PROTEINS

Possible Role for the *Glu-Dl* Locus with Respect to Tolerance to Dough-Quality Change After Heat Stress

C. BLUMENTHAL,[1,2] P. W. GRAS,[1] F. BEKES,[1] E. W. R. BARLOW,[2] and C. W. WRIGLEY[1]

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In recent years, considerable research attention has centered on the glutenin subunits 5+10 and 2+12 because the presence of either has provided correlations to dough strength or weakness (respectively) in many sets of wheat genotypes, although there have been significant exceptions for some sets of wheats (reviewed by Payne 1987 and by MacRitchie et al 1990). We now report an additional association for these alleles: wheats having the 2+12 subunits tend to be more sensitive than those with the 5+10 subunits to the dough-weakening effects of heat stress during grain filling. (In this set of experiments, dough weakening has been assessed as reduced time to peak or increased breakdown in the Mixograph). This unexpected conclusion was deduced from a surveying the heat sensitivity of a diverse set of 44 genotypes (to be more fully described in a subsequent paper). This set of wheats, selected on the basis of reputations for heat tolerance or sensitivity, included 34 Australian cultivars representing most pedigree groups, six cultivars from other countries, and four unnamed genotypes from the Hot Climate nursery at CIMMYT, Mexico.

The experiment involved growing these wheats under controlled conditions of 13°C night (16 hr) and 18°C day in pots in the CSIRO Division of Plant Industry's Phytotron in Canberra. Pots were moved around periodically to avoid the possibility that positioning might affect results. At 30 days after anthesis, half the pots of each genotype were subjected for three days to a regime of 40°C for 10 hr (day) and 25°C overnight. Grain was harvested from mature plants (two sites) to provide duplication of both treatments for all the genotypes, giving a total of 176 grain samples (2 treatments × 2 sites × 44 wheats) each of 50–100g.

The grain was milled to flour in a Brabender Quadrumat Junior Mill and grain protein content was determined by the Dumas method. Dough properties were determined in replicated analysis in the two-gram direct-drive Mixograph (Rath et al 1990) and expressed as the time to the peak (mix time in seconds), dough breakdown (as the % drop in resistance 3 min after the peak), and as the height at peak resistance. The proportions of glutenin and gliadin were determined by size exclusion high performance column chromatography of a sonicated sodium dodecyl sulfate-phosphate extract of the flour by the method of Batey et al (1991). Allelic constitutions for the *Glu-1* A, B, and D loci for high molecular weight (HMW) glutenin subunits were obtained from published reports, where available, particularly from the GeneJar software of Cornish et al (1993), or by electrophoretic analysis (Gupta and Shepherd 1990). In the few cases, where a grain sample was polymorphic for a particular locus, the predominant allele was recorded.

There was a wide range of mixing properties for the 44 control samples (not heat stressed) ranging in mix time from 80 to 440 sec. Table I shows quality attributes split into means for each of the two groups of *Glu-D1* alleles. There were no significant differences in the control samples between the *a* and *d* alleles with respect to protein content, mix time, resistance breakdown, or glutenin-gliadin ratio. The set of 5+10 *(Glu-D1d)* wheats (controls, 19 genotypes) showed significantly greater breakdown than did the 2+12 set (25 genotypes). In this respect (only), the 5+10 genotypes were weaker than the 2+12 ones for this set of wheats, indicating that *Glu-D1* allele identity cannot universally predict all aspects of dough properties.

The effects of the heat-stress episode on the means of all these attributes are shown in Table I as the difference of heat shock minus control. There was a highly significant weakening of dough properties, after heat stress, for the 2+12 lines, but not for those with 5+10 subunits, based on mix time and dough breakdown as shown by mean values (Table I) and by the reactions of most genotypes in either allelic group (*a* or *d*) for the mix time (Fig. 1). The weakening of the *Glu-D1a* group was accompanied by a greater decrease in the glutenin-gliadin ratio, compared to the *Glu-D1d* group. This dough weakening occurred for the *Glu-D1a* group despite an increase in its mean protein content (Table I). On the other hand, there was a significant increase in peak resistance for the *Glu-D1a* group after heat stress, but this might be expected given the relationship ($r = 0.60$) for this attribute to protein content. On the other hand, mix time and resistance breakdown results were not significantly correlated with protein content.

When similar statistical analyses were performed with respect to the *Glu-1* alleles in the A and B genomes, there were no significant relationships between allelic compositions for these loci, and the effect of heat stress on Mixograph properties. Our observation with respect to *Glu-D1a* and *Glu-D1d* was reinforced when groups of genotypes having the same HMW glutenin subunits in the A and B genomes were compared (Fig. 2). In

TABLE I
Means for Grain Quality Values for Control Samples and for Differences Due to Heat Stress[a] Together with Significance of Differences Between Allelic Groups, *P* Values, and Least Significant Differences (LSD)

Attribute	*Glu-D1a*	*Glu-D1d*	*P*	LSD
Grain protein, %				
Control	12.1	12.8	0.18	1.0
HS−C	2.4	1.7	0.05	0.7
Mix time (sec)				
Control	263	235	0.22	45
HS−C	−44	−4	0.001	26
Breakdown				
Control	14.4	20.0	<0.001	2.9
HS−C	4.5	−0.2	<0.001	2.2
Peak resistance				
Control	331	332	1.00	24
HS−C	37	11	0.01	19
Glutenin–gliadin				
Control	0.76	0.71	0.16	0.07
HS−C	−0.05	−0.03	0.04	0.02

[a]Heat stress minus control (HS−C). Grouped according to the alleles *a* (subunits 2+12) or *d* (5+10).

[1]CSIRO Division of Plant Industry, North Ryde NSW 2113 Australia.
[2]University of Western Sydney, Richmond NSW 2573 Australia.

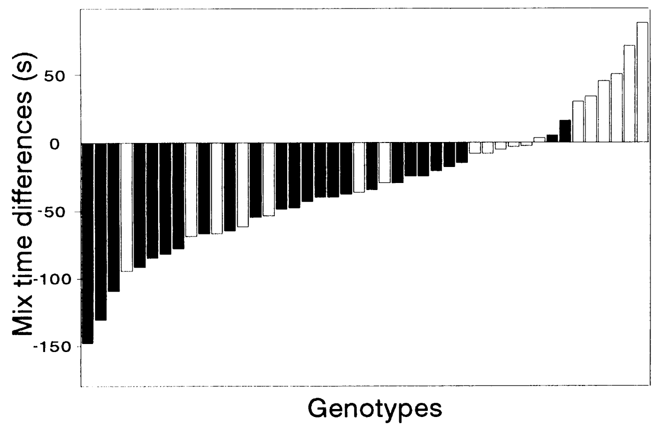

Fig. 1. Differences (heat-shock minus control) in mix time (time to Mixograph peak in seconds) for all 44 genotypes arranged in order of increasing tolerance to heat stress. Black bars indicate genotypes with the *Glu-D1a;* white bars are for the *Glu-D1d* allele.

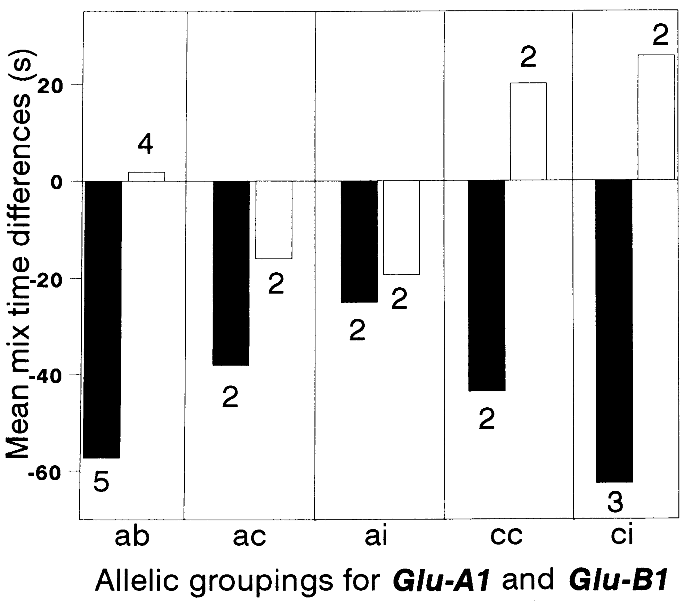

Fig. 2. Mean differences (heat-shock minus control) in mix time (as for Fig. 1) for subgroups of genotypes sharing the same high molecular weight glutenin alleles in A and B genomes (indicated below each pair of bars). Black and white bars indicate *a* and *d* alleles for the *Glu-D1* locus, respectively. Numbers indicate how many genotypes are averaged for each bar. For example, the first pair of bars indicates 9 genotypes, all having *Glu-A1a* and *Glu-B1b* alleles (subunits 1, 7, and 8), 5 with *Glu-D1a,* 4 with *Glu-D1d.* Genotypes in the second and subsequent pairs of columns have subunits 1, 7, and 9, subunits 1, 17, and 18, subunits 7 and 9, and subunits 17 and 18, respectively.

each case where this comparison was possible (admittedly small sample numbers), the 5+10 genotypes were more tolerant to heat than those with subunits 2+12, compared in the same *Glu-1* background.

These results suggest that 2+12 subunit genotypes may be more sensitive to the effects of heat stress on dough quality than are 5+10 genotypes, although this conclusion needs to be further examined in an even wider range of genotypes. A retrospective examination of the results reviewed by Blumenthal et al (1993) indicates that their observations of dough weakening after heat stress were mainly based on cultivars of the 2+12 type (particularly Sunelg, Vulcan, Sunco, Songlen, Cook, Kite, Eagle, and Oxley). A few other cultivars in these studies that showed little change after heat stress have 5+10 subunits (e.g., Suneca and Egret). The observation of dough weakening for "hot" seasons in the Australian Prime Hard crop (Blumenthal et al 1990) is also consistent with the above conclusion, since many of the cultivars grown for this grade have 2+12 subunits. On the other hand, a dough-weakening effect was not found as a result of heat stress by Bernardin et al (1994), based on glutenin and gliadin analysis, but the five varieties studied were all 5+10 types. Furthermore, when Stone and Nicholas (*in press*) chose two extreme genotypes from a survey of many wheats that had been heat stressed, the one that was tolerant to heat (based on change in glutenin-gliadin) was a 5+10 genotype (Egret) and the susceptible one (Osprey) was a 2+12. If this further relationship for the *Glu-D1* alleles is confirmed by further studies, it will provide further impetus for wheat breeders to focus on these genes in selecting for consistency in grain quality.

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