Journal of Agricultural Science; Vol. 4, No. 7; 2012
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Genotype-based Stability of Dough Quality in Wheat from Different Growth Environments

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Received: February 29, 2012 Accepted: March 19, 2012 Online Published: May 22, 2012

doi:10.5539/jas.v4n7p41 URL: http://dx.doi.org/10.5539/jas.v4n7p41

Abstract

Consistency of dough properties is an important requirement of millers and bakers. Attempts to achieve this aim by prior testing (prediction) of grain samples might become unnecessary if varieties could be identified that are largely tolerant to the effects of growth conditions on dough quality. Three commercial Australian wheat varieties (Janz, EGA Gregory and LongReach Guardian) were grown in four different locations in New South Wales, Australia. Their dough quality was evaluated by several small-scale methods to determine the extent to which the varieties differed in dough quality due to variations in growth conditions. Of these varieties, LongReach Guardian showed stability of dough quality irrespective of growth conditions as indicated by the results of SIG testing, of ten-gram Mixograph, extension testing and of fundamental dough testing, based on G(1) and Hencky strain. This pilot-scale experiment indicates that there is significant promise in breeding varieties for tolerance to the effects of growth conditions on dough quality.

Keywords: wheat dough, rheology, uniaxial extension, stress relaxation, genotype, environment

1. Introduction

Millers and bakers require consistent quality for wheat and flour consignments. ‘No nasty surprises’ may be a general expression of this requirement. ‘Nasty surprises’ are exemplified by foreign objects that could damage milling equipment and by contaminants such as ergot and tainting weed seeds. ‘Nasty surprises’ that are more difficult to detect, to anticipate and to counteract include variations in dough quality, which can lead to complications in the plant bakery (Cauvain, 2012; Hajselova & Alldrick, 2003; Wrigley, 2009).

The potential for dough-processing quality to be suited to the wheat grade or class is ‘built in’ by the breeder, by selection of appropriate progeny (Ross & Bettge, 2009). However, growth environment exerts considerable influence on the quality of the harvested grain, due to the vagaries of weather, soil nutrition, farming practices and harvest conditions (Gooding, 2010). The specific contributions of genotype (G, the variety that has been planted) and of the growth environment (E) are different for each of the various aspects of grain quality (Wrigley & Batey, 2003; Delwiche, 2010). For example, growth conditions have less effect on grain hardness than on grain protein content in many growth regions.

On the other hand, genotype makes little or no contribution to the moisture content, or to the presence of contaminants and weed seeds in the harvested grain (Wrigley & Batey, 2003). The attribute of sprouting is considered to present a problem only if the environmental factor of rain at harvest is present. The effect of genotype is very important for this attribute because breeders have been able to provide genetic tolerance to the effects of rain at harvest especially for red wheats (Ross & Bettge, 2009; Mares & Mrva, 2001). In this case, there is strong interaction between genotype and environment (‘G x E’) (Ross & Bettge, 2009; Wrigley & Batey, 2003). The ability of such sprout-tolerant genotypes to overcome this adverse environmental factor might be expressed as ‘G x e’.

Dough strength appears to be affected by equal contributions from genotype and growth conditions for dough quality (Wrigley & Batey, 2003, Caffe-Treml et al, 2011), indicated above as a potential source of 'nasty surprises' for millers and bakers. It is well accepted that many aspects of growth environment affect dough quality (Gooding, 2010, Caffe-Treml et al, 2011). Heat stress (a few days of >35^{o}C) during grain filling has been reported to cause dough weakness in the resulting grain (Corbellini, 1998; Blumenthal, 1995). In addition, some genotypes have been reported to be more tolerant than others to the dough-weakening effects of heat stress (Blumenthal, 1995); this example of genotype-based tolerance (G x e) is presumably similar in principle to the well-established tolerance of sprout-resistant varieties to rain at harvest (Mares & Mrva, 2001).

Leaving aside extreme environmental influences such as heat stress, little attention has been given to the possibility that better stability of dough properties might be provided by selecting genotypes that provide grain with dough quality that is affected to only a minor extent by normal variations in growth conditions. This paper describes a pilot study to explore the possibility of identifying genotypes (existing varieties or advanced breeding lines) that may provide significant genotypic tolerance to the effects on dough quality of variations in growth conditions. The extent of this pilot study was restricted to three varieties (Table 1), each grown under four contrasting sets of growth conditions (Table 2), but differing no more than would be encountered in normal commercial practice. The resulting dough properties were tested by a range of established methods plus some less-traditional methods that would provide fundamental information about the resulting dough rheology. Sample treatment (simple milling and sieving of small grain samples to produce flour) and testing methods were selected to be appropriate to the small-scale needs of breeding practice, in anticipation of subsequent application of any of the methodology for this purpose (Ross & Bettge, 2009).

Table 1. Genotypes used in this project and their glutenin alleles

	Janz*	**EGA Gregory***	**LongReach Guardian****
Glu-1 alleles	a, b/u, a	a, u, a	a, u, d
HMW-GS subunits	1, 7+8/7*+8, 2+12	1, 7*+8, 2+12	1, 7*+8, 5+10
LMW-GS subunits	b, b, b	c, b, c	b, b, b

* From Wrigley et al. (http://www.aaccnet.org/grainbin/)

** Personal communication with LongReach Breeding staff.

Table 2. Growth conditions and flour quality

	Coolah	**Canowindra**	**Spring Ridge**	**Wagga Wagga**
Growth Locations				
Latitude	31°50′0″South	33°16'60"South	32°16'0"South	35°7'0"South
Longitude	149°43′0″East	151°13'60"East	149°21'0"East	147°22'0"East
Region	Northern NSW	Central NSW	Northern NSW	Southern NSW
Growth conditions				
Soil N in 0-10 cm layer, measured at sowing time	14.4 mg/kg	32.0 mg/kg	33.3 mg/kg	42.9 mg/kg
Soil P in 0-10 cm layer, measured at sowing time	25 mg/kg	50 mg/kg	18 mg/kg	41 mg/kg
Soil organic C in 0-10 cm layer, measured at sowing time	1.5%	0.9%	1.6%	0.6%
Sowing time	Late May to early June 2008			
Harvest time	Late November or early December 2008			
Average rainfall during grain filling in mm per week	20	14	26	8
Average daily maximum temperature during grain filling	26.7^{o}C	25.8^{o}C	26.1^{o}C	26.1^{o}C

In previous studies (Uthayakumaran et al, 2007), we interacted with a large Australian milling company to develop tests that could be applied to define the dough quality of grain consignments prior to milling. In this case, 'dough quality' was mainly defined by the specifications of the flour customers in terms of Rmax, the height of the Brabender Extensograph curve. For the purposes of this project and its potential for extension into breeding, smaller-scale testing is required. Therefore, the present study employed a small-scale version of the Extensograph; this version has been shown to produce Rmax results similar to the full-scale equipment, while also providing more basic information about dough rheology (Uthayakumaran et al, 2000; Rath et al, 1994). In the previous study (Uthayakumaran et al, 2007), the Swelling Index of Glutenin (SIG) test (Wang & Kovacs, 2002), was found to be a useful indicator of dough strength, for small flour or wholemeal samples. This test was also used in the present studies.

The varieties selected for this study (Table 1) are commonly grown in the wheat belt of New South Wales (Australia). All three are similar in their low-molecular-weight glutenin subunits (LMW-GS) alleles and similar in the A & B genomes for the high-molecular-weight glutenin subunits (HMW-GS) (Wrigley et al, http://www.aaccnet.org/grainbin). However, contrasting HMW-GS were selected for the D-genome; two varieties (Janz & EGA Gregory) have the allele (*Glu-D1a*) for subunits 2+12. The Australian variety LongReach Guardian, distinct from the U.K. variety named Guardian, has the allele (*Glu-D1d*) for subunits 5+10, coming from the parent variety Krichauff. The growth locations were selected to represent the range of NSW wheat-growing regions as well as contrasting soil nutrition, thus providing a range of protein contents in the samples.

2. Materials and Methods

2.1 Grain and Growth Conditions

Wheat grain samples (varieties Janz, EGA Gregory and LongReach Guardian, Table 1) were grown in the 2008 National Variety Trials of the Australian Grains Research and Development Corporation (http://www.nvtonline.com.au/) (Table 2).

The harvested grain was milled into whole meal flour using a Udy Mill. The flour was sieved to pass through a 500 μm screen sieve and stored in Falcon tubes at 4° C until analysed. The protein and moisture contents of the wheat flour samples were determined respectively, by near infrared reflectance analysis (NIR) and by oven moisture testing with Approved AACC Methods 39-11 and 44-19 (AACC Methods, 2002).

2.2 Dough Testing

2.2.1 Simple Dough-quality Testing

The test for the Swelling Index of Glutenin (SIG) (Wang & Kovacs, 2002), was performed as modified slightly by Uthayakumaran et al, 2007.

2.2.2 Traditional (small-scale) Dough-Mix Testing

Water absorption values for all sieved samples were determined using a micro-dough-LAB four-gram Z-arm mixer (Newport Scientific Pty. Ltd., Warriewood NSW, Australia), (Bason et al, 2007). Results were expressed as the amount of water required (as a percentage, based on the amount of flour) for the dough to achieve a mixing resistance up to the 115 mN mark.

To determine the mixing time (*MT*, in minutes) required to reach peak dough development, flour and water were mixed in a Mixograph^{TM} (TMCO, Lincoln, NE, USA), (Cavanagh et al, 2010), suited to a dough sample consisting of about ten grams of flour. Results were collected and analysed using MixSmart software, version 1.0.484 (AEW Consulting, Lincoln, NE, USA).

Small-scale dough-extension testing was carried out according to the equipment and method of Cavanagh et al. (2010). Results were expressed as maximum force (*Fmax* in Newtons) at peak dough development and as the distance to this point (*Dist* in mm).

2.2.3 Basic Rheological Tests

Uniaxial dough elongation

Dough-elongation measurements were carried out on an Instron 5564 Universal Testing Machine at a constant elongation rate of 0.01 s^{-1}. Previous work (Tanner, 2007) has shown that the choice of elongation rate is not crucial. The specimen was stretched between two parallel plates 26.2 mm in diameter. The lower plate was a fixed in position and the upper plate was fixed to the load cell and moving crosshead of the Instron. After mixing to the time of peak dough development in the ten-gram Mixograph (Cavanagh et al, 2010), the dough sample was loaded into a plastic cylinder with an inside diameter of 26.5 mm and a height of 14 mm; it was stored in a sealed bag to

relax for 20 min. Before testing, the Instron was zeroed without any loading, and thin coats of superglue were applied to the lower and upper plates. The plastic cylinder holding the dough was fitted vertically on the lower plate, and the upper plate was brought down until it fully contacted the dough cylinder. The plastic cylinder was slid down over the lower plate. The free cylinder of dough was pressed by hand along the edge so that a thin dough layer could wrap around the lower and upper plates, thus avoiding separation from the plates. A thin layer of Vaseline was applied to the external surface of the dough sample to prevent moisture loss. The mounted sample was compressed to 10 mm, and allowed to relax for a further 20 min to allow any built-up residual stress to decay. During testing, the sample was stretched until it was physically broken. The specimen diameter (and thus the cross-sectional area) was measured using a digital camera that downloaded the results to a computer as a movie. Figure 1a shows traces from some elongation tests. The elongational stress (in Pa, vertical axis of Figure 1a) at any Hencky strain (horizontal axis) could be calculated by dividing the Instron's force/load applied by the area measured. The Hencky strain ε_H is a logarithmic strain, and in simple elongation it is given by

$$\varepsilon_H = \ln[\text{ final length/initial length}]$$

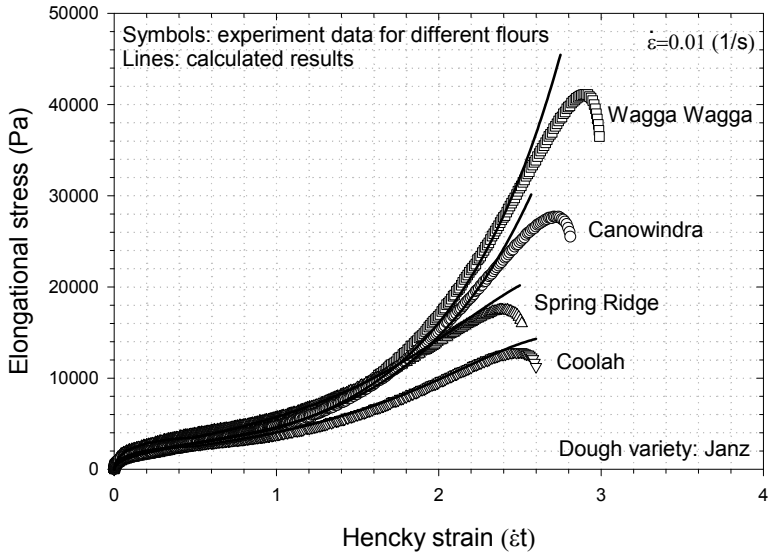


Figure 1a. Effect of growth location on elongational stress and Hencky strain for Janz dough

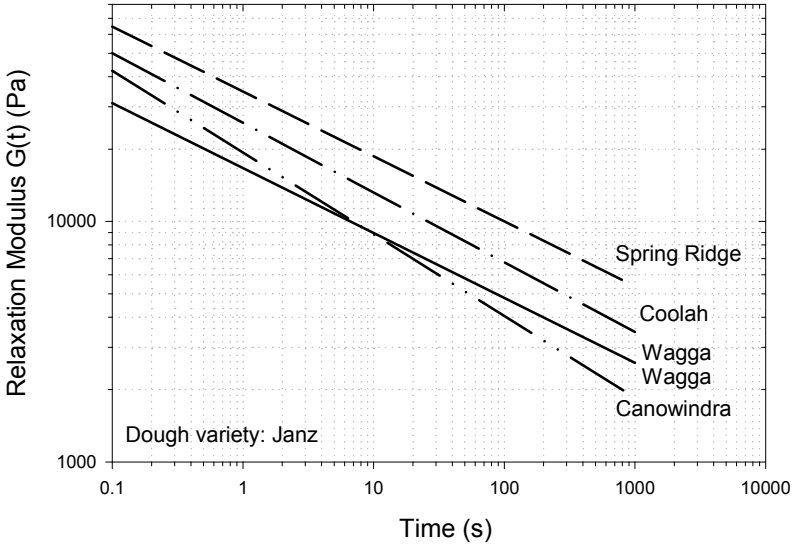
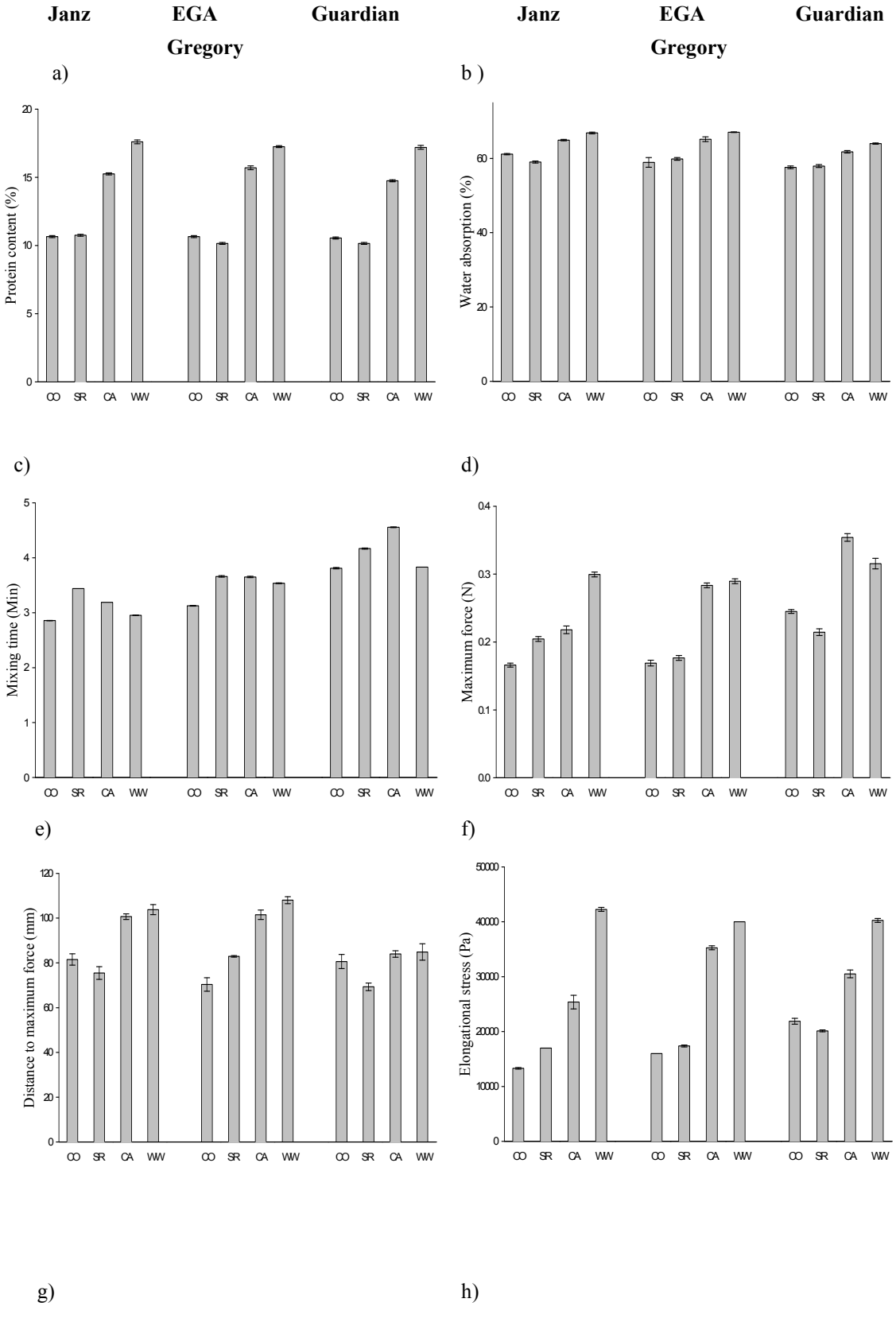


Figure 1b. Effect of growth location on the relaxation modulus for Janz dough

Here ln denotes the natural logarithm (base *e*).

For each flour sample, results were recorded for the elongational stress and the Hencky strain at the point of dough breakage (the maximum stress) (Figure 2).



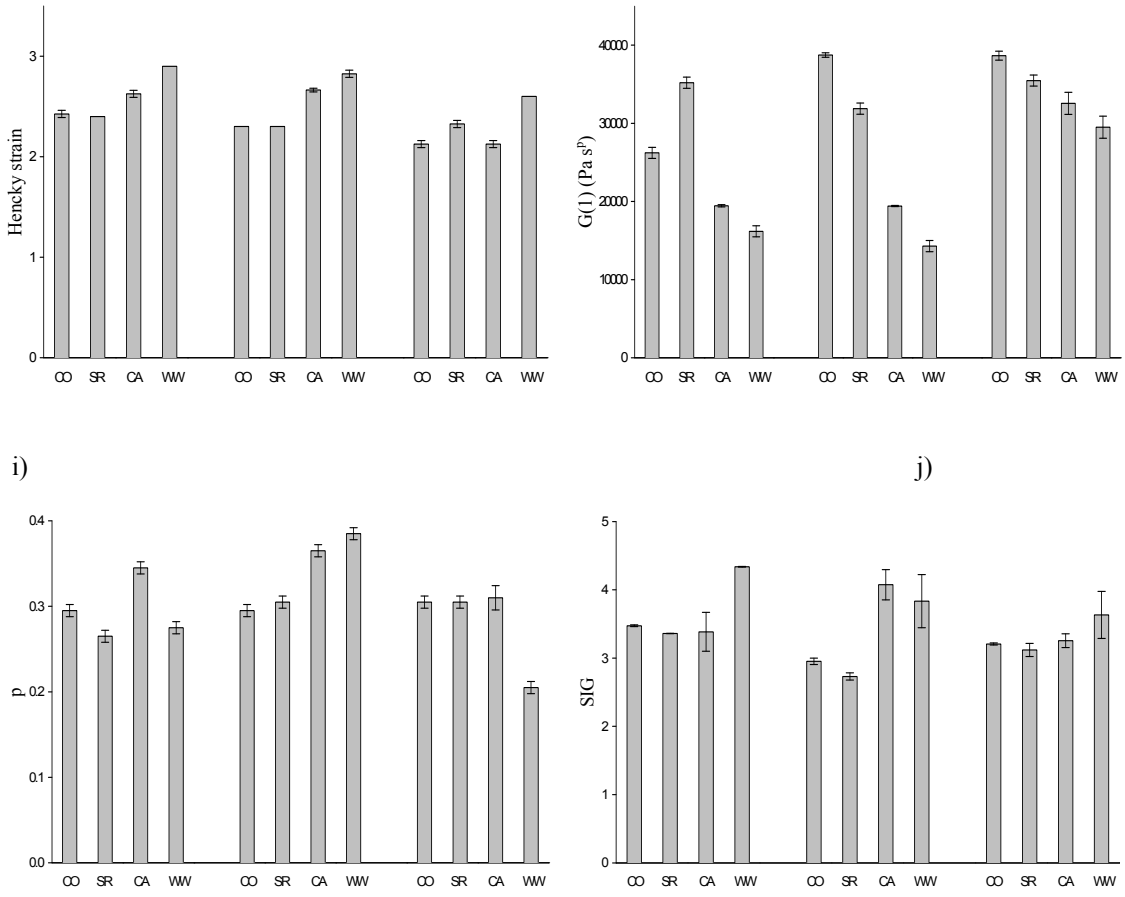


Figure 2. Results for the three varieties for the five locations, side-by-side in the sequence (left-to-right) Coolah (CO), Spring Ridge (SR), Canowindra (CA) and Wagga Wagga (WW). The groups of bars (left to right) are for Janz, EGA Gregory and Guardian. a. Protein content; b. Water absorption; c. Mixing time; d. Fmax; e. Distance to Fmax; f. Elongational strain; g. Hencky strain; h. G(1); i. p; j. SIG.

Shear relaxation measurements

For relaxation measurements (Safari-Ardi & Phan-Thien, 1998), the dough sample (about 3 g) was stored in a sealed bag after mixing in the ten-gram Mixograph (Cavanagh et al, 2010) to peak dough development and it was allowed to relax for 20 min. The relaxation experiments were carried out on a Paar Physica MCR301 shear rheometer. Parallel plates with a diameter of 25 mm were used, and the gap was set to 2 mm for the measurements. Slippage during testing was prevented by gluing fine sandpaper to the parallel plates. The rheometer was calibrated before each test. The sample was mounted on the lower plate, and compressed between the plates by moving the upper plate down to a set gap. Excess dough was trimmed off, the edge of the sample was coated with a thin layer of Vaseline to prevent moisture loss, and the sample was allowed to relax for a further period of 20 min. Relaxation tests were conducted at a small initial shear strain of 0.1%. This strain was applied rapidly in about 20 ms. The results (Figure 1b) were expressed as the variables G1 and p which were calculated as described below.

The resistance of the dough to deformation could be described by the damage function model, which contains few parameters (Tanner, 2007). For small strains, of the order of 0.1%, the model gives the complete linear viscoelastic behaviour in terms of only two parameters: G(1) and p. To explain these parameters, suppose a small shear strain of magnitude γ is suddenly applied to the sample at the initial time (t = 0), and that the decay of the shear stress (τ) –stress relaxation- is then measured. To a close approximation, we find, for t>0,

$$\tau(t) = \gamma\, G(1)\, t^{-p}$$

From the measured shear stress response, for a fixed, small γ, we can find G(1) and p. The G(1) is a constant with the dimension Pa s^p and the value equals the shear modulus (equal to τ/γ) of the dough when t=1s., and so is a

direct measure of the initial stiffness of the dough mix; the larger G(1) is the stiffer is the dough. The p-parameter describes the slope of the logarithmic plots of the decay of τ versus time; the larger p is the quicker is the decay of stress.

2.3 Statistical Analysis

The data compiled was submitted to an analysis of variance (ANOVA) using GENStat Software (Release 13, PA, USA). Values represent means of four replicates.

3. Results

3.1 The Range of Growth Conditions

The four locations differed modestly in growth conditions (Table 2). The times of sowing and harvest were approximately uniform. The temperatures during grain filling were similar and moderate, with no very hot days. On the other hand, there were considerable differences between growth sites with respect to rainfall and soil nutrition. As a result there was a relatively wide range of grain protein content in the harvested grain samples, namely, from 10.1% (Guardian at Spring Ridge) to 17.7% (Janz at Wagga Wagga) (Figure 2a). All samples from the Wagga Wagga site were well above the others in protein content, due to the combination there of higher rainfall and higher soil nitrogen (Table 2). Nevertheless, the samples from the same site were very similar in protein content, indicating that this attribute is governed more by growth environment rather than by genotype.

3.2 Protein Content and Dough Quality

Protein content is acknowledged to relate closely to protein quality and thus to dough strength (Gooding, 2010, Wrigley & Batey, 2003, Uthayakumaran et al, 2000). Protein content is readily monitored at harvest (Delwiche, 2010) and thence further down the grain chain. However, other factors contribute to dough strength and it was the aim of this project to identify the role of such factors.

Water absorption is acknowledged to be largely (but not wholly) determined by protein content (Carson & Edwards, 2009), as was indicated by the close correlation between these two attributes ($r^2 = 0.84$) for this set of samples. Nevertheless, the results for Guardian covered a narrower range in water absorption (6 percentage points) than for the other two varieties (more than 8 percentage points).

Variations in protein content might also be expected to appear as a factor controlling the results of conventional (small-scale) empirical dough testing. These were:

- mixing time (***MT***, in minutes) to achieve maximum resistance to mixing (in the ten-gram Mixograph, Figure 2c), probably equivalent to development time in the full-scale Brabender Farinograph (Dobraszczyk, 2004);
- force at maximum resistance to mixing (***Fmax***, in Newtons, in the small-scale extension tester) (Figure 2d), probably equivalent to Rmax in the full-scale Brabender Extensograph (Dobraszczyk, 2004);
- distance at *Fmax* (***Dist*** in mm, in the small-scale extension tester) (Figure 2e), probably equivalent to extensibility in the full-scale Brabender Extensograph.

However, the correlation coefficients (r^2) with protein content for these empirical attributes were low or modest (0.00, 0.61 and 0.65, respectively). Thus, millers and bakers would be disillusioned to the extent that they might depend solely on protein content to indicate dough strength according to these attributes. An exception to this statement was the range of *Fmax* values for the varieties Janz and EGA Gregory. Whereas the relationships to growth environment were clearly evident for protein content and water absorption, an environment-relevant relationship was not evident for mixing time (*MT*) and distance (*Dist*) to *Fmax*.

On the other hand, a contribution from genotype for these three empirical attributes was seen in the stronger dough quality for Guardian (despite being similar in protein content to the other two varieties) with respect to mixing time and *Fmax*. Its *Dist* results (distance to *Fmax*) were shorter, and importantly much narrower in range compared to the other varieties. To the extent that we were seeking to identify varieties that might vary less with changes in growth environment, there was initial evidence that Guardian may fulfil this requirement.

3.3 Fundamental Rheological Tests

In an attempt to 'measure the forces required to produce controlled deformations', we used two fundamental rheological test systems:

- Uniaxial extension testing. Figure 1a shows how the dough sample under test stretches progressively until it eventually breaks, just after the extension force falls. The two resulting parameters are elongational stress (ES) and Hencky strain (HS).

- The shear relaxation experiments show how the dough sample relaxes with time after the imposed rotational strain. G(1) and p are the two main parameters derived from the shear relaxation testing (Figure 1b).

Elongational stress and Hencky strain (Figures 2f and 2g) were well correlated with protein content (r^2 coefficients of 0.90 and 0.60, respectively), although the range of values was much narrower for Hencky strain, and the differences between sites were less for Guardian. Elongational stress, especially, is presumably influenced strongly by growth environment, thus constituting another case of g x E. The results from shear relaxation (Figure 2g) showed that the derived function G1 (Figure 2h) was generally related to protein content (negatively) (r^2 = 0.60), but the p value (Figure 2i) was not (r^2 = 0.004). The range of G1 values was again much narrower for Guardian.

3.4 Simple Dough-quality Testing

The SIG test was shown by Uthayakumaran et al, (2007) to be a valuable indicator of Brabender Rmax, and thus a practical basis for the commercial segregation of harvest samples to meet the flour specifications of customers. For this set of samples (Figure 2j), SIG values were not significantly correlated to protein content (r^2 = 0.36). Once again, the range of results was narrow for Guardian (no more than the ranges of the error bars), and less than for the other two varieties. Overall, the variety Janz seemed to be more susceptible to environmental influences and Guardian was the least affected. It thus appeared that there is some promise in pursuing this approach of attempting to identify genotypes that will demonstrate dough-quality stability and less-than-normal variation due to growth environment.

4. Discussion and Conclusion

Wrigley and Batey, 2003 considered dough strength as being influenced about equally by genotypic and environmental factors. However, our study indicates that genotypes may differ in the relative contributions of growth environment to dough strength. In particular, the Australian variety LongReach Guardian showed stability of dough quality irrespective of growth conditions, especially as indicated by the results of SIG testing, G(1), Hencky strain, Fmax, Dist and water absorption.

Presumably it is significant that LongReach Guardian differed mainly from the other two varieties in respect of gluten-protein composition by having the HMW-GS 5+10, in contrast to the 2+12 subunits of the other two (Table 1). The 5+10 combination (*Glu-D1d* allele) has been reported on many occasions to relate to stronger dough properties (Ross & Bettge, 2009, Wrigley et al, 2009), as well as relating to tolerance to the dough-weakening effects of heat stress (Blumenthal et al, 1995). The preliminary results now provided add to the apparent beneficial effects of the *Glu-D1d* allele on wheat quality. Breeders are thus alerted to a possible new attribute for this allele.

However, despite the established significant influence of the HMW glutenin subunits on dough quality, a wider range of genes is involved in the regulation of dough quality, as indicated by studies with quantitative trait loci (Kerfel et al, 2010). Nevertheless, our findings provide a new approach to G X E research and an avenue for further study.

An approach, previously espoused by Uthayakumaran et al, 2010, as a means of providing the baker with stable dough quality, was to screen grain samples by a simple test such as the SIG method, thus providing the grain buyer with the tools to see that grain of suitable quality reaches the mill. In the present project, we examined the possibility of moving the emphasis further up the line of the grain chain to the stage of selection in breeding. If quality-stable genotypes could be bred, buyers could select grain according to variety with more confidence about predictable dough quality. If new varieties are thereby made more attractive to grain buyers, the extra selection effort may be justified. Furthermore, this step of selection might be relatively simple, since in the late stages of breeding, advanced lines are commonly grown in a range of relevant sites, thereby offering the opportunity to seek the few that showed the least variability in dough quality (i.e., an interaction of G x e with respect to this trait). Because of the accent on genotype in this approach, we can expect new findings to be helpful with the discovery of more information about the genes (Wrigley et al, 2009) for dough strength and their interaction with growth environment.

Acknowledgement

We are grateful to the Australian Research Council for support by Grant DP 1095097, and the GRDC for providing the grain samples.

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