# Wheat Intercultivar Differences in Susceptibility of Glutenin Protein to Effects of Bug (*Eurygaster integriceps*) Protease

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**ABSTRACT**

Preharvest bug damage to wheat can cause significant losses in breadmaking quality. One of the most prevalent forms of bug damage which frequently occurs in most countries of the Middle East, Eastern Europe and North Africa can be attributed to *Heteropterous* insects, particularly *Eurygaster* spp. Intercultivar differences in the susceptibility of glutenin to proteolytic degradation by the bug *Eurygaster integriceps* were investigated using six breadwheat cultivars of Turkish origin. Crude enzyme extract was prepared with distilled water from bug-damaged wheat. The freeze-dried extract was blended with sound samples of ground wheat, and the mixture was incubated in distilled water for 30 and 60 min at 37°C and subsequently freeze-dried. The proteolytic effects of bug damage were determined on large polymeric glutenin. The latter was measured as 50% 1-propanol insoluble (50PI) glutenin extractable with 50% 1-propanol in reductant dithiothreitol. The decreases in the amount of 50PI glutenin and the high and low molecular weight subunits were quantified using reversed-phase HPLC. There was a substantial and progressive decrease in the quantity of 50PI glutenin and its subunits with increasing incubation time. Intercultivar differences were observed that were unrelated to intrinsic levels of proteolytic activity. After 60 min of incubation, the relative decrease in 50PI glutenin compared with control samples ranged from 43% (cv. Ankara) to 65 % (cv. Kirkpinar). Some cultivars (Lancer, Ankara and Gün) with similar levels of intrinsic proteolytic activity showed significantly different responses to bug protease. One cultivar (cv. Kirkpinar) with the lowest proteolytic activity was the most susceptible. High quality breadwheats (cvs. Bezostaya, Lancer, Kiraç and Gün) were generally more resistant to the bug protease, although Ankara, with both intermediate protease activity and breadmaking quality, was the most resistant cultivar. While the 50PI glutenin test was very effective in quantifying the damaging effects of bug protease on wheat protein quality, the nature of the intercultivar differences was unclear.

One of the most important forms of preharvest damage to wheat in European, North African, Middle Eastern countries and New Zealand is that caused by some species of *Heteropterous* insects, i.e. *Eurygaster* spp., *Aelia* spp. and *Nysius huttoni* (Paulian and Popov 1980; Cressey et al 1987). This damage is commonly known as bug damage and the resulting bread typically has low volume with poor crust and crumb characteristics (Kretovich 1944; Matsoukas and Morrison 1990; Every 1992; Every et al 1998; Hariri et al 2000). The nature of the bug damage effect is a specific bug protease that hydrolyzes gluten proteins with high specificity and glutenin in particular (Sivri and Köksel 1996; Sivri 1998; Sivri et al 1998, 1999). The bug (*Nysius huttoni*) protease in New Zealand wheats also appeared to have high specificity for the high molecular weight glutenin subunits (HMW-GS) (Cressey and McStay 1987; Cressey et al 1987). It was reported that high quality bread wheats were less susceptible to the adverse effects of bug protease in baking than the poor quality bread wheats (Matsoukas and Morrison 1990; Every 1992; Every et al 1998).

We previously observed that electrophoretic patterns of gliadin and glutenin proteins of bug (*E. maura*) damaged wheat cultivars with the same proteolytic activity were affected differently (Sivri and Köksel 1996; Sivri 1998; Sivri et al 1998). In a subsequent study, various bread wheat samples were colonized with equal numbers of *E. maura* at the milk ripe stage of grain development in nylon gauze cages (Sivri et al 1999). The decrease in the amount of large polymeric glutenin in those samples due to enzymatic degradation was quantified by reversed-phase HPLC (RP-HPLC) of reduced 50% 1-propanol insoluble glutenin (50PI). Bug damage caused substantial decreases (>80% on average) in the amount of 50PI glutenin, and some cultivars showed very different patterns of degradation as evidenced by differences in the ratio of HMW-GS to LMW-GS between sound and bug-damaged samples. In this study, we extend our earlier work on wheat that has been bug-damaged in the field, to study the effects of protease from a different wheat bug, *E. integriceps* on a range of sound bread wheats, for their resistance to the hydrolyzing effects. *E. integriceps* is the most important insect species in Eastern Europe and the Middle East in terms of its effect on wheat quality (Paulian and Popov 1980).

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## MATERIALS AND METHODS

### Materials

Six bread wheat cultivars, Bezostaya, Lancer, Kirkpinar, Ankara, Kirac and Gun, were selected to represent different grain characteristics and breadmaking quality (Table I). The HMS-GS compositions of these wheats at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively, were Bezostaya (2*, 7+9, 5+10), Lancer (2*, 7+9, 5+10), Kirkpinar (2*, 7, 2+12), Ankara (2*, 7+8, 5+10), Kirac (2*, 7+8, 5+10), and Gun (2*, 17+18, 5+10). The wheat samples were grown in experimental plots of the Field Crops Improvement Center, Ankara, and each plot was covered with a nylon gauze cage (1 × 1 × 1 m) at the milk ripe stage of grain maturity to prevent insect damage. Wheat kernels were visually examined after harvest and no indication of damage was observed. The wheat samples (10 g) were ground with a coffee grinder (Moullinex model 505) and the whole meal was sifted through a 65-mesh sieve.

### Crude Enzyme Extract

Crude enzyme extract was obtained from a heavily bug-damaged wheat sample (>50%, assessed visually) by *E. integriceps*, which is the most common wheat bug in the Trace region of Turkey. The method for preparation of crude enzyme extract is outlined in Fig. 1. The bug-damaged wheat was ground on a laboratory hammer mill (model 120, Falling Number AB Stockholm, Sweden). The whole meal (300 g) was extracted twice with distilled water (1,500 mL) by magnetic stirring for 48 and 24 hr at 4°C and centrifuged for 10 min at 15,000 × *g* at 4°C. The supernatants were pooled and freeze-dried. The resulting dry material was the crude enzyme extract.

### Preparation of Insoluble Glutenin for HPLC

Crude enzyme extract (50 mg) was blended with whole meal samples (250 mg) and suspended in distilled and deionized water (200 µL). After a brief vortexing, the samples were incubated for 30 and 60 min at 37°C in a shaking water bath. The slurries were frozen and freeze-dried at the end of incubation. Samples were prepared from the freeze-dried controls (no enzyme extract added) and enzyme-treated samples by the 50% 1-propanol (v/v) extraction

procedure (Sapirstein and Fu 1998). In this procedure, large polymeric glutenin is isolated in reduced form as the fraction of endosperm protein that is insoluble in 50% 1-propanol but soluble in a solution of 50% 1-propanol plus reductant (1% dithiothreitol, DTT). This extraction procedure yields a glutenin fraction of high purity but does not extract total 50% propanol insoluble (50PI) protein. As previously reported (Sapirstein and Fu 1998), the 50PI-DTT insoluble residue contains a significant amount of nonglutenin protein that was not related to dough mixing requirements or bread loaf volumes.

## RP-HPLC

For quantitative analysis, HMW-GS and LMW-GS of 50PI glutenin were prepared and analyzed as previously described (Fu and Sapirstein 1996; Sivri et al 1999). HMW-GS and LMW-GS eluted at 24–48 min and 48–82 min, respectively. A representative chromatogram of reduced glutenin subunits was previously reported (Sivri et al 1999). 50PI glutenin was measured as the sum of integrated areas of HMW-GS and LMW-GS.

## Analytical Methods

Moisture and protein (N × 5.7) of ground wheat samples were determined according to Approved Methods 44-15 and 46-11, respectively (AACC 2000).

## Protease Activity

Protease activity of the sound wheat samples was determined as described by Sivri et al (1998), and was adapted from a procedure which measures the concentration of large polymeric (50PI) glutenin by UV absorbance (Sapirstein and Johnson 2000). The percentage decrease in 50PI glutenin content of a control sample of sound wheat (Canadian bread wheat cultivar Katepwa) after 1 hr of incubation with enzyme extracts of test samples was taken as the measure of the proteolytic activity. For calculating intercultivar differences to the effects of bug protease, reductions in the amounts of 50PI glutenin, HMW-GS and LMW-GS after 30 and 60 min of

**TABLE I**
**General Properties of the Wheat Cultivar Samples**

| Cultivars | Bread-making Quality[a] | Grain Characters: Color | Hardness | Texture | Protein Content (14% mb) |
|---|---|---|---|---|---|
| Bezostaya | High | Red | Hard | Vitreous | 14.0 |
| Lancer | High | Red | Hard | Vitreous | 14.8 |
| Kırkpinar | Low | White | Semihard | Opaque | 13.8 |
| Ankara | Medium | White | Semihard | Intermediate | 16.2 |
| Kırac | High | White | Soft | Intermediate | 14.8 |
| Gun | High | Red | Hard | Vitreous | 15.0 |

[a] Atli et al (1990).

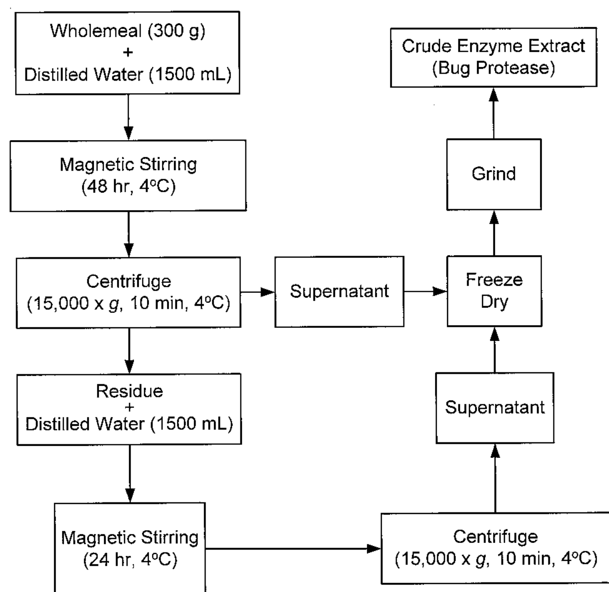


**Fig. 1.** Preparation of crude bug protease enzyme extract.

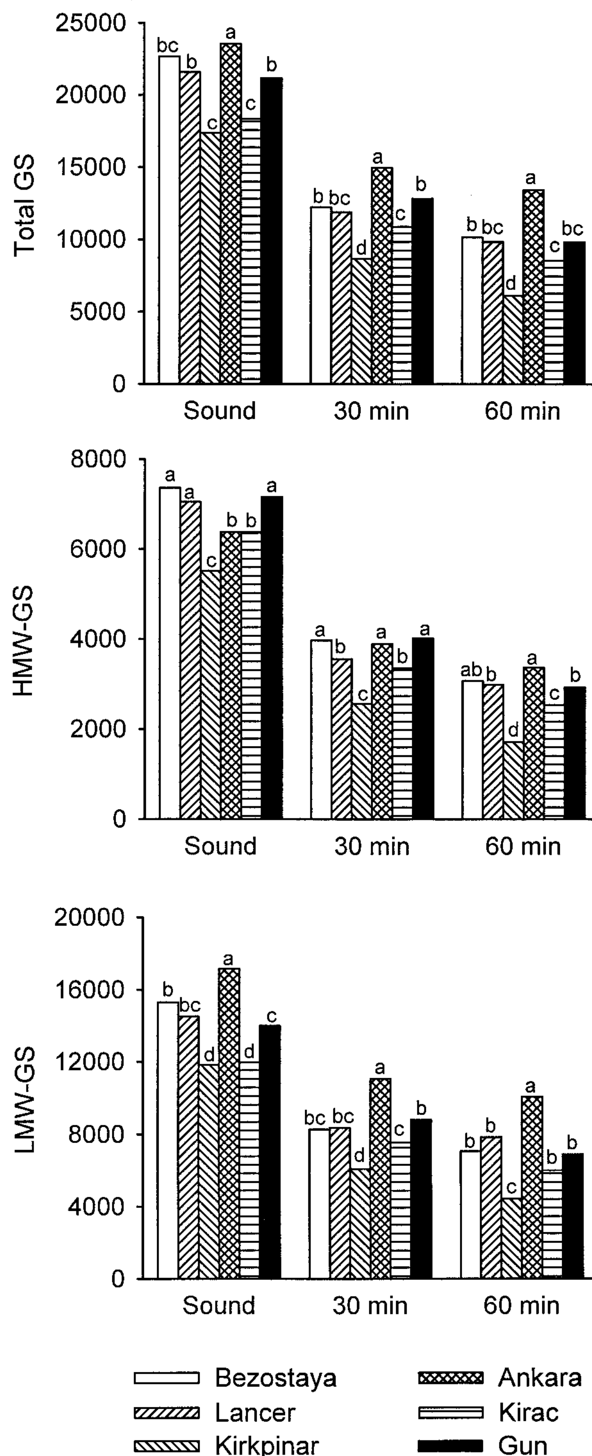


**Fig. 2.** Effect of bug (*Eurygaster integriceps*) protease on amount of total glutenin subunits (GS), HMW-GS, and LMW-GS determined by reversed-phase HPLC of reduced 50% 1-propanol insoluble glutenin isolated from six cultivar samples. For each cultivar and glutenin parameter, data are grouped according to treatment including a control (sound wheat). Within each treatment group, the same letter indicates a treatment that is not significantly different ($P < 0.05$).

incubation were calculated as the percent of the respective fractions of the sound sample of the corresponding cultivar.

**Statistical Analysis**

All experiments were performed at least in duplicate and average values are reported. The protease activity and RP-HPLC results were statistically evaluated by the one-way analysis of variance procedure using the MSTAT-C statistical analysis program (Michigan State University, Lansing, MI). The least significant difference (LSD) test was applied when the analysis of variance indicated significant differences in mean values.

## RESULTS AND DISCUSSION

Bug protease from *Eurygaster integriceps* significantly affected the amounts of 50PI glutenin and constituent HMW-GS and LMW-GS (Fig. 2, Table II). Similar to results we previously reported for the wheat bug *Eurygaster maura* (Sivri et al 1999), the amounts of the 50PI glutenin, HMW-GS and LMW-GS decreased with increasing incubation time for all the wheat cultivars. As there were significant differences in the initial amounts of 50PI glutenin, HMW-GS and LMW-GS among cultivars before incubation, relative reductions were calculated to facilitate intercultivar comparison of the bug protease effects (Table II). For incubation times of 30 and 60 min, the relative decreases in these three protein parameters were 36–46% and 41–69%, respectively, depending on genotype. Some cultivars differed significantly ($P < 0.05$) in the relative reductions of 50PI glutenin and subunits. Whereas Kirkpinar was the most susceptible cultivar, Ankara was the least susceptible. These differences in the degradation of 50PI glutenin and HMW-GS and LMW-GS were more obvious at 60 min of incubation.

To investigate the possibility that these intercultivar differences arose from genotypic differences in proteolytic activities, sound samples of these cultivars were assayed for protease activity. We found that the intrinsic level of protease activity was not related to glutenin degradation by *E. integriceps*. For example, of the three cultivars with intermediate protease activity (Table II, Lancer, Ankara and Gun), Ankara had the lowest relative reduction in 50PI glutenin, HMW-GS and LMW-GS contents. Although, Kirkpinar had a lower level of intrinsic proteolytic activity compared with Kirac, Ankara, Gun, and Lancer, Kirkpinar was the most susceptible cultivar to the effects of protease as indicated by the higher relative reductions in 50PI glutenin, HMW-GS and LMW-GS contents. On the other hand, Kirac had the highest proteolytic activity among the six cultivars, while the relative reduction in its 50PI glutenin, HMW-GS and LMW-GS contents were not significantly different from other cultivars with intermediate proteolytic activity (Gun and Lancer, Table II). Conversely, for Bezostaya with distinctly low proteolytic activity, the relative reduction in 50PI glutenin and subunit fractions was comparable with those of Lancer, Kirac, and Gun. These results indicate that the differences in glutenin susceptibility of the six cultivars did not arise from differences in intrinsic proteolytic activity.

The intercultivar differences observed in this study were generally comparable with previously reported (Sivri et al 1999) protease effects of a different *Eurygaster* bug on glutenin prepared from the same set of cultivars. For incubation times of 30 and 60 min, the correlation coefficients between the levels of decrease of 50PI glutenin as affected by *E. integriceps* (Table II, this study) and total GS prepared by RP-HPLC of reduced 50PI glutenin affected by *E. maura* (Table I, Sivri et al 1999) were 0.76 and 0.84, respectively. Evidently, there can be marked yet consistent intercultivar differences in glutenin susceptibility to the effects of wheat bug damage.

The results also showed that the relative reductions in the HMW-GS contents of all cultivars were higher than the relative reductions in the LMW-GS; these differences were more noticeable in the samples incubated for 60 min. These RP-HPLC results are consistent with those previously reported (Sivri et al 1999), as well as analogous results obtained by electrophoresis of reduced glutenin subunits (Cressey and McStay 1987; Sivri and Köksel 1996; Sivri et al 1998). These decreases are clearly reflected in the decreases for all the cultivar samples in the ratio of HMW-to-LMW-GS in 50PI glutenin with increasing incubation time (Fig. 3). These latter results confirm earlier observations with *E. maura* protease (Sivri et al 1999) and likewise indicate a higher specificity of *E. integriceps* protease for glutenin of larger average molecular size. Owing to the very close relationship between the quantity of large polymeric glutenin in flour and dough strength (Gupta et al 1993; Sapirstein and Fu 1998), the effects of bug damaged wheat on most technological quality indicators of gluten strength (farinograph, mixo-

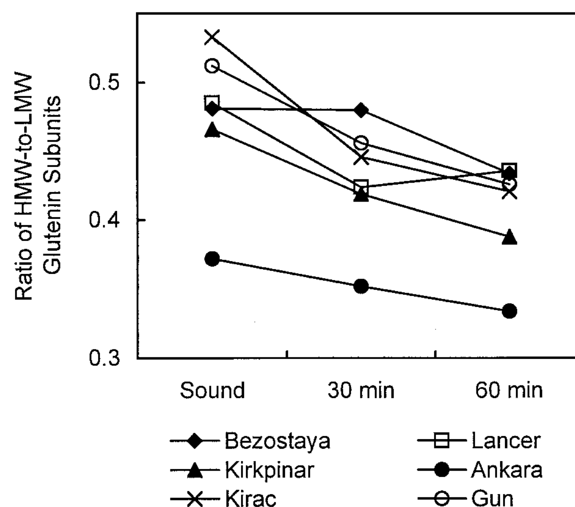


**Fig. 3.** Effect of bug (*Eurygaster integriceps*) protease with increasing incubation time on ratio of HMW-GS to-LMW-GS determined by reversed-phase HPLC of reduced 50% 1-propanol insoluble glutenin isolated from six wheat cultivar samples.

**TABLE II**
**Effect of Bug Protease (*E. integriceps*) on Whole Meal Samples**

| Cultivar | Decrease in 50 PI Glutenin(%) | | Decrease in HMW-GS(%) | | Decrease in LMW-GS(%) | | Protease Activity[b] |
|---|---|---|---|---|---|---|---|
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min | |
| Bezostaya | 45.9ab | 55.1b | 46.0b | 58.1b | 45.9a | 53.7b | 7.8d |
| Lancer | 44.8abc | 54.4b | 49.5ab | 57.6b | 42.4ab | 52.9b | 20.9b |
| Kirkpinar | 50.1a | 64.6a | 53.6a | 68.8a | 48.5a | 62.6a | 15.3c |
| Ankara | 36.4d | 42.8c | 39.0c | 47.2c | 35.4b | 41.2c | 23.2b |
| Kırac | 40.5bcd | 53.4b | 47.3b | 60.2b | 36.9b | 49.7bc | 29.5a |
| Gun | 39.3cd | 53.6b | 43.9bc | 59.1b | 37.0b | 50.9b | 21.0b |
| LSD($P < 0.05$) | 6.48 | 7.40 | 6.12 | 5.14 | 7.33 | 8.57 | 4.65 |

[a] Reported values are means of duplicate RP-HPLC analyses. For each cultivar, values with the same letter within a column are not significantly different ($P < 0.05$).
[b] Decrease in 50PI glutenin content (%) of substrate flour after 1 hr of incubation with whole meal of cultivar sample. Higher values denote higher levels of protease activity.

graph, and alveograph properties, sedimentation test volume, wet gluten content, wheatmeal fermentation time) can be substantial (Karababa and Ozan 1998; Hariri et al 2000).

Regarding intercultivar differences in wheat susceptibility to bug damage, Matsoukas and Morrison (1990) reported that the loaf volumes of high quality hard wheats were less affected by *E. integriceps* damage in baking. Kretowich (1944) also reported similar results using a simple test of dough extensibility. In New Zealand, Every et al (1998) also showed that susceptibility to the bug (*N. huttoni*) protease was significantly different among cultivars and the high quality bread wheats were less susceptible to the effects of the protease in baking than the poor baking quality wheats. Our results support the conclusion that wheat cultivars differ in their resistance to the effects of bug enzyme. There is some basis for surmising that stronger mixing wheats are generally less susceptible to the effects of bug protease than weaker mixing counterparts based on results for the group of Bezostaya, Lancer, Ankara and Gun, compared with Kirkpinar, which have higher and lower dough mixing requirements as well as higher and lower 50PI glutenin contents, respectively. However, what distinguishes Ankara as the least susceptible strong cultivar, and Kirkpinar from Kirac, which are both weak mixing cultivars with comparably low 50PI glutenin contents, is not known. Clearly, further work is necessary to gain a better understanding of these intercultivar differences in glutenin degradation due to the effects of bug damage.

## CONCLUSIONS

Bug (*Eurygaster integriceps*) protease as a crude extract prepared from a common source of damaged wheat was incubated with ground wheat of undamaged cultivars varying in breadmaking quality and protein quality. The effects of bug protease were evaluated on large polymeric glutenin measured as 50% 1-propanol insoluble protein extracted with 50% 1-propanol and 1% DTT. There was a significant and progressive decrease in large polymeric glutenin after 30 and 60 min of incubation. There were significant intercultivar differences in the degradation of 50PI glutenin and relative decreases in the quantity of constituent HMW-GS and LMW-GS. Results of this study and previous ones indicated that glutenin of largest average molecular size was most susceptible to the proteolytic effects of the bug enzyme. There seemed to be no relationship between intrinsic proteolytic activities of the sound wheat and the relative reductions in 50PI glutenin and HMW-GS and LMW-GS contents of the six cultivars that were studied. While the nature of the intercultivar differences to bug damage remains elusive, breeding wheat for resistance to bug protease should be possible using 50PI glutenin measurement as a screening procedure.

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