this purpose CIMMYT works in the development of new wheat germplasm that can be used by national partners to improve their own germplasm or be released directly as varieties when appropriate. The priorities of the breeding program of CIMMYT are high grain yield, disease resistance, tolerance to abiotic stresses such as drought and heat, and desirable quality. Thousands of new breeding lines are evaluated annually in the three main field stations of the Program (Ciudad Obregon and Toluca in Mexico, and Njoro in Kenya). These evaluations lead to the formation of a set of best lines targeted to different environments (irrigated, semi-arid, high rain fed, etc.), which are distributed to national partners as international nurseries. Following this approach CIMMYT germplasm is extensively used worldwide, particularly in developing countries (Lantican et al., 2016).

As above mentioned, wheat quality is an integral part of this breeding process. Wheat Chemistry and Quality Laboratory has been an important component of the Global Wheat Program since its creation. As CIMMYT has a global mission and end-use quality of all bread wheat products developed worldwide are diverse, the strategy of the program has been mainly to guarantee good gluten quality (diverse levels of gluten strength combined with good extensibility) at medium protein content levels, in semi-hard or hard grains. This set of quality parameters is preferred for most products in developing countries. The laboratory has been continuously evolving to be able to analyze the largest number of samples possible, in the shortest time, at lowest cost, in order to deliver data on diverse quality traits on time to the breeders for making selections for advancement in the breeding pipeline. Currently for bread wheat, the following analyses and traits are routinely tested for the samples of the breeding program: grain image analysis (test weight and thousand kernel weight), visual grain inspection (color), grain analysis by NIR (hardness, protein and moisture content), milling (flour yield), flour analysis by NIR (protein, ash and moisture content; water absorption for mixograph, alveograph and bread-making based in Guzman et al., 2015), SDS-sedimentation, dough rheology (mixograph for optimum mixing time and torque, and alveograph for gluten strength and extensibility) and end-use product testing (baking puff loaf for volume and crumb structure). The methodologies used for these analysis are based on the official protocols of the AACC (American Association of Cereal Chemists, 2010), although several modifications have been implemented in both equipment and procedures to gain higher throughput and greater genetic diversity (Peña et al., 1990; Guzman et al., 2015, 2016a). This is necessary to characterize around 2500 samples in five months’ time, deliver data on time, and allow breeders to select based in both field and quality traits.

For a better understanding and use of the wheat quality data generated, samples are classified in five different potential end-use types (1–5), using an index of the phenotypic data (Table 1). In each type there can be also subtypes based on protein content (a for medium-high and b for medium-low). An overview of the typical uses for each category is found in Table 2. Briefly, type 1 are the white and red grain lines suitable for pan type breads in mechanized industry; type 2a (above 11.5% of protein content) are for leavened breads produced in semi-mechanized industry (baguette, supermarket breads, etc.), two-layer flat breads (baladi), and dry & fresh noodles (alkaline, white, instant), while type 2b are for single-layer flat breads (chapatti) and steamed bread (North-China style); type 3 are for lines used to develop handmade products including dense and flat breads and some kind of noodles; type 4a (above 11.5% of protein content) are for steamed bread (South-China style) and white-salted noodles while type 4b are for

| Table 1 | Bread wheat gluten and end-use type classification to facilitate BW breeding at CIMMYT. |
|-------------------|---------------------------------|-----------------|
| Hard wheat | Gluten typea | End-use typeb |
| Hard-white and hard-red | Strong (W > 300; P/L < 1.3) | 1a, 1b |
| Hard-white and hard-red | Medium strong (W = 200–300; P/L < 1.2) | 2a, 2b |
| Hard-white and hard-red | Medium weak (W = 150–200; P/L < 1.1) | 3a, 3b |
| Soft wheat | Strong and medium-weak | 4a |
| Soft-white and soft-red | Weak | 4b |
| Household or utility wheat | Tenacious (P/L > 1.3) or weak in not soft endosperm (W < 150) | 5 |

Type 1a should have grain protein above 12.5% (12.5% M. B.);
Type 2a and 3a should have grain protein above 11.5% (12.5% M. B.);
Type 4a should have grain protein above 11.0% (12.5% M. B.);
Type 5 has no differentiation regarding protein content.

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For a better understanding and use of the wheat quality data generated, samples are classified in five different potential end-use types (1–5), using an index of the phenotypic data (Table 1). In each type there can be also subtypes based on protein content (a for medium-high and b for medium-low). An overview of the typical uses for each category is found in Table 2. Briefly, type 1 are the white and red grain lines suitable for pan type breads in mechanized industry; type 2a (above 11.5% of protein content) are for leavened breads produced in semi-mechanized industry (baguette, supermarket breads, etc.), two-layer flat breads (baladi), and dry & fresh noodles (alkaline, white, instant), while type 2b are for single-layer flat breads (chapatti) and steamed bread (North-China style); type 3 are for lines used to develop handmade products including dense and flat breads and some kind of noodles; type 4a (above 11.5% of protein content) are for steamed bread (South-China style) and white-salted noodles while type 4b are for...
biscuits and cakes; at last type 5 are for the lines unacceptable for any product, sold as utility or feed wheat. The main factors to define these types are grain hardness and gluten properties determined by the alveograph. Hard or semi-hard endosperm lines can be suitable for end-use types 1, 2, and 3, while soft endosperm lines are the ones than can be classified as type 4. About gluten properties, lines included in end-use type 1 should have alveograph W (× 10^{-4}) value above 300 and P/L ratio below 1.3—meaning strong, but not tenacious gluten; end-use types 2 W higher than 200 and P/L ratio below 1.1—meaning medium strong gluten and acceptable-good extensibility; and end-use types 3 W between 150 and 200 and P/L ratio below 1.0—demonstrating weaker gluten strength but extensible. For end-use type 4 the range of values is not well defined, but biscuits and cakes W should not be higher than 125 and P/L below 0.5—meaning weak, but very extensible gluten. The hard or semi-hard lines showing tenacious or weak gluten, and the soft ones with tenacious gluten are automatically classified as end-use type 5, and should be discarded from the breeding program. This is a simple and probably simplistic classification criterion but works well as a selection tool to have an overall idea of the potential of each line in terms of quality. National partners conduct more specific quality analysis, including end-use tests, to further assess if wheat lines match with their quality requirements in their specific region.

In the spring bread wheat program the participation of wheat quality analysis/selection is mainly carried out in two stages of the breeding process: evaluation of the parental lines for new crosses and advanced lines in preliminary and elite yield trials (Fig. 1). The best way to ensure good quality in the breeding lines is to use the appropriate parental lines for crosses. For this reason, annually, all the lines that are part of the crossing block are characterized for the above mentioned quality traits. These lines are additionally analyzed for glutenin composition at the Glu-1 and Glu-3 loci, using SDS-PAGE to have a more complete genetic profile in terms of quality of these lines. The presence of other genes with effects on quality traits as Pin-D1 (Morris, 2002), Wx-1 (Guzman and Alvarez, 2016) or wbn (Guzman et al., 2016b). Based on that information parental lines are recommended to the breeders, who include in each cross at least one line donor of good quality traits and desirable glutenins. Following parental assessment, quality analyses are not conducted until lines reach preliminary yield trials (approximately seven generations). There are three main reasons for this: 1) analyzing quality in the heterozygous or heterogenous state is not indicative of quality in the pure-line; 2) high throughput, accurate methodologies are not available to analyze the extremely high number of entries present in early and middle generations of the breeding pipeline; and 3) probably more important, early testing for quality must not compromise the other priorities of the Program (high grain yield, disease resistance, tolerance to abiotic stresses), which must run with finite testing resources.

Due to these reasons, quality analysis are only performed in those lines already selected for agronomic traits and yield from the preliminary yield trials, resulting in approximately 1400 lines for quality testing. The quality data generated will be used to select which best yielding and highest quality lines will advance to the yield trials in next cycle. After this trial is complete, approximately 500 best performing lines from the second year yield trial are also analyzed for quality. Thus, breeders have two years of quality data to decide which lines should be discarded based on quality traits or which ones can be distributed with partners through international nurseries. Following this approach, the progress in terms of quality in spring bread wheat CIMMYT germplasm has been good in the last ten years (Fig. 2), since the percentage of lines showing poor quality (end-use type 5) is currently much lower than ten years ago.

### 3. Genomic selection for wheat quality improvement

Fast developments in genotyping and sequencing technologies have prompted breeders to use dense genotypic information for genomic selection (Meuwissen et al., 2001). Furthermore, practical evidence obtained from plant and animal breeding data have demonstrated that genomic selection (GS) overcomes the prediction accuracy of models based on a reduced number of loci or that of pedigree-based methods (de los Campos et al., 2009, 2010; Crossa et al., 2010, 2011; Heslot et al., 2012; Pérez-Rodríguez et al., 2012). Genomic selection models were originally developed for a single trait evaluated in a single environment, and most analyses published so far are based on within-environment analyses.

Genomic selection has been proposed to predict quantitative traits (Meuwissen et al., 2001) and is applicable to multiple quantitative traits in a breeding pipeline by retaining historical phenotypes (which the program is already conducting) and adding high-density genotypic information. Due to advances in sequencing technology, genome-wide single nucleotide polymorphism (SNP) markers are available through genotyping-by-sequencing (GBS) at a cost conducive to application for genomic selection (Poland and Rife, 2012; Poland et al., 2012). Currently, the cost of genotyping all ~10,000 first year yield trials lines is equivalent to phenotyping 2000 lines for processing and end-use quality (Battenfield et al., 2016). Thus, genomic selection, for quality alone will assist in selecting for quality at CIMMYT since all first-year yield trials can be predicted, rather than only the yield trial winners. In addition, several other quantitative traits are being predicted.

Several studies have proposed using GS models that accommodate G × E; for example, Burgueño et al. (2012) was the first to extend the single-trait-single-environment GBLUP model to a multi-environment framework reporting important gains in prediction accuracy relative to single-environment analysis. More recently, Heslot et al. (2014) and Jarquin et al. (2014) modeled G × E using both genetic markers and environmental covariates. Several studies also showed that modeling G × E can give substantial gains in prediction accuracy.

In GS, phenotypic and marker data on the base training population are fitted together in a statistical model in order to estimate all marker effects. These estimates can then be used to compute the genomic estimated breeding values (GEBVs) that are predictors of the breeding values of unobserved genotypes for selection in the testing population for which there is only marker information (Heffner et al., 2009; Lorenz et al., 2011). Heffner et al. (2011) and Bernardo and Yu (2007) have shown that selection based on genomic predictions can lead to greater genetic gain for complex traits.

Several methods for predicting marker effects and breeding values have been developed (VanRaden, 2008; Goddard, 2009; de los Campos et al., 2013; Gianola, 2013) and used for wheat traits prediction at CIMMYT (Crossa et al., 2016; Mondal et al., 2016). The standard method is the Ridge-Regression Best Linear Unbiased Predictor (or its equivalent, the Genomic BLUP). Methods such as Bayes A and Bayes B assume that the variance of marker effects has an a priori inverse chi-
squared distribution (Meuwissen et al., 2001) that produces shrinkage as well as variable selection. The difference among several Bayesian models is how they specify the prior distribution of the parameters of interest. In general, when the random marker effects have a multivariate normal distribution and the size of the training population and the number of markers is large, all methods produce GEBVs that are highly correlated with the true breeding values of the candidates for selection (Hayes et al., 2009; Verbyla et al., 2010).

We know some loci control large portions of trait variances; for example, hardness is controlled primarily by Pin-D1 genes (Lillemo et al., 2006; Morris, 2002), and dough rheology traits have large impact from high and low molecular weight glutenins (Payne et al., 1987). However, not all genes controlling these traits have been identified, and in any cases clear “best” ideotypes of quality genes have not been identified to allow marker-assisted selection a clear path regarding quality traits, and finally breeding programs must cross outside of...
ideal quality for diversity in other traits of interest such as yield and disease resistance.

Battenfield et al. (2016) applied genomic selection to all of the processing and end-use quality traits regularly tested in the CIMMYT spring wheat breeding program as listed above. These traits have variable levels of prediction accuracy, however, they demonstrated that most expensive traits, dough rheology and baking levels of prediction accuracy, however, they demonstrated that most

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The next challenge in implementing genomic selection for multiple processing and end-use quality traits is determining best methods for making selections in the breeding program. Since there are several end-use type classifications and both upper and lower thresholds on many quality traits, “good quality” may mean keeping the population consistent. In this regard, genomic selection predictions have been returned to breeders along mean and standard deviation of trait GEBVs. Wheat lines with trait GEBV one and two standard deviations above and below the mean are identified. Predicted end-use classification on the total predicted quality profile is examined like the empirical phenotypic end-use type profile. For example, two standard deviations above mean for predicted Alveograph P/L is predicts tenacious gluten, Alveograph W one or two standard deviations below mean predicts very weak compared to other material in the program. This system does not specifically predict end-use quality type, but is still useful for breeders who will be able to discard the lowest quality lines of each cycle, advancing lines which are outside of a given quality profile.

Well-known check variety lines have also been included in genomic selection predictions of the first year trial yield. These lines were chosen for a study of genetic yield potential improvement, and represent some of the mega-varieties released from the CIMMYT international nurseries (some examples include Seri M 82, Pavon F 76, Attila, and PBW343, to newer entries such as Roelfs 2007 and Bolauga 100) (Guzman and Alvarez, 2016; Velu et al., 2016). Among these lines are good examples of good and poor bread making quality, and lines commonly used for making chappati. The predicted quality profiles of the check varieties were used as both a validation of using the mean ± 1 and 2 standard deviation methods, and also demonstrated what various quality profiles would look like among the predictions.

In conclusion, genomic selection is being revealed as an efficient approach to support wheat quality improvement in breeding programs. However, genomic selection is not a complete replacement for phenotypic selection. In the CIMMYT program, currently genomic selection is applied to filter for quality selection among all ~10,000 first-year yield trials lines. This allows a first pass to filter out lines that would be very poor in processing and end-use quality, mostly end-use type 5. Yield is still the first metric of performance in the CIMMYT program, so top yield performers (~top 10%) will remain in the program regardless of quality. Genomic selection is applied in the next set of materials (11–25% top yielding) to break ties among performance, where lower quality materials are not advanced, allowing CIMMYT to better utilize resources in the breeding pipeline. Phenotypic selection is still used on all materials advanced to make the empirical judgement of lines advancing in the breeding pipeline.

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