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EFFECT OF RUST INFECTION ON THE PROTEIN COMPONENTS OF WHEAT

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Key Word Index—*Triticum aestivum*; *Puccinia striiformis*; *Puccinia recondita*; wheat; yellow rust; brown rust; electrophoresis; glutenin; gliadin.

Abstract—The three wheat varieties KSML-3, HD-2009 and WG-377 were grown in rust-free plots and in plots infected with the rusts *Puccinia striiformis* and *P. recondita*. Total protein extracted from the grain samples was analysed quantitatively by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) to determine effects of rust infection on the protein components. In varieties HD-2009 and WG-377 rust infection increases the proportion of albumin-globulin and gliadin proteins while decreasing the high M_r glutenins. The proteins of variety KSML-3 were less affected by rust infection than were those of the other two varieties. The consequent possible effects on the functional quality of the grain could be a weakening of dough mixing resistance and an increase in dough extensibility. The difference in varietal response to rust infection could be important in selection of seed for cultivation in rust prone locations.

INTRODUCTION

Disease influences economic crop production through its effects on both quantity and quality of the ultimate product [1]. While the effect of disease on grain yield has been extensively investigated and reported, the role that diseases play in altering the quality aspect of the grain has not been studied in great detail. The few reports available are mostly concerned with protein content, but little is known about protein constituents [2–4]. Therefore this paper attempts to improve the understanding of the effects of *Puccinia striiformis* (yellow rust) and *P. recondita* (brown rust) infection on the pattern of components as seen by quantitative SDS-PAGE of the total grain protein.

RESULTS AND DISCUSSION

Figure 1 shows the patterns from one of our SDS-PAGE runs of the rust-free and rust-infected samples of the three varieties studied. The advantage of this SDS-mercaptoethanol-PAGE system is that all protein of the grain can be solubilized. A possible disadvantage is that the resulting mixture is one of reduced protein subunits rather than of intact molecules as they exist in the flour or in wet processing steps where functionality is of importance. However, grain protein can be adequately quantitated by SDS-PAGE and densitometry as demonstrated in previous work [5], and the relationship between the subunit ranges and the classical albumin, globulin, gliadin and glutenin fractions of wheat flour is well known [6]. The highest M_r subunit region, Area 1 in our densitometric tracings (Figs 2–4), is mostly from high M_r glutenins. Area 2 contains subunits from low M_r glutenins and some high M_r gliadins (ω-gliadins), Area 3 is high M_r gliadins with some low M_r glutenin subunits, Area 4 contains gliadins, and Area 5 is the albumin and globulin subunit fraction. Because the glutenins and gliadins are the proteins which determine functional properties of the flour, it can be an advantage to have them spread into several areas in studies relating individual subunit components to function.

Figures 2–4 show the densitometric curves (solid lines) of the protein subunit separation patterns for each of the rust-infected varieties studied, with the curve of the pattern from the corresponding rust-free sample superimposed as a dashed line.

Area differences

The most consistent difference between the protein classes of the rust-infected and rust-free samples is the increased level of albumin-globulin fraction (A5) found in the presence of infection. The increase is most apparent in samples of HD-2009 and WG-377 with the difference in KSML-3 being slight, as Figs 2–4 show. Table 1 gives the quantitative data averaged from seven such runs. Only the difference between the albumin-globulin fractions of the HD-2009 samples is significant at the 95% confidence level. For the seven electrophoresis runs quantitated, the average confidence limits for all areas are $\pm$ 1.8 at the 95% level. The smaller areas such as A1 and A2 had narrower limit values with A4 and A5 values somewhat larger. Other readily apparent differences include A1, the high M_r glutenin subunits, also in both HD-2009 and WG-377 samples. Each variety shows a decrease in these glutenin subunits when rust infected. Again only the difference in the HD-2009 samples is significant at the 95% level.

The A2 and A3 regions, containing lower M_r glutenin and high M_r gliadin subunits, of all three varieties is decreased with rust infection, but none of the differences is statistically significant. On the graphs shown in the figures, the differences appear larger than the averages of

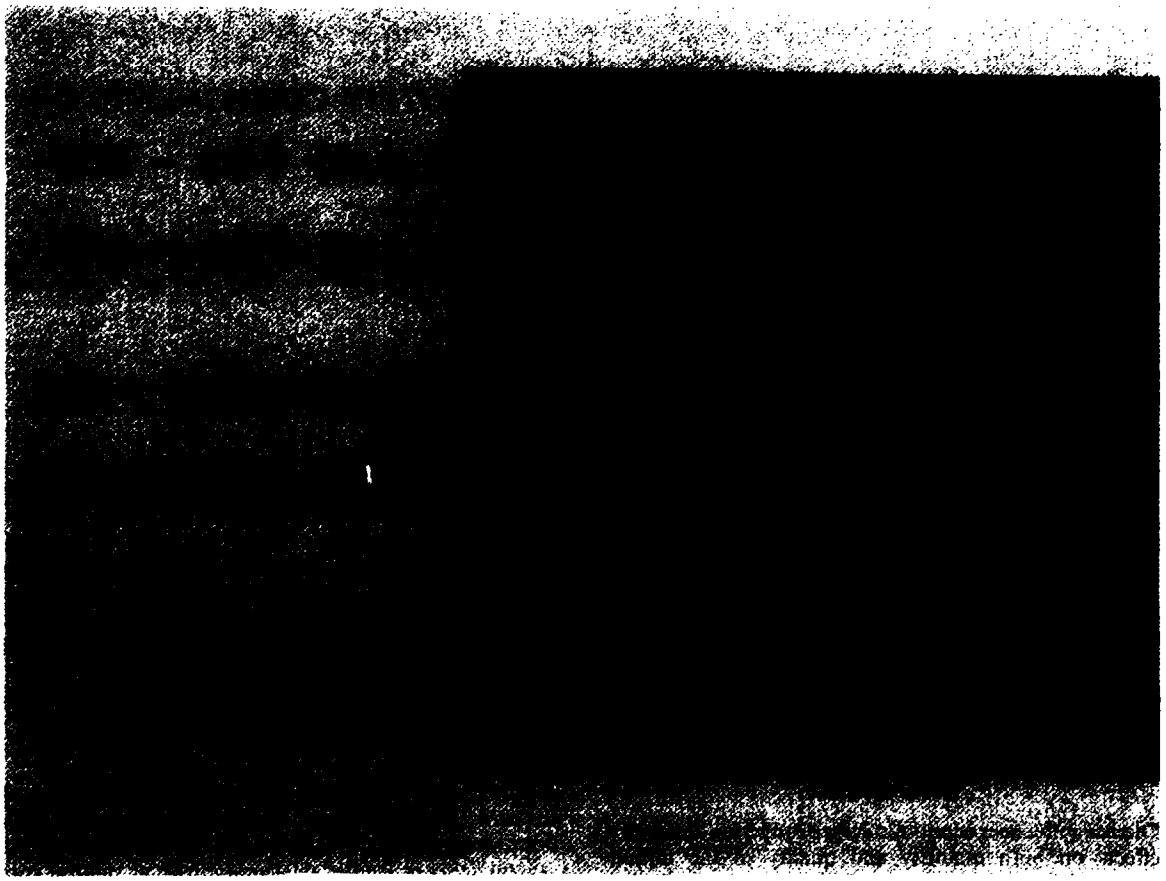

Fig. 1. SDS-polyacrylamide gel electrophoresis patterns for total protein extracts from rust-free (R.F.) and rust-infected (R.I.) samples of wheat varieties WG-377, HD-2009 and KSML-3. Migration is from left to right with the high M_r components at the left or origin side of the gel.

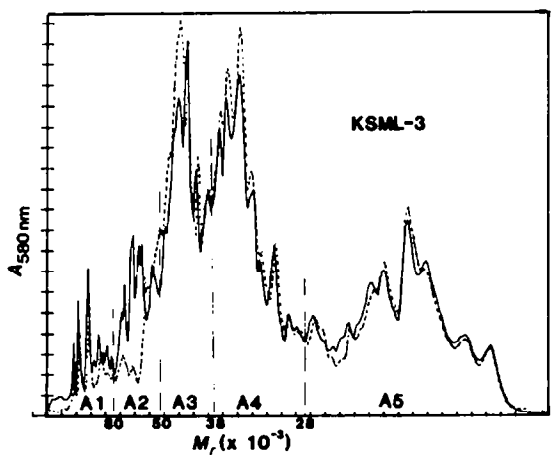

Fig. 2. Densitometric tracings of electrophoretic patterns of protein from rust-infected (——) and rust-free (– – –) samples of wheat variety KSML-3.

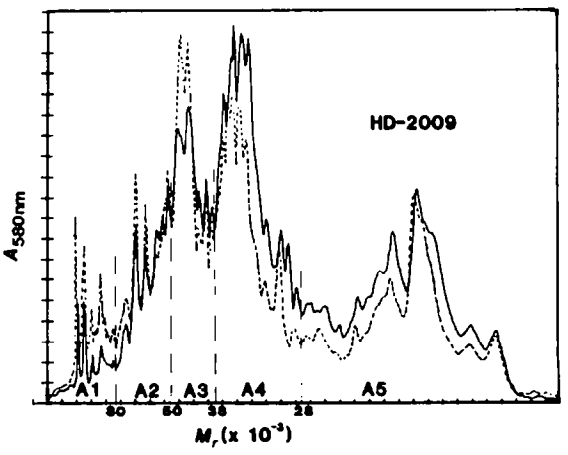

Fig. 3. Densitometric tracings of electrophoretic patterns of protein from rust-infected (——) and rust-free (– – –) samples of wheat variety HD-2009.

all the runs, especially in A2. Densitometric tracings for the figures were chosen, all from one run for best comparability, and from the one run which best represented the average. The A2 region of variety KSML-3 in the tracing shown is especially divergent from the average of all runs in showing a difference opposite to the average. It must be remembered that the numbers in Table 1 are percentages of total area for the sample rather than

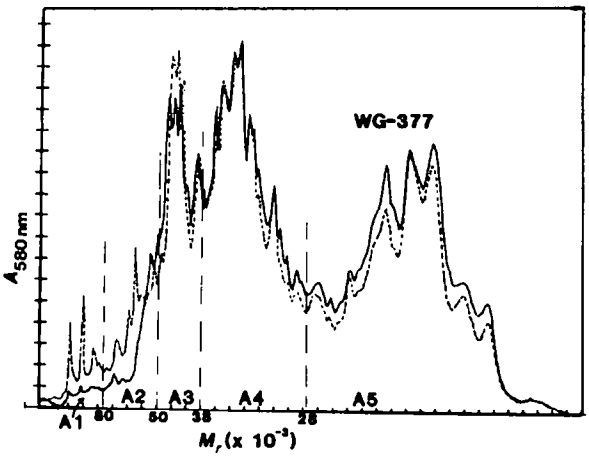

Fig. 4. Densitometric tracings of electrophoretic patterns of protein from rust-infected (——) and rust-free (– – –) samples of wheat variety WG-377.

Table 1. Percentages of total densitometric tracing area for the proteins of rust-infected (R.I.) and rust-free (R.F.) wheat varieties

	Percentage of tracing area				
	A1	A2	A3	A4	A5
KSML-3 (R.I.)	4·2	10·3	19.4	29·8	36·3
KSML-3 (R.F.)	3·7	10·8	21·5	30·7	33·2
HD-2009 (R.I.)	3·4*	13·4	16·6	30·8†	35·8*
HD-2009 (R.F.)	6·6*	13·9	19·9	27·9†	31·4*
WG-377 (R.I.)	1·3	6·4	14·4	32·4	45·2
WG-377 (R.F.)	3·3	8·3	14·7	29·8	43·9

*Difference significant at the 95% confidence level.
†Difference significant at the 99% confidence level.

absolute area quantities. They are therefore dependent on the quantities of protein in other portions of the pattern. The tracings reflect better the absolute amounts of protein in each region. For this reason both the table and the tracings should be examined, as the comparisons made are somewhat different in the two formats.

The A4 region, principally gliadins, of the HD-2009 samples showed an increase with rust infection which is quantitatively significant at the 99% confidence level. The WG-377 samples show a similar but smaller difference in A4, while the A4 region of KSML-3 decreases an insignificant amount with rust infection.

Band differences

The above mentioned area or protein class differences are brought about by quantitative differences in individual protein subunits within the groupings of electrophoretic bands. In a few instances qualitative differences are seen between the rust-infected and rust-free patterns. In all patterns produced from WG-377 there was a disappearance with rust infection of the middle range components in Area 2, components which are possibly ω-gliadins. This is not seen in the case of HD-2009, and the opposite case appears in most extracts of KSML-3, which show bands in this region in the rust-infected sample which are not present in the absence of rust. The only other indication of appearance of subunit components upon rust infection is in the leading edge of the A4 region for HD-2009. Grebenchuk *et al.* [7] have reported the appearance of such a band in mildew-resistant wheat in the water-soluble fraction upon fungus infection.

Implications for quality

Although much research is presently underway in attempts to correlate individual protein subunit components of wheat flour to functionality in baking, it is at this time still difficult to assign quality correlations to particular components. Proposals have been made connecting quality with the highest M_r proteins of the gluten or glutenin fractions [8–10]. If this is indeed the case rust infection would be expected to degrade baking quality in varieties HD-2009 and WG-377 by its deleterious effect on the proportions and amounts of highest M_r components, but possibly not the quality of variety KSML-3 in which the highest M_r components are not reduced. Although we cannot say on the basis of a study of three varieties how widespread such a reduced effect of rust infection might be, this case does demonstrate that not all varieties will be degraded equally in functionality by rust infection, and that by careful selection of variety in rust-prone locations a minimal effect on wheat functional quality might be achieved. The principal gliadin portion of the patterns (A4) follows this trend also, in that the variety KSML-3 shows less effect from rust infection on the proportion of this fraction as shown in Table 1, although the absolute amount of A4 protein is reduced as shown by Fig. 2.

The proportions of the various classes of protein, or areas of the tracings, have been correlated with dough quality parameters by Wrigley *et al.* [11] for a series of flours of the variety Olympic differing in sulphur content. For this series dough extensibility had a high negative correlation with the amount of protein in A1 and A2, and resistance was found to correlate positively with the proportions of protein subunits in A1 and A2. The opposite was found for proteins in A4 and A5; a greater proportion of protein in these classes produced more extensible, less resistant doughs. The albumin-globulin proteins which make up A5 are not generally thought to directly affect dough properties to a great extent: the correlation comes about because of the inverse relationship generally found between the amounts of the higher M_r proteins and the A5 proteins in a given sample. During protein deposition in the seed it is the storage protein (A1–A4) which is being formed while the enzymic or biosynthetic protein (the albumins and globulins of A5) does not increase, so that the proportion of A5 decreases.

If these findings for the variety Olympic can be extrapolated to other wheats one would then expect rust infection in the varieties HD-2009 and WG-377 to produce flours with more extensibility and less resistance to mixing because of the effects of infection on the A4 and A5 protein, and also doughs with less strength or stability because of the reduction of A1 protein. The flour of variety KSML-3 would be expected to show the least effect on quality from rust infection. Pelshenke gluten test values were determined for previously produced crops of each of the three varieties grown under rust-free and rust-infected conditions [12] and showed decreases for each variety. Because we have not directly tested quality

parameters for the present set of samples we can rely only on extrapolation from these previous samples to estimate general effects on quality.

EXPERIMENTAL

Materials. The trial growth plot was sited in October 1982 in a field with recommended doses of fertilizers and consisted of three randomized blocks. Non-experimental lines of oats were sown as buffer rows to ensure the arrest of drift and lateral transposition of rust spores from rust-infected plots and of chemical protectants from the rust-free plots. The wheat varieties chosen for investigation were as follows: KSML-3, a multiline comprised of six near isogenic components mixed in equal proportions. HD-2009, A semi-dwarf variety, an outcome of the cross between Lerma Rojo 64 and Nainari 60. WG-377, A semi-dwarf variety developed from the cross (WG 145 × USA 255) × Kalayansona.

Disease development. Plots meant for development of artificial infection with yellow and brown rusts were sprayed with a knapsack sprayer dispensing virulent rust spores suspended in distilled water, *ca* 60 days after sowing the grain. After about another 14 days, rust spores were mixed with talcum powder and dusted with muslin cloth onto the plants in order to ensure epidemic infection. The plots which were to remain rust-free were sprayed with Plantvax *ca* 1.0 l./ha to control yellow rust and RH 124 *ca* 500 ml/ha to control brown rust.

Disease was scored on a scale of 100. All the plots intended for rust epidemic scored 80–90% rust development. Plots meant to be rust free had rust incidence of 5–10% which is negligible for affecting overall protein composition.

Extraction of proteins and SDS-PAGE. Proteins were extracted for the electrophoresis runs from three seeds of each grain sample by the procedure previously outlined [13] in 0.062 M Tris–(hydroxymethyl)aminomethane–HCl (pH 6.8) that included 2% SDS (Sigma), 5% 2-mercaptoethanol and 0.01% Pyronin Y tracking dye. Solid sucrose (100 mg/ml) was added to the mixture after grinding the grain and extraction buffer (1 ml/30 mg grain). After about 1 hr the mixture was centrifuged and 10–30 μl (an amount to give *ca* equal protein loading in each sample well) was taken for electrophoresis. The SDS-PAGE method of ref. [10] as modified [13] was used in a vertical gel apparatus (Hoeffer Scientific Instrument Co., San Francisco) for 16 hr runs. Separation gels were 25 × 16.5 cm × 1.5 mm and contained 17.5% acrylamide and 0.08% *N,N'*-methylene-bis-acrylamide. Gels were stained for 24 hr with 0.02% Coomassie brilliant blue R250 in H_2O–MeOH–HOAc (80:20:7) containing 6% trichloroacetic acid (TCA). Destaining was in 6% TCA for 48 hr with one change of TCA at 24 hr. Gels were soaked overnight in H_2O, then washed with a non-ionic detergent soln to remove surface dye before photographing and scanning.

Photography and scanning of gels. Gels were photographed wet with Polaroid type 55 (black and white) film through a Wratten G-15 yellow–orange filter. The gel was then sliced into strips corresponding to each sample slot and track and placed into a 21 cm cuvette for densitometric scanning in a Gilford apparatus [5]. The digital output of the densitometer was recorded on computer tape and the data was handled with a Tektronix 4052 computer for quantitation of scan areas. Data were recorded at the rate of two points per second during scans of 2 cm/min, or *ca* 1200 points for each 20 cm scan. The computer was used also for redrawing of densitometric curves to equivalent areas, for determination of area averages among sets of runs and scans, and for confidence level calculations.

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