Application of a 2-Gram Mixograph to Early Generation Selection for Dough Strength[1]

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ABSTRACT

Cereal Chem. 69(3):254–257

A direct-drive, 2-g mixograph was used to examine the heritability of mixing characteristics in wheat flours. A good range of variation in mixograph parameters (each with acceptable errors of determination) was found for flours derived from the seed of F_2, single-plant selections and in their F_3 progeny. The results agreed well over years for the important variables of time to maximum resistance (mixing time) and tolerance to mixing (resistance breakdown), as evidenced by the medium-to-high offspring-parent heritability within and over crosses. These results demonstrate that the 2-g mixograph meets the requirements for use in early generation selection for wheat quality. The value of the instrument was confirmed by simulated selection based on guideline values for two wheat grades and the response to selection as measured using realized heritability estimates.

Cereal laboratories involved with wheat breeding programs continually develop and evaluate new procedures that can be applied to early generation selection for wheat quality. Such procedures must be simple, rapid, and reliable, and they must use only a small quantity of seed, have high correlations with end-use properties, and retain their predictive capacity independent of the location or year of growth. The advantages of such early generation testing procedures are that they result in more efficient use of limited cereal laboratory and breeding resources. Subsequent costly tests for field plot disease resistance, yield, milling, physical dough properties, and baking need only be conducted on lines that have an enhanced probability of having acceptable end-use quality.

Early generation quality tests are many and varied in type, ranging from sedimentation tests of wheat meals and flour (e.g., Zeleny volume, sodium dodecyl sulfate volume, alkaline water-retention capacity) or yeasted wheat meal or flour dough balls (e.g., Pelshenke time) to varied flour-water dough mixing tests (e.g., mixograph [TMCO, Lincoln, NE] and farinograph [Brabender, Duisberg, Germany]), and microscale test baking procedures (MacRitchie and Gras 1973, Shogren and Finney 1984). The results of testing for kernel hardness, protein content, and rate of dough development have been shown to provide adequate prediction of test loaf quality (Fowler and de la Roche 1975a). Each of these parameters showed considerable, although population dependent, genetic variation (Fowler and de la Roche 1975b). Similarly, response to early generation selection has been reported for the use of measures of kernel hardness, protein content, and small-scale tests correlated with dough strength such as sodium dodecyl sulfate volume, percent residue protein, Zeleny volume, and Pelshenke time (Fischer et al 1989, O'Brien et al 1989).

The use of a mixograph employing 10 g of flour (Finney and Shogren 1972) has enabled breeders to select directly for physical dough properties in early generations. The need to mill at least 15 g of seed to produce the flour for this purpose may have restricted its application in early generations in many breeding programs. A smaller recording mixer utilizing 5 g of flour has been reported recently (Finney 1989).

A test that required even smaller sample sizes (2 g of flour obtained from 3–5 g of seed) would have many applications. It could be widely used in laboratories for basic research on wheat quality where quantity of sample is a major limitation. However, the most widespread application would be for quality evaluation in the early generations of wheat breeding programs, allowing early generation testing for physical dough properties to be applied to any breeding program. Seed from single plants, progeny rows, or yield plots could be evaluated.

The applicability of an early generation test relies not only on its correlation with standard measures of wheat quality, but also on the response measurable within segregating breeding populations in the generation following selection. This article reports the use of a direct-drive 2-g mixograph (Rath et al 1990) to determine the physical dough properties of flours from F_2 single-plant selections. The accuracy and effectiveness of the procedure was evaluated on the resulting F_3 progeny.

MATERIALS AND METHODS

Single plant-selections from the F_2 generation of three crosses, Suneca/Sunfield (SS), Vasco/Hartog//Vasco (VH), and Sunbird/Dollarbird (SD), and their derived F_3 lines were used in this study. In the F_2 generation single plants were selected from space-planted rows on the basis of having acceptable straw strength, lodging resistance, height, flowering time, and maturity for the target region of the breeding program (northern NSW, Australia).

Seed of the single plants was stored in a seed room for a minimum period of three weeks until it had equilibrated to approximately 12% moisture content. Ten grams of seed was preconditioned to 15% moisture content for 24 hr before being milled with a Quadrumat Junior flour mill (Brabender, Duisberg, Germany) fitted with a sieving screen covered with 9XX silk mesh. The milling procedure followed was similar to that described by Whan (1974). In the F_3 generation the lines were sown in separate two-replicate experiments with randomized complete block designs, one for each cross. Each experiment included the parents of the cross and the varieties Sunco, Hartog, and Sunelg as checks. Plots were harvested with a plot combine, and a 10-g subsample of each replicate was milled to flour following the same procedures as for the F_2 generation.

Flour protein contents were determined by near-infrared reflectance spectrometry, using a calibration based on Kjeldahl analysis (protein = N × 5.7). All flours were equilibrated for 72 hr at 22°C with air at 60% relative humidity to obtain a moisture content of approximately 12.8% before mixograph testing (Bushuk and Winkler 1957). Water additions for mixing tests were calculated according to a formula relating water addition to flour moisture and protein content (AACC 1983).

Mixing parameters were obtained with a prototype recording dough mixer (TMCO, Lincoln, NE) employing 2 g of flour per measurement (Rath et al 1990). Mixing parameters were obtained by automated interpretation of the recorded data using specially written software (Gras et al 1990). Mixing parameters were measured from the center line of the recorded data after

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[1] This is the written version of a paper presented at the 76th annual meeting of the AACC, held in Seattle, WA, October 13–16, 1991.

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smoothing, using a 25-sec moving mean. Parameters measured were time to maximum resistance and time to maximum bandwidth (both in minutes), maximum resistance (maximum height of the mixing curve), bandwidth at maximum resistance, breakdown in resistance, breakdown in bandwidth, and maximum bandwidth (reported in a 100-unit scale, where 0 and 100 correspond to the minimum and maximum deflection on a standard 35-g mixograph). Resistance and bandwidth breakdowns were defined as the decreases in resistance and bandwidth, respectively, measured 3 min after maximum resistance. The limited quantity of seed available from the F_2 single-plant selections, combined with the predetermined quantity needed to plant the F_3 generation, did not permit remilling of additional samples for repeats of mixographs that were discarded because of operator error. This resulted in a lower number of paired samples (F_2 to F_3) for the SD cross.

Statistical analyses were performed by standard procedures using the MSUSTAT package of statistical analysis programs.

RESULTS AND DISCUSSION

In each generation, a wide range of values was found for most of the dough properties measured by the 2-g mixograph. The means and ranges for these parameters and the flour protein content in the F_2 and F_3 generations are shown in Table I. The skewnesses of the data were usually less than one with the exception of the data for the mix time of the F_2 generation of the VH cross, where the skewness was 1.16. These results indicate that these crosses were a suitable sample to evaluate the effectiveness of the 2-g mixograph for early generation testing of dough-mixing properties.

The duplicate mixographs of the F_2 generation flours indicate that in most cases the measurements of dough properties have acceptably low errors of determination (Table I). A wide range of values and low errors of determination are both prerequisites for the application of the 2-g mixograph to early generation selection.

An analysis of variance of the duplicate mixographs of each field replicate for all lines of all crosses in the F_3 generation indicated significant differences at the 0.001 level of probability between lines for the estimates of time to maximum resistance (mixing time), maximum resistance, resistance breakdown (tolerance to overmixing), and time to maximum bandwidth. Of these, time to maximum resistance and resistance breakdown are the most meaningful measures of dough quality (Hoseney and Finney 1974, Fowler and de la Roche 1975a). Significant block effects (field replication) were apparent for a number of variables, but these, like the few interactions between line and block, were not considered important because of the consistent differences between lines for mixograph properties in each cross.

Offspring-parent heritability estimates (Frey and Horner 1957) for flour protein content and the mixograph parameters (Table II) indicated that medium-to-high values were obtained for a number of measures. For the population pooled over crosses, significant heritabilities were obtained for all parameters. However, some estimates, although statistically significant, were so low that reduced rates of genetic gain from early generation selection would be expected. No significant skewness was found in the residuals from any of the heritability calculations.

The measures of dough development time (time to maximum resistance and time to maximum bandwidth) had medium-to-high heritability, except for the time to maximum resistance in the SD cross, where a lower estimate was obtained. This was in part due to the reduced range for time to maximum resistance in this cross. Mixing tolerance as measured by resistance breakdown had medium heritability values. The remaining mixograph parameters showed consistently less significant heritabilities and

TABLE I
Means, Ranges, and Within-Line Standard Errors for Flour Protein Content and Mixograph Parameters for Three Crosses in the F_2 and F_3 Generations[a]

	F_2				F_3			
	Mean	Minimum	Maximum	SE[b]	Mean	Minimum	Maximum	SE
Suneca/Sunfield (29 lines)								
Flour protein, % as is	13.0	12.1	14.9	...	12.6	11.5	13.2	...
Mixing time, min	4.6	2.9	7.2	0.6	4.2	2.4	6.5	0.5
Maximum resistance, MU[c]	47.1	34.0	54.0	2.5	62.2	52.5	67.6	2.8
Bandwidth at maximum resistance, MU	39.3	32.0	48.0	2.9	32.2	26.1	39.3	5.1
Resistance breakdown, MU	5.9	1.7	10.3	1.3	8.3	4.1	14.5	2.4
Bandwidth breakdown, MU	13.8	5.9	20.1	2.9	10.8	6.0	15.1	5.0
Time to maximum bandwidth, min	3.9	2.2	6.1	0.7	3.1	1.7	5.2	0.3
Maximum bandwidth, MU	44.3	38.9	52.6	2.6	40.1	34.7	44.0	3.3
Sunbird/Dollarbird (20 lines)								
Flour protein, % as is	12.2	11.1	13.3	...	11.8	11.0	12.6	...
Mixing time, min	6.1	4.6	7.2	0.3	5.5	4.5	6.7	0.8
Maximum resistance, MU	51.5	41.8	59.2	1.9	61.6	57.6	66.5	0.3
Bandwidth at maximum resistance, MU	39.1	30.9	50.3	3.1	33.5	27.9	41.7	1.6
Resistance breakdown, MU	11.1	4.0	19.5	1.1	8.4	5.0	12.6	3.6
Bandwidth breakdown, MU	11.6	7.8	18.9	2.5	14.6	9.9	19.5	1.1
Time to maximum bandwidth, min	4.7	3.3	5.7	0.3	4.2	3.5	5.1	0.3
Maximum bandwidth, MU	48.1	40.9	54.6	2.0	45.2	42.2	49.1	2.0
Vasco/Hartog//Hartog (30 lines)								
Flour protein, % as is	11.0	9.7	12.3	...	11.7	10.7	12.8	...
Mixing time, min	4.1	2.7	7.3	0.3	4.3	2.8	6.3	0.2
Maximum resistance, MU	55.5	42.2	75.6	2.8	60.5	52.0	68.3	2.1
Bandwidth at maximum resistance, MU	38.3	30.1	47.1	3.1	33.4	27.8	40.7	3.3
Resistance breakdown, MU	9.1	4.7	13.4	1.7	9.9	4.7	15.6	1.6
Bandwidth breakdown, MU	14.5	0.1	24.4	2.8	15.1	12.3	19.8	2.8
Time to maximum bandwidth, min	3.1	2.1	5.6	0.3	3.4	2.1	5.4	0.2
Maximum bandwidth, MU	45.0	36.9	53.2	3.2	42.9	38.4	48.0	2.1

[a] Protein contents are the results of single determinations in the F_2 and F_3 generations. All other F_2 values are means of duplicate mixographs of each flour. F_3 values are means from duplicate mixographs of flours from each field replicate.

[b] SE = standard error.

[c] MU = mixograph units. Maximum resistance, bandwidth at maximum resistance, resistance breakdown, bandwidth breakdown, and maximum bandwidth all reported using a 100-unit scale, where 0 and 100 correspond to the minimum and maximum deflection on a standard 35-g mixograph. Breakdowns were calculated as the change in the parameter in the 3 min after maximum resistance.

appear to be of little use for early generation selection.

In part, the low heritability values for maximum resistance and bandwidth at maximum resistance were due to the method used to determine water additions. The procedure followed was based on the protein and moisture content (AACC 1983), which produces doughs with near-optimum properties. This has the effect of reducing the range and consequently the heritability of measures of maximum resistance and bandwidth at maximum resistance.

Heritability for flour protein content varied from cross to cross, with the highest value being obtained for the SD cross. The heritability for flour protein content was such that had water additions not been adjusted on a protein basis, the genetic variation for protein content between lines could have confounded the dough property data.

To undertake early generation selection, guideline values for selection are essential. These can be determined by considering generalized values for grades of wheat destined for specific end uses and adjusting them for seasonal variation using varieties representative of those grades. Simulated early generation selection was possible with the current set of lines because the above conditions could be satisfied, and all lines grown in the F_2 generation were grown in the next generation. In this way the effectiveness of the F_2 decisions in the simulated selection procedure could be evaluated.

The two main grades of wheat produced in the breeding program's target region are Australian Prime Hard and Australian Hard. These grades differ by having different generalized protein and dough properties (Table III). These generalized values and the type varieties have been set out as guidelines for breeding programs (summarized by O'Brien and Blakeney 1985). The use of numerous check varieties and the parents of the respective crosses enabled computation of guideline values for use in simulated selection. The analysis of variance of the check varieties gave standard errors of 0.50 minutes (time to maximum resistance), 2 units (resistance breakdown), and 0.4% for flour protein content. The generalized guidelines were then adjusted to account for the margins of error. The actual values used for selection by sequential independent culling, where selection for the subsequent traits was exercised only on the lines remaining after each cull, are given in Table IV.

TABLE II
Offspring-Parent Heritability Estimates (%) for Each Cross and the Population Pooled over Crosses

Parameter	Cross SS[a]	Cross SD[b]	Cross VH[c]	Pooled Crosses
Flour protein content	24.0	77.2[d]	35.0	63.9[d]
Mixing time	68.2[d]	34.2	65.9[d]	72.5[d]
Maximum resistance	35.8	22.3	10.9	23.8[f]
Bandwidth at maximum resistance	20.4	67.4[e]	29.3	36.5[e]
Resistance breakdown	58.8[d]	44.8[f]	56.4[e]	60.3[d]
Bandwidth breakdown	48.8[e]	23.7	28.3	22.6[f]
Time to maximum bandwidth	84.4[d]	52.1[f]	77.5[d]	75.0[d]
Maximum bandwidth	6.8	51.7[f]	26.8	37.2[e]

[a] SS = Suneca/Sunfield.
[b] SD = Sunbird/Dollarbird.
[c] VH = Vasco/Hartog//Vasco.
[d] Estimate significantly different from zero at the 0.001 level of probability.
[e] Estimate significantly different from zero at the 0.01 level of probability.
[f] Estimate significantly different from zero at the 0.05 level of probability.

TABLE III
Generalized Specifications and Type Varieties for Australian Prime Hard and Australian Hard Wheat Types

	Grade Prime Hard	Grade Hard
Wheat protein content, %	>12.8	>11.5
Mixing time, min	4–6	3–4
Resistance breakdown, MU[a]	<10	6–12
Type varieties	Hartog Sunco Suneca	Dollarbird Sunelg Vasco

[a] MU = mixograph units. Calculated as the change in the parameter in the 3 min after maximum resistance, where 0 and 100 correspond to the minimum and maximum deflection on a standard 35-g mixograph.

Of the total F_2 population of 79 lines, 55 had flour protein content acceptable for the prime hard grade. Among them 34 had acceptable times to maximum resistance (mixing times), and the same lines had acceptable resistance breakdown. When these 34 lines were assessed in the F_3 generation to see whether they met the prime hard requirements, 29 of them met the guidelines, giving a correct F_2 to F_3 prediction rate of 85%. For F_2 lines suited for Australian Hard, 46 had acceptable protein content, 22 also had acceptable time to maximum resistance, and of these, 18 had acceptable resistance breakdown. In the F_3 generation 12 of these 18 lines met the Australian Hard guidelines, a correct prediction rate of 67%.

A further way of assessing the effectiveness of the mixograph for early generation selection is to measure actual response to selection. This can be done by computing the ratio of the response in the F_3 generation to the selection differential (i.e., mean of the selected group minus the mean of the unselected group) for each measure, scaled as necessary. This ratio is the realized heritablility resulting from selection, and values within each cross were medium to high for protein content, mix time, and resistance breakdown (Table V). The exception was the resistance breakdown for selection of Australian Hard, in the VH cross where there was virtually no selection differential among the lines for these three crosses.

CONCLUSIONS

The direct-drive, 2-g mixograph meets the requirements for use in early generation selection for wheat quality. A good range of variation in mixograph parameters combined with acceptable errors of determination of them was found for flours derived from the seed of F_2 single-plant selections and in their F_3 progeny. The results agreed well over years for the important mixograph variables of mixing time and tolerance to mixing as evidenced by the medium-to-high offspring-parent heritability within and over crosses. Simulated selection based on guideline values for two wheat grades and response to selection as measured using

TABLE IV
Values of Flour Properties Used for Simulated Selection Within Each Wheat Grade

	Grade Australian Prime Hard	Grade Australian Hard
Flour protein content, %	>11.6	10.3–12.4
Mixing time, min	3.5–6.5	2.5–4.5
Resistance breakdown, MU[a]	<10.8	5.2–12.8

[a] MU = mixograph units. Calculated as the change in the parameter in 3 min after maximum resistance, where 0 and 100 correspond to the minimum and maximum deflection on a standard 35-g mixograph.

TABLE V
Realized Heritability Estimates (%) for Within Wheat Grade Selection for Each Mixograph Measure from F_2 to F_3

	Grade Australian Prime Hard	Grade Australian Hard
Protein content	55.2	45.8[a]
Mixing time	65.5[a]	65.3[a]
Resistance breakdown	55.3	17.6[a]

[a] Scaled data was used for parameters where the quality specification for the desired types was within a finite range (Tables III and IV).

realized heritability estimates also confirmed the usefulness of the instrument.

As little as 3–5 g of seed is required to be milled to give an adequate quantity of flour for a single analysis with the 2-g mixograph. Such a small sample size makes it an ideal instrument for use in research programs and in breeding programs, where seed quantity for testing is always a consideration.

ACKNOWLEDGMENTS

The authors wish to express their appreciation of Nikola Jancic for technical assistance. This research was partly funded by the Wheat Research Council and the Wheat Research Committee for New South Wales. MSUSTAT version 4.10 was developed by Richard E. Lund, Montana State University, Bozeman, MT 59717.

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[Received May 31, 1991. Accepted September 5, 1991.]