Use of rapid tests to predict quality traits of CIMMYT bread wheat genotypes grown under different environments.

Carlos Guzmán*, Suchismita Mondal, Velu Govindan, Jorge E. Autrique, Gabriel Posadas-Romano, Fausto Cervantes, Jose Crossa, Mateo Vargas, Ravi P. Singh, Roberto J. Peña

Global Wheat Program, CIMMYT, Apdo Postal 6-641, Mexico, D.F.

* Author for correspondence (Phone: +52 595 952 1900 ext. 2280; Fax:+52 555 804 7558; E-mail: c.guzman@cgiar.org)

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Abstract

At the International Maize and Wheat Improvement Center (CIMMYT), wheat quality improvement is an important goal of breeding. CIMMYT scientists develop germplasm, which is diverse for quality traits intended for use in the preparation of different wheat-based products. The integration of quality traits is complex due to the high cost of conducting traditional quality tests. One option for tackling this problem is the use of such rapid-small-scale methods as Solvent Retention Capacity (SRC), SDS Sedimentation (SDSS) and Swelling Index of Glutenin (SIG) to predict flour performance. The objectives of this study were to investigate the effect of genotypes, contrasting environmental conditions and their interactions (GxE) on different rapid-small-scale tests, and to identify their suitability for use in prediction of quality traits. A significant GxE effect was observed for all three methodologies. Overall, SIG was found to be the best predictor of gluten strength across different environments. It was also best at determining bread-making quality in some environments, followed by SDSS for bread making. SRC was found to be useful to select for gluten strength, but for extensibility and bread-making more grain data is needed.

Keywords: wheat quality; solvent retention capacity; SDS sedimentation; swelling index of glutenin, alveograph; bread-making.

1. Introduction

Successful adoption of new wheat varieties is largely dependent on the grain yield and grain quality demands of average consumers and industrial food manufacturers (both semi-mechanized and mechanized industrial) in a given region. Due to these complex and multifaceted needs, International Maize and Wheat Improvement Center (CIMMYT) scientists focus on the core breeding challenges of simultaneous improvement of wheat production and quality for global distribution. With the estimated growth of the bakery industry at 6% globally, a need for improved quality varieties has increased, but the integration of quality traits in a breeding program remains a challenge. The focus is often on traits with more direct importance for farmers such as grain yield or disease resistance. Additionally, high costs and time limitations restrict the use of traditional quality tests conducted with the mixograph, farinograph, alveograph, or end-use quality tests in large breeding programs where thousands of genotypes are evaluated annually. Often there is not enough grain in early generations to conduct such analyses. The absence of quality selection tests in the early or middle generations of a breeding program could result in the development of advanced lines unsuitable for release due to related shortcomings of poor processing and end-use quality.

Small-scale, high-throughput methods for predicting flour performance, allow researchers to make a broad selection, discard lines with insufficient quality, keeping those with improved quality. The development of small scale dough testing equipment has been successful in several cases (see Bekes, Lukow, Uthayakumaran, & Mann, 2003, for a good review). Several types of equipment have been developed to work with small samples, including the two-gram mixograph (standard mixographs use 35 g of flour) (Rath, Gras, Wrigley, & Walker, 1990), the micro Z-arm mixer (4 g of flour), analogous to the farinograph (50-250 g of flour) (Haraszi, Gras, Tömösközi, Salgó, & Bekes, 2004), which shows high correlations with standard equipment. Near infrared (NIR) spectroscopy also has a great deal of potential to predict quality traits (Osborne, 2006), but is a costly, difficult for many breeding programs to afford. An economical and time saving alternative is to use simple chemical tests, which result in correlated processing and end-use quality traits. Sodium dodecyl sulfate sedimentation (SDSS), a commonly used traditional quality test, gives an overall idea of gluten quality and a fair prediction of bread-making (Blackam & Gill, 1980; Peña, Amaya, Rajaram, & Mujeeb-Kazi, 1990). The Swelling Index of Glutenin (SIG), developed by Wang and Kovacs (2002a), is a newer high throughput evaluation method, based on the same principle as SDSS (glutenin swelling capacity and insoluble glutenin content) and has revealed the capacity to predict quality traits in bread wheat (Li, Wu, Hernandez-Espinosa, & Peña, 2015; Wang and Kovacs 2002b). Finally, Solvent Retention Capacity (SRC) is another significant means of measuring quality to determine which micro-methods have already been developed (Bettge, Morris, Demacon, & Kidwell, 2002; Guzman, Posadas-Romano, Hernandez-Espinosa, Morales-Dorantes, & Peña, 2015). SRC, originally developed by Slade and Levine (1994), determines the capacity of flour to hold four solvents: water, associated with the overall water holding capacity of all flours constituents; 50 g/L sodium carbonate, related to the damaged starch content of the flour; 500 g/L sucrose, associated with the concentration of arabinoxylans; and 50 g/L lactic acid , associated with the glutenin swelling capacity (Gaines, 2000). This method develops a flour-quality profile that defines the contribution of individual grain components (Kweon, Slade, & Levine, 2011). This method has been widely used in soft wheats for cookie-making (Duyvejonck, Lagrain, Pareyt, Courtin, & Delcour, 2011; Gaines (2004); Guttieri, Bowen, Gannon, Brien, & Souza, 2001;

Pasha, Anjum, & Butt, 2009; Zhang, Zhang, Zhang, He, & Peña, 2007) and in hard wheat germplasm for other products (Colombo, Pérez, Ribotta, & León, 2008; Duyvejonck, Lagrain, Dornez, Delcour, & Courtin, 2012; Li et al. 2015; Xiao, Park, Chung, Caley, & Seib, 2006). However, most of the aforementioned studies, which used hard bread wheat were undertaken with a limited number of genotypes and/or under a single set of environmental conditions. More SRC data from diverse genetic backgrounds and environmental conditions are needed to validate the value of this test in breeding programs and to understand its use relative to SDSS and SIG.

This study aimed mainly to investigate the effect of genotype (G), contrasting environmental (E) conditions and their interactions (GxE) on SDSS, SIG and SRC. It also aimed to identify the suitability of those methods for use in the prediction of quality traits in a set of CIMMYT bread wheat lines grown worldwide.

2. Materials and methods

2.1 Plant Materials and Field Trials

A trial consisting of 54 CIMMYT bread wheat lines, including advanced lines, historical and modern varieties (Electronic Supplementary Material 1), were sown in the 2012-2013 and 2013-2014 crop seasons in Ciudad Obregon (Mexico). The trial was set up in a lattice square design with three replications and sown under six different environmental conditions: optimum irrigation with drip (control environment); flat sown with basin irrigation; reduced irrigation or moderate drought stress; severe drought stress; medium heat stress and severe heat stress. More details of the trial are illustrated in Guzman, Autrique, Mondal, Singh, Govindan, Morales-Dorantes, et al. (2016)

2.2 Grain and flour parameters

Thousand kernel weight (g) and test weight (g/L) were evaluated with the digital image system SeedCount SC5000 (Next Instruments, Condell Park, Australia). Grain protein (g/kg), hardness (%) and moisture content were determined by near-infrared spectroscopy (NIR Systems 6500, Foss, Hillerød, Denmark) calibrated based on official American Association of Cereal Chemists (AACC) methods 39-10 and 46-11A (AACC, 2010). Grain samples previously conditioned at 140-160 (g/kg) of moisture were milled into flour using Brabender Quadrumat Jr (C. W. Brabender OHG, Duisburg, Germany).

Measurement of SDSS volume was carried out according to Peña et al. (1990). SIG was determined with lactic acid according to the second variant of the method used by Wang and Kovacs (2002a). SRC was carried out according to Guzman at al. (2015) with four solvents: water, sodium carbonate, sucrose and lactic acid. All data from these tests are available in Electronic Supplementary Material 1.

2.3 Rheological and baking tests

Dough development properties were determined by Mixograph of Swanson (National Mfg., Lincoln, U.S.A.) using 35 g of flour (AACC method 54-40A), obtaining dough development time and %Torque*min. The Chopin Alveograph (Trippette & Renaud, Villeneuve-la-Garenne, France) was used to determine dough tenacity, extensibility, strength (ALVW) and tenacity/extensibility ratio (ALVP/L) (AACC 54-30A) using 60 g of flour. The bread-making process was conducted using the direct dough method (AACC method 10-09) and bread loaf volume was determined by rapeseed displacement using a volumeter.

2.4 Statistical Analysis

Pearson correlation coefficients (r) and the significance of each comparison in the study were obtained using SAS.

Combined analyses of variance (ANOVA) across environments for grain and other quality traits were performed using procedure Proc Anova of the SAS statistical software (SAS, 2014).

The means of genotypes in each environment throughout the two-year period during which the trials were undertaken were used in the variable selection stepwise procedure using an alpha level of 0.0001 (Proc Stepwise, SAS version 9.4, 2014). All multiple regression equations are detailed in Electronic Supplementary Material 2.

3. Results and discussion

3.1 Grain and flour characteristics

The data of both cropping cycles were quite similar (data not shown), explained by the high heritability revealed in all traits (Table 1). A wide range in grain characteristics was observed in genotypes across different environments. Test weight and particularly thousand kernel weight grain morphology parameters showed great variability, between and within each environment. The range of values for grain hardness was somewhat smaller (32-55%), without any samples showing real soft texture (>55%). For grain protein content, the variation was also important (107-175 g/kg) and larger in such highly stressed environments as severe drought stress or severe heat stress. Compared to the optimum environment (110-141 g/kg) protein content was high in severe drought (12-17.1%) and heat stress environments (121-175 g/kg). Across environments, test weight and thousand kernel weight showed a negative association with grain protein content, r = -0.48 and -0.52, respectively, ($p<0.0001$), due to a dilution or concentration effect depending on grain size. In SRC tests, lactic acid SRC showed the highest variation and the lowest was shown in sodium carbonate SRC, with water SRC and sucrose SRC (also showing smaller ranges than lactic acid SRC. The range of lactic acid SRC in control environment (105-162.3%) was similar to that found by Duyjevonck et al. (2012), Li et al. (2015) and Xiao et al. (2006) (studies conducted using hard wheat). Lactic acid SRC is related to gluten strength (Gaines 2000) and thus higher values are expected as this study included hard or semi-hard bread wheat lines often used for products that require medium-strong gluten compared to soft wheat or cookie-making that requires weak gluten content (Guttieri et al. 2001; Zhang et al. 2007). Lactic acid SRC significantly increased in drought-stressed environments, most likely due to the increase in grain protein content (*r* value between lactic acid SRC and grain protein content in the whole trial was 0.33, $p<0.0001$), although no such effect was seen in medium and severe heat-stress environments. The increase in grain protein content in heat-stressed environments is done together with qualitative change in protein composition (a decrease in glutenin-to-gliadin ratio) that influenced the lactic acid SRC values (and weaker gluten). Previous studies have reported such observations (Blumenthal, Bekes, Gras, Barlow, & Wrigley, 1995). A similar trend was found also in SDSS and SIG. A significant correlation was observed between lactic acid SRC and SDSS and SIG ($r = 0.32$ and 0.48, respectively, $p<0.0001$), and SDSS with SIG ($r = 0.76$, $p<0.0001$). The same fact was observed by Duyvejonck et al. (2012) with Zeleny sedimentation and lactic acid SRC.

Sodium carbonate SRC is related with grain hardness ($r = -0.55$, $p<0.0001$) because sodium carbonate SRC is related to flour starch damage. Both high and low sodium carbonate SRC values were observed, higher values indicating hard texture (Duyvejonck et al. 2012; Xiao et al. 2006) and lower values soft texture (Bettge et al. 2002; Guttieri, Souza, & Sneller, 2008). Higher sodium carbonate SRC values were found in drought environments, associated to lower grain hardness values (hard grain), and lower in severe heat-stress environment, associated to softer texture. The same trend was found in sucrose SRC, which is related to the pentosans and somewhat to gliadin content, and water SRC (Gaines 2000). SRC, SDSS and SIG values were affected by specific irrigation treatments and temperature regimes similar to those reported by Walker, Campbell, Carter, & Kidwell (2008), Zhang et al. (2007) and in contrast to results reported by Guttieri, McLean, Lanning, Talbert, & Souza (2002). As grain protein content, sucrose SRC showed an inverse correlation with test weight and thousand kernel weight (*r* = -0.22 and -0.31, respectively, $p<0.0001$), probably because pentosans are located in the cell wall, which is more concentrated in the grain.

Therefore, the samples appear to represent a wide spectrum of grain and quality traits. This was confirmed with further rheological analysis (mixograph, alveograph and bread-making) (data not showed).

3.2 Analysis of variance

For any methodology to be suitable for use as a wheat-quality improvement selection tool, it must detect significant differences among genotypes and have low GxE effects for consistent selection. This has been already studied for SRC in soft wheat, with several studies reporting high genotypic and limited or no significant GxE effect (Guttieri and Souza 2003; Guttieri et al. 2001, 2002; Pasha et al. 2009). In contrast, significant GxE effects were found in a study by Walker et al. (2008) and by Zhang, Zhang, & He, (2008) in a study involving soft spring and winter wheat lines grown in different locations of Washington state in the United States.

In the current study, genotype was the most important source of variation (Table 2) for water SRC, sodium carbonate SRC, sucrose SRC and SDSS, and the second one, lactic acid SRC and

SIG. This is most likely due to the large diversity for quality traits that the set of lines used had and for the high heritability of those traits. Environmental effect was significant, particularly for lactic acid SRC and SIG, explaining more than 40% of the variation found in those traits. This strong environmental effect was due to highly contrasting field management conditions used in each trial, particularly in severe drought and heat-stress environments. The result for lactic acid SRC is in contrast to that observed by Guttieri et al. (2001, 2002) where no significant environmental effect was observed. On the contrary, Walker et al. (2008) found a significant environmental effect on spring wheat lactic acid SRC values. A significant environmental effect implies that for accurate measurements and efficient selection of genotypes with the rapid tests, control genotypes must be evaluated in diverse environments. This applies to SIG and SDSS tests as well, based on the results in this study. The strong environmental effect on the three traits was somewhat expected as all of them depend on some way in protein content, which is well known to be highly influenced by different environmental conditions.

The year effect was minor. All the interactions related with the genotype (GxE, GxY and GxExY) were highly significant and together explained around 12-19% of the variation, except for lactic acid SRC (9%). This result agrees with Walker et al. (2008), who also found significant interactions for SRC tests in samples produced in a wide range of environments. Previously, Guttieri et al. (2001, 2002) did not find significant GxE for SRC tests. In our study, although the variation explained by interaction effects is low, their significance serves as a recommendation that multiple environments should be used for selection. This approach should be used in breeding programs with a multiregional or global focus similar to CIMMYT´s, which involves the use of contrasting growing conditions in different environments and the use of diverse germplasm that could potentially adapt to each of them.

Among lactic acid SRC, SDSS and SIG, SDSS showed stronger genetic control, thereby suggesting its efficiency in selection for better quality genotypes in breeding programs. Other SRC solvents (water, sodium carbonate, sucrose) also had high genotype effect, making them suitable for selection, although high GxE interactions reveal the need for selection in multiple environments.

3.3 Prediction models for processing and end-use quality traits

In breeding for wheat quality, there are other traits that are taken into consideration in addition to bread-making while evaluating lines, which means that flour quality cannot be expressed by a single trait, but depends on the interaction of several factors. Among them, the most important are dough strength and extensibility, which can be measured by the mixograph (dough development time and Torque) and by the alveograph (balance between tenacity and extensibility, ALVP/L, gluten strength, ALVW). The above mentioned traits together with grain hardness are used in the CIMMYT breeding program to classify the lines evaluated in five different end-use types (Guzman, Medina-Larqué, Velu, González-Santoyo, Singh, Huerta-Espino, & Peña, 2014). Well-trained operators and a large quantity of flour are required for the aforementioned tests. Therefore, the development of simple tests that use just a few grams of flour is essential for early generation testing in breeding programs. . Stepwise multiple regression models were deployed using six different sets of wheat grain and flour quality parameters. The first set included data from SRC tests carried out with the four different solvents; second and third sets only included the data from SDSS and SIG, respectively. The next sets (fourth, fifth and sixth) were equivalent to the first three tests, but in this case data of test weight, thousand kernel weight, grain hardness and grain protein content were added to all of them. Those parameters are routinely and easily obtained with such high-throughput methodologies as image analysis and NIR (Osborne, 2006), which require a small amount of grain. M aking different sets to build prediction models was intended to use as few parameters as possible to really save resources and time, so that only highly significant traits ($p<0.0001$) to each model were included. SRC, SDSS and SIG were not combined in the same set to make clear which of the three popular tests is the best option for analysis of lines from the breeding program. Regression models were first built based on all environmental data of the trial and subsequently for each specific environment (data from both years). The *r* values of each model to predict different quality traits are shown in Table 3. In Electronic Supplementary Material 2 all multiple regression equations are included.

From the first three sets of parameters (SRC, SDSS or SIG) we obtained models that explained much of the variation in target traits. In models developed with all trial data, SIG was found to be the best predictor of gluten strength, with *r* values of 0.81 and 0.90 for torque and alveograph gluten strength (ALVW), respectively. SRC (in this case only lactic acid SRC as the other solvents were not found significant for prediction; see Electronic Supplementary Material 2) was also a very good predictor for those traits (r = 0.85 for ALVW) followed by SDSS (r = 0.77 for ALVW). To predict alveograph gluten tenacity and extensibility, the models built with both SDSS and SIG were unable (not significant) to predict the important alveograph tenacity/extensibility ratio (ALVP/L) value. Predictions with lactic acid SRC for ALVP/L improved slightly ALVP/L (r = 0.58) although they were far from accurate. For alveograph tenacity and alveograph extensibility, the combination of sodium carbonate SRC and water SRC, respectively, with lactic acid SRC, gave acceptable values of prediction. When predicted values of alveograph tenacity and alveograph extensibility were obtained and used to calculate

ALVP/L, the prediction of this trait is improved (*r* = 0.64). For bread-making, SIG and SDSS (*r* 0.67 and 0.66) were remarkably better than lactic acid SRC (0.51). Overall, trial data revealed that SIG was the most useful test to predict end-use quality and gluten strength, and for selection of gluten extensibility lactic acid SRC, sodium carbonate SRC and water SRC should be carried out.

The next research priority was to determine if models built with data from a specific environment would have the same potential to predict quality traits in that environment. In environments with better field conditions lactic acid SRC and SIG were both excellent predictors of gluten strength, being lactic acid SRC slightly better. SDSS was also acceptable but did not reach higher *r* than 0.67 for ALVW. In drought-stressed environments results were the same, but SDSS showed better prediction than previously in medium drought stress environments (*r* = 0.78) and something less in severe drought stress environments (*r* = 0.73). In heat-stressed environments, lactic acid SRC lost some prediction power but water SRC gained significantly in medium heat-stress environments to reach SRC *r* value of 0.74 and the same in severe heat-stress environments, where of all SRC solvents only lactic acid SRC again played a significant role. SDSS predictions for gluten strength were improved in medium heat-stress environments and even more in severe heat stress environment, but SIG fared better (*r* = 0.84). Again, results indicated that SIG is the best rapid test to predict gluten strength consistently across different environments. Wang and Kovacs (2002b), and Li et al. (2015) reached the same conclusions after conducting similar experiments. The ability of SIG to predict gluten strength compared to other tests is due to its higher association with the insoluble glutenin fraction in flour (Wang and Kovacs 2002a).

For alveograph tenacity/extensibility ratio (ALVP/L) prediction SIG and SDS models results were completely insignificant. Lactic acid SRC data was also insignificant. This could be due to the interaction of other factors, apart from the insoluble glutenin fraction, which affect ALVP/L, reflecting the balance between dough tenacity and extensibility. However, other SRC solvents had a certain amount of prediction power. For example, sodium carbonate SRC (r = 0.61 and 0.68) revealed this characteristic when sown flat with basin irrigation and medium heat-stress environments, respectively. Duyvejonck et al. (2012) also observed the association of sodium carbonate SRC with alveograph tenacity. Dexter et al. (1994) observed that starch with higher damage resulted in more water retention by flour, leading to stiffer dough and related increased dough resistance. Although the r values were moderate, experiments showed they may not lead to accurate prediction of ALVP/L.

For bread-making predictions, SIG was found to be the best in most of the environments (r not higher than 0.64), excluding flat sown with basin irrigation and severe heat-stress environments were SDSS was the best one (r = 0.64 and 0.72, respectively). Lactic acid SRC was only near SDSS and SIG in predicting bread loaf volume in the control environment, so that test is not highly recommended to predict loaf volume if it is not complemented with other data. This finding is in disagreement with Xiao et al. (2006), who found lactic acid SRC better than SDSS when predicting loaf volume (r of 0.83 vs. 0.76) and with Colombo et al. (2008) (r of 0.72 vs. 0.51). Study results are in partial agreement with Li et al. (2015), who showed SDSS to be the best predictor for bread loaf volume sown flat with basin irrigation, severe drought and heat-stress enviroments, in comparison to lactic acid SRC or SIG. Wang and Kovacs (2002b) found SIG and SDSS of equal benefit to predicting bread loaf volume (r = 0.54).

When additional test weight, thousand kernel weight, grain hardness and grain protein content data was added to the original sets of SRC, SDSS and SIG data, the predictions were significantly increased for some traits and in some specific environments. For gluten strength traits, overall predictions were not increased to a great degree, although in some cases progress was evident. For example, SDS + grain hardness in full irrigation environments or lactic acid SRC + thousand kernel weight in severe heat stress environment. SIG alone or with such other parameters as grain protein content in full irrigation environments, continued being the most useful trait to predict gluten strength. With regard to ALVP/L, in most circumstances new data added to the models did not involve an increase in the prediction with SDSS or SIG. Therefore, both rapid tests should be discarded if the objective is to make selection for gluten extensibility. In the case of SRC, a significant increase in prediction for ALVP/L was achieved when other traits were added to the model. In the model showing all data, lactic acid SRC + sodium carbonate SRC+ test weight explained 65% of the ALVP/L variation. In the control environment, the use of lactic acid RC + sodium carbonate SRC + grain protein content resulted in *r* of 0.61, in areas flat sown with basin irrigation environment lactic acid RC + grain protein content + test weight resulted in *r* of 0.7, while in severe heat stress environment *r* of 0.6 (and of 0.69 if ALVP/L is manually calculated from predicted alveograph tenacity and extensibility) was obtained with water SRC + thousand kernel weight. Those values, although not very high, could be sufficient to undertake a broad selection for the purpose of discarding tenacious breeding lines. Additionally, in medium heat stress environment the prediction for ALVP/L reached *r* of 0.78 (sodium carbonate SRC + test weight + grain protein content), which was sufficient to make a more accurate selection.

Finally, for bread-loaf volume prediction, the addition in the model of more grain traits data resulted in very important increases, particularly for SRC. Different combinations of two solvents (lactic acid SRC + sodium carbonate SRC or sucrose SRC) + grain protein content + test weight or thousand kernel weight led to predictions with *r* of 0.78 in full irrigation environments, higher than those of SDSS or SIG in combination with other grain traits. For drought-stressed environments, better predictions are given by SIG + thousand kernel weight, but similar results are obtained with SRC or SDSS. For heat-stressed environments, SDS in combination with grain protein content + thousand kernel weight or with test weight had the higher values. Therefore, for bread making there are various choices of rapid tests – some better than others – depending on the environment and the availability of other grain traits. It seems unsuitable to select only one rapid test for all the environments, but if the priority of the breeding program is to improve gluten strength and bread-making, research shows that SIG is overall probably the best test. The results from our models for predicting loaf volume are slightly inferior to those from Xiao et al. (2006) using similar sets of data (*r* of 0.83 or 0.87), probably because they also include milling and mixograph parameters in the model. Using those parameters, lactic acid SRC, combined with other traits, produced better result than SDSS combined with other traits, which for this study occurred in full irrigation environments.

As expected, grain protein content data was incorporated as a significant factor into several of the predictions models, as it is well known that processing and end-use quality traits are highly dependent on protein quality and quantity. Test weight and thousand kernel weight were significant in some models because they explained the concentration or dilution of other grain components that affect quality. Grain hardness was also significant in some models due to its profound effect on dough water absorption, which can influence several rheological and end-use quality tests.

4. Conclusions

In the current study, a large and diverse set of hard bread wheat germplasm was analyzed with different rapid tests. Research confirmed that the three methodologies are under strong genetic control, although environmental effects and GxE interactions were significant in all cases with an impact significant enough to consider the necessity of multi-location evaluation. The prediction efficiency for several quality traits of each rapid test was evaluated in different environments and in combination with other grain traits, an important component to help breeding programs select the best methodology depending on field conditions and traits of interest. In addition to these discoveries, when choosing a rapid-small-scale test to screen for quality attributes in a breeding program, research revealed the importance of considering the number of samples that can be analyzed per unit time, the cost of that activity and the equipment required to do it. In this regard, SRC tests and SIG, performed as described by Guzman et al. (2015) and Wang and Kovacs (2002a) require more expensive equipment (thermomixer and centrifuge for small test tubes), which also ensure high repetitiveness of the analysis done. Besides, both methodologies imply the use of Eppendorf test tubes, which could be discarded after running the test or be cleaned, although in the case of SRC this is extremely time-consuming because the pellets remain strongly adhered to the bottom of the tube. The SDSS performed as described by Peña et al. (1990) requires 25 ml test tubes that need to be cleaned after the test. The three methodologies are high-throughput and hundreds of samples can be analyzed in one day. SDSS is faster because it requires a higher flour amount (1g), making the sample weighting process much faster than for LARC or SIG, for which 0.3g and 0.02g must be accurately weighted in a precision balance. Evaluating these methodological characteristics is also important when selecting a methodology to be implemented in the breeding program.

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Table 1. Mean values, value ranges and heritability for grain traits and rapid tests found for 54 bread wheat genotypes in the whole and in each environment of the trial.

	Total			**Full drip irrigation**		**Full basin irrigation**		**Mild drought stress**		**Severe drought stress**		**Mild heat stress**		**Severe heat stress**	
	Mean	**Range**	**Heritability**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Grain traits															
Test weight (g/L)	802	679-846	0.88	812	767-832	811	756-841	815	751-842	800	742-828	802	744-830	773	679-807
Thousand kernel weight (g)	41.6	23.5-59.3	0.97	46.5	29.4-56.7	43.4	28.1-55.1	45.8	30.6-57.6	37.6	26.7-52.5	42.6	30.8-59.3	33.3	23.5-45.9
Grain hardness (%)	42.6	32-55	0.9	42.2	36-50.8	43.2	36-52.4	40.0	35.0-48.8	41.5	32-51	43.3	36-53.4	45.5	38-55
Grain protein content (g/kg)	135	107-175	0.83	124	110-141	120	107-138	137	117-158	150	120-171	129	114-152	150	121-175
Rapid tests															
Water SRC (%)	70.2	56.4-83	0.92	69.8	58.2-78	68.8	56.4-77.3	72.1	61.5-83.1	72.8	63.3-81.4	69.8	60.5-77.3	68.0	57.3-77
Sodium carbonate SRC (%)	79.1	62.4-94.3	0.94	79.5	67.1-90	78.2	65.5-88.6	81.4	69.2-94.3	81.1	69.1-89.4	78.4	67-89.7	75.9	62.4-86.5
Sucrose SRC (%)	94.1	80.3-110.6	0.92	91.4	81.2-101.7	89.9	80.3-98.5	96.4	82.2-109.8	98.9	88.7-110.7	92.9	83.9-105.5	95.3	82.4-108-8
Lactic acid SRC (%)	128.4	87.4-196.2	0.95	125.6	105-162.3	122.2	92.7-163.1	139.1	108.4-177.5	146.9	119.6-196.2	120.5	91.8-160.9	116.3	87.4-152.2
SDSS (ml)	15.7	8-23.0	0.96	13.9	8-21.5	13.8	8-20.5	15.7	10-21.5	18.7	11.5-23	15.0	9-22.5	17.1	9-22.0
SIG	5.9	4.2-7.9	0.92	5.6	4.6-6.6	5.5	4.2-6.5	6.0	5-7.4	6.6	5.1-7.9	5.7	4.7-7.3	6.1	4.9-7.4

SRC: solvent retention capacity; SDSS: SDS sedimentation; SIG: swelling index of glutenin.

Table 2. Effects of genotype, environment and year and their interactions on the rapid tests. Sum of squares and percentages of sum of squares respect to the total sum of squares obtained from ANOVA analysis are showed.

		SDSS		**Water SRC**		**Sodium carbonate SRC**		**Sucrose SRC**		**Lactic acid SRC**		**SIG**	
	DF	SS	% SS	SS	% SS	SS	% SS	SS	% SS	SS	% SS	SS	% SS
Genotype	53	7861	54	8411	48	13934	54	15295	41	125491	40	145	34
Environment	5	4024	28	3711	21	4570	18	12075	33	152620	49	178	41
Year	1	15	0.1	177	1	62	0.2	74	0.2	7	0**	23	5
GxE	265	948	6	1961	11	2587	10	4257	12	15018	5	30	7
GxY	53	211	1	265	1	504	2	433	1	2843	1	6	1
YxE	5	607	4	1152	6	1545	6	1355	4	4026	1	10	2
ExGxY	265	669	5	1057	6	1592	6	1664	4	9434	3	25	6

*All the effects were highly significant (p<0.0001), except **.

SDSS: SDS sedimentation; SRC: solvent retention capacity; SIG: swelling index of glutenin.

Table 3. *r* correlation values obtained with different multiple regression equations to predict processing and end-use quality traits using rapid tests and grain traits in the whole trial and in each environment.

Total (six environments)	**Dough development time**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L***	**Loaf volume**
Set 1: SRC	0.63	0.74	0.85	0.58	0.78	0.70	0.64	0.51
Set 2: SDSS	0.66	0.73	0.77	NS	NS	0.62	NS	0.66
Set 3: SIG	0.73	0.82	0.91	NS	0.52	0.52	NS	0.68
Set 4: SRC + GT	0.63	0.74	0.85	0.64	0.81	0.77	0.69	0.78
Set 5: SDS + GT	0.66	0.73	0.79	NS	NS	0.71	0.51	0.74
Set 6: SIG + GT	0.73	0.82	0.91	0.48	0.65	0.69	0.51	0.80

Full drip irrigation	**Dough development**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L***	**Loaf volume**

	time							
Set 1: SRC	0.60	0.72	0.83	0.50	0.71	0.57	0.52	0.58
Set 2: SDS	0.60	0.65	0.64	NS	NS	0.57	NS	0.57
Set 3: SIG	0.62	0.71	0.79	NS	0.48	0.52	NS	0.59
Set 4: SRC + GT	0.60	0.72	0.83	0.62	0.77	0.62	0.58	0.78
Set 5: SDS + GT	0.64	0.68	0.73	NS	NS	0.64	0.48	0.73
Set 6: SIG + GT	0.68	0.75	0.81	0.47	0.65	0.65	0.50	0.74

Full basin irrigation	**Dough development time**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L***	**Loaf volume**
Set 1: SRC	0.67	0.76	0.84	0.62	0.77	0.67	0.68	0.57
Set 2: SDS	0.61	0.64	0.68	NS	NS	0.64	NS	0.65
Set 3: SIG	0.67	0.73	0.82	NS	0.52	NS	NS	0.58
Set 4: SRC + GT	0.76	0.82	0.85	0.70	0.77	0.84	0.71	0.81
Set 5: SDS + GT	0.71	0.75	0.73	0.57	0.57	0.77	0.55	0.77
Set 6: SIG + GT	0.76	0.73	0.84	0.57	0.71	0.73	0.59	0.75

Mild drought stress	**Dough development time**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L***	**Loaf volume**
Set 1: SRC	0.66	0.77	0.85	0.50	0.67	0.66	0.57	0.61
Set 2: SDS	0.66	0.73	0.78	NS	NS	0.62	NS	0.64
Set 3: SIG	0.66	0.77	0.85	NS	NS	0.52	NS	0.65
Set 4: SRC + GT	0.66	0.77	0.85	0.50	0.67	0.66	0.57	0.71
Set 5: SDS + GT	0.66	0.73	0.78	NS	NS	0.66	NS	0.71
Set 6: SIG + GT	0.66	0.77	0.85	NS	NS	0.62	NS	0.71

Severe drought stress	**Dough development time**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L***	**Loaf volume**
Set 1: SRC	0.68	0.78	0.86	NS	0.62	0.56	0.45	0.49
Set 2: SDS	0.63	0.69	0.73	NS	NS	NS	NS	0.58
Set 3: SIG	0.75	0.82	0.89	NS	NS	NS	NS	0.65
Set 4: SRC + GT	0.68	0.78	0.86	NS	0.71	NS	0.50	0.71
Set 5: SDS + GT	0.63	0.69	0.73	NS	NS	NS	NS	0.75

Set 6: SIG + GT	0.59	0.69	0.75	NS	NS	NS	NS	0.75
Mild heat stress	**Dough development time**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L****	**Loaf volume**
Set 1: SRC	0.63	0.69	0.75	0.69	0.76	0.66	0.71	0.45
Set 2: SDS	0.62	0.62	0.75	NS	NS	0.58	NS	0.59
Set 3: SIG	0.59	0.69	0.75	NS*	NS	NS	NS	0.65
Set 4: SRC + GT	0.58	0.66	0.77	0.79	0.79	0.79	0.79	0.69
Set 5: SDS + GT	0.71	0.72	0.78	NS	NS	NS	NS	0.73
Set 6: SIG + GT	0.69	0.74	0.78	NS	NS	NS	NS	0.77
Severe heat stress	**Dough development time**	**Torque**	**Alveograph W**	**Alveograph P/L**	**Alveograph tenacity**	**Alveograph extensibility**	**Alveograph P/L***	**Loaf volume**
Set 1: SRC	NS	0.61	0.74	0.51	0.76	0.54	0.54	0.46
Set 2: SDS	0.68	0.75	0.79	NS	NS	0.66	NS	0.72
Set 3: SIG	0.65	0.75	0.84	NS	0.57	NS	NS	0.62
Set 4: SRC + GT	0.58	0.71	0.82	0.61	0.79	NS	0.69	0.72
Set 5: SDS + GT	0.68	0.75	0.79	0.51	0.57	0.73	0.51	0.77
Set 6: SIG + GT	0.65	0.75	0.84	0.51	0.67	0.65	0.55	0.75

*NS: not significant; the rest of *r* values were highly significant (p<0.0001); **ALV P/L calculated using predicted alveograph tenacity and alveograph extensibility.

SDSS: SDS sedimentation; SRC: solvent retention capacity; SIG: swelling index of glutenin; GT: grain traits, which could include test weight, thousand kernel weight, grain hardness and grain protein content.