the kernel. In general, it is rather difficult to interpret the physical meaning of the second peak. The distribution curves (not shown) of the second peak for hard and soft wheats are not as distinguishing as the first peak or the first valley.

Ratio of the First Valley to the First Peak

This ratio is a measure of the pattern of the first peak and first valley. A high ratio indicates a small dip and little breakdown of kernel structure. A small ratio (below 0.5) indicates a large dip and a pronounced collapse of kernel structure. The ratio for most HRW wheat Hawk kernels was below 0.5 and for most SRW wheat Pike kernels above 0.5 (Fig. 12). When 0.5 is used as the cut-off ratio to distinguish hard from soft wheat, 10 of 102 Hawk kernels were atypical, and 14 of 112 Pike kernels were atypical. The reasons for the atypical kernels are unknown. There may be heterogeneity within a variety or presence of kernels from other classes. Microheterogeneity is likely to affect much more the results of testing single kernels than of testing bulk samples in which the results of many kernels are compensated and averaged. Still, the ratio of the first valley over the first peak seems to be potentially the single best distinguishing characteristic to differentiate between hard and soft wheats.

Maximum Positive Slope

This slope is a measure of the maximum rate of force increase per unit time. The maximum positive slope dictates the steepness of the curve for the force vs. time relation. The distribution curves for kernels of HRW wheat and SRW wheat Pike overlapped. Maximum positive slope is therefore an unsatisfactory criterion to distinguish soft from hard wheat.

Maximum of the Absolute Value of the Negative Slope

The rate of decline in force vs. time is measured by the maximum absolute value of the negative slope, which indicates how fast the kernel collapses. Several factors (probably including kernel size and shape, resilience, and overall texture) affect this parameter, and its physical meaning is difficult to interpret. Moreover, the distribution curves (not shown) of the hard and soft wheats overlapped to such an extent that it was impossible to use this parameter as the sole criterion for determining hardness of a single kernel.

In summary, the operator-selected six methods of calculation and evaluation differed in their effectiveness in differentiating hard from soft wheats. The ratio of the first valley over the first peak was the most reliable single parameter, although no single parameter differentiated perfectly between kernels of soft and hard wheat. A combination of parameters may be needed to improve differentiation between kernels that vary in hardness and the reliability of prediction of composition of mixtures of hard and soft red winter wheats. A definitive evaluation is predicated on the availability of a reference method to determine the basis of differences in hardness characteristics among individual kernels. As stated previously, such a reference method is not available at this time. Moreover, deviations from expected hardness characteristics and polyacrylamide gel electrophoretic patterns were shown both for kernels differing in morphology and among kernels considered typical of a cultivar on the basis of grain morphology and texture (Lookhart et al 1985).

The results summarized in Table II are based on the single criterion of the ratio of the valley over the first peak. Using this single criterion, we predicted the percentage of soft wheat with more than 90% accuracy. Results of the two bulk methods showed that grinding time in the Brabender microhardness tester decreased as hardness increased, whereas the Stenvert tester, hard wheats required longer grinding time than soft wheats. The linear correlation coefficient between percentage of HRW as determined by FGIS and determined by the CASK-HaT method was 0.826. That correlation was lower than between the FGIS determination and the Brabender microhardness tester on bulk samples (0.968) and almost equal to the correlation between the FGIS determination and the Stenvert hardness test on bulk samples (0.868). A major factor in the case of the single kernel test is the large sampling error.

CONCLUSIONS

The CASK-HaT was developed to distinguish between soft and hard red winter wheats. Stress-strain relations at a rate of 15 kernels per minute were recorded for individual kernels by the tester and were used to establish patterns typical of hard and soft wheat kernels. Those patterns were the basis of a computer system, including software, that predicted with better than 90% accuracy the compositions of mixtures of hard and soft red winter wheat kernels.

ACKNOWLEDGMENT

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top). That band is also present in the Sage patterns. Less than 4% of the HRW variety Arkan classified by FGIS possessed atypical morphological characteristics (Table I).

Arkan kernels selected by FGIS, having morphological characteristics of Sage (ARK-SA), exhibited three different patterns. The patterns of ARK-SA 2 and 4 were similar to the typical Sage patterns Sage 2, 3, and 4. Among the differences were the absence of the light fifth band (mobility 24) in ARK-SA 2 and 4 that was present in the Sage pattern and two bands in the high mobility region (70–85 relative mobility [RM] units) where the ARK-SA types had a band at 74 RM that was absent in Sage and the dark band in Sage at 76 RM that was absent in ARK-SA types.

TABLE I
Wheats Used in the Study

Class Variety	Weight (g) Major	 Minor
Soft red winter		
Arthur	9.89	...
Hard red spring		
Stoa	10.76	0.32-HRW[a] (2.88)
Guard	10.93	0.76-HRW (6.50)
Hard red winter		
Sage	9.98	...
Ram	10.21	0.31 HRS, 0.54 SRW (2.80) (4.88)
Ute	11.42	1.56 HRS (12.02)
Newton	9.91	...
Eagle	10.00	...
Arkan	10.16	0.42 (HRW-Sage) (3.97)

[a] Value in parentheses is percent of minor class on the basis of morphological characteristics.

Another difference in band patterns of ARK-SA kernels was found in ARK-SA 1, where the upper fourth of the gel, 0–25 RM (low mobility), had Arthur type bands instead of Arkan or Sage bands and the lower fourth exhibited some bands that were not present in Arkan, Arthur, or Sage.

A third pattern type in ARK-SA was found in ARK-SA3, which has a pattern identical to bulk Arkan. Thus, kernel morphology seems effective (in three of four cases) in separating ARK-SA kernels having major electrophoretic differences, because the four ARK-SA kernels exhibited three different PAGE patterns, one of which was identical to Arkan but none to Sage.

Patterns of the four Sage kernels appear identical except for the presence of a minor band in Sage 1, at 38 RM units. The patterns of Sage 2, 3, and 4 were identical to Sage electrophoregrams previously analyzed (Jones et al 1982). The four Arthur patterns were all identical and were typical of previously analyzed Arthur patterns.

Single seed electrophoregrams of four seeds each of Newton and Eagle are shown in Figure 3. Newton patterns 1 and 4 appear identical to each other but different from Newton 2 or 3, which are also different from each other. All of the differences among Newton samples were in the top half (10–25 RM region) of the gel. None of these single seed electrophoregrams was identical to the typical (bulk) pattern of Newton (Jones et al 1982). The gliadin patterns of all four Eagle kernels (Fig. 3) appear identical to each other and to the typical pattern of other bulk Eagle samples previously analyzed in our laboratory (Jones et al 1982). Eagle was expected to be very homogeneous, as it was originally selected from an F_{16} single plant, whereas most of the other cultivars studied were selected from F_4 or F_5 single plants.

The electrophoretic patterns of four single kernels each of the major Ute fraction, Ute M, and of Ute kernels with morphological characteristics of hard red spring wheat (Ute-HRS) are shown in Figure 4. All patterns of the individual kernels of the HRW wheat Ute, including kernels with HRS morphological characteristics,

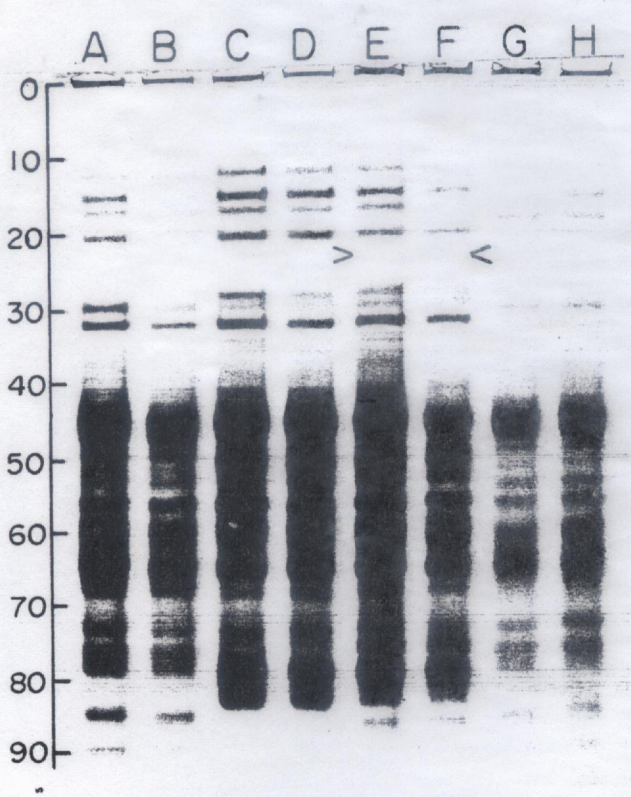

Fig. 1. Gliadin electrophoregrams of A, Arkan 1; B, Arkan 3; C, Arkan with Sage characteristics (ARK-SA 2); D, ARK-SA 4; E, Sage 1; F, Sage 3; G, Arthur 2; H, Arthur 3. Carets indicate light bands not easily seen.

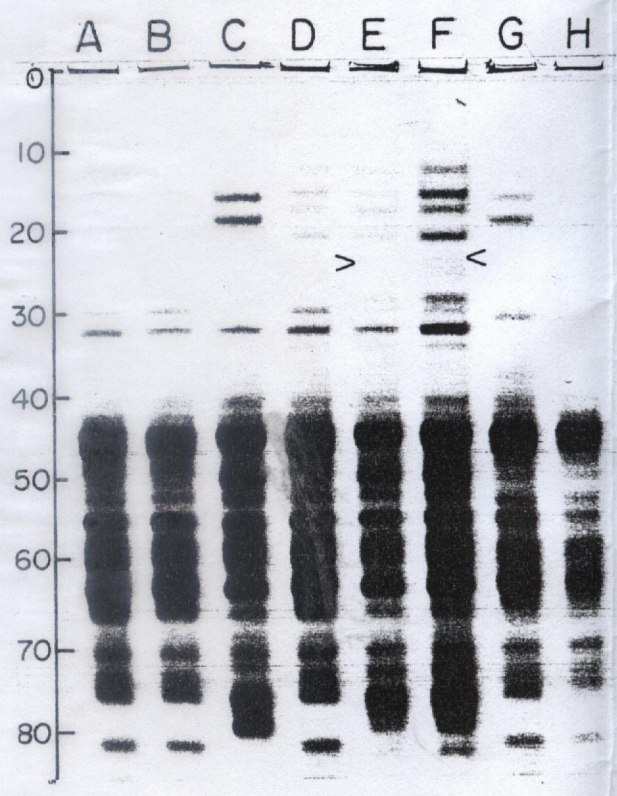

Fig. 2. Gliadin electrophoregrams of A, Arkan 2; B, Arkan 4; C, Arkan with Sage characteristics (ARK-SA 1); D, ARK-SA 3; E, Sage 2; F, Sage 4; G, Arthur 1; H, Arthur 4. Carets indicate light bands not easily seen.

exhibited gliadin patterns identical to each other and to bulk Ute samples previously analyzed.

Gliadin patterns of four kernels of the major kernel type in the HRS wheat Guard, Guard M, and of four of the kernels with morphological characteristics of hard red winter, Guard HRW, are shown in Figure 5. The HRS variety Guard contained 6.5% of the HRW type kernels (Table I). All patterns are identical to the typical Guard bulk pattern except for Guard M-3, in which the third fastest band has higher mobility (the normal 74 RM band appears at 77 RM). Because the gliadin patterns of the Guard kernels having typical HRW morphology appear identical to the typical PAGE patterns of the major fraction, differences in kernel morphology appear unrelated to the electrophoregrams of Guard.

Electrophoretic patterns of four single kernels each of the major kernel type, in the HRS wheat Stoa (Stoa M) and of kernels having HRW characteristics (Stoa HRW) are shown in Figure 6. All gliadin patterns are identical to the typical pattern of a bulk Stoa sample previously analyzed. Thus, Stoa kernels having HRS and HRW morphology do not differ electrophoretically.

Gliadin patterns of four kernels each of the major HRW wheat fraction of the cultivar Ram (Ram M) and kernels having HRS characteristics (Ram HRS) and soft red winter (Ram SRW) characteristics are shown in Figure 7. Gliadin patterns of eight of the 12 kernels are identical to the typical bulk electrophoretic pattern of Ram. The electrophoretic patterns of the other four kernels, Ram M-1, Ram HRS-1, Ram HRS-4, and Ram SRW-1, exhibit a slight change in the mobility of a single band (marked by an arrow) near the center of the gel, which is the only difference between their patterns and the typical pattern of a bulk sample. Again, differences in kernel morphology were not expressed in the gliadin patterns. This effect is particularly striking because these gliadin patterns were nearly invariant for kernels having HRW, SRW, and HRS morphological characteristics. These data support the concept that the gliadin proteins, which are coded for by only six complex loci (Payne et al 1984), are inherited independently from other genotypic and phenotypic characteristics.

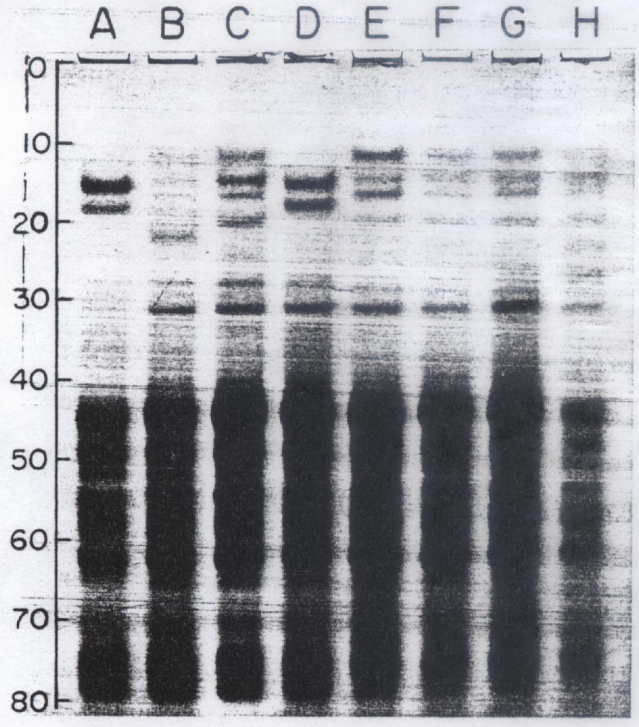

Fig. 3. Gliadin electrophoregrams of **A**, Newton 1; **B**, Newton 2; **C**, Newton 3; **D**, Newton 4; **E**, Eagle 1; **F**, Eagle 2; **G**, Eagle 3; **H**, Eagle 4.

Hardness

The method for determining hardness of individual kernels of hard and soft wheat was described by Lai et al (1985). For a hard wheat, there is a distinct drop in crushing force after the first peak, whereas for a soft wheat, the drop is gradual and relatively small. To establish the characteristic pattern of each curve, we measured the magnitude of the first peak and first valley and calculated the ratio of first valley over first peak (Lai et al 1985). The results of those measurements on each of the 60 kernels are given in Table II. The predicted hardness value (PHV) is determined from a combination of the ratio of the first peak to the first valley and the magnitudes of each. If the ratio is less than 0.25 or greater than 0.45, the PHV is determined as hard or soft, respectively. However, if the ratio is between 0.25 and 0.45, additional criteria, including the magnitude of the first peak and first valley, are considered. We found that soft wheats normally have a first peak to first valley ratio greater than 0.4 and hard wheats less than 0.3.

Relation of Electrophoretic and Hardness Patterns

All kernels of Arkan and Arkan (Sage type) were predicted to be hard. The ratios for Arkan kernels 2 and 3 were higher than for kernels 1 and 4 (Table II); they were also grouped by similar electrophoretic patterns.

The PHV for Sage kernel 1 was soft. The hardness pattern for that kernel was distinctly different from the patterns of the other Sage kernels. The size of the first peak was unusually high, which caused the ratio to be high and therefore to grade soft. The overall intensities of the bands in the electrophoretic pattern of Sage 1 were greater than those in the other Sage kernels' patterns, which implies a higher protein content. Therefore, high protein content may affect hardness determination of single kernels.

The PHV of Arthur kernel 1 was hard. The first valley was low compared to those of the other three kernels, which may have affected the PHV. The intensities of the electrophoretic bands of

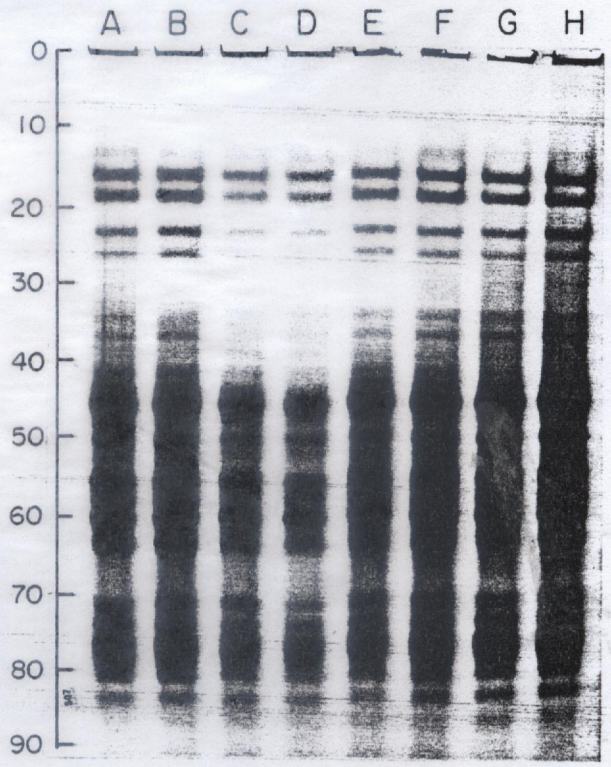

Fig. 4. Gliadin electrophoregrams of **A**, Ute major fraction (Ute M-1); **B**, Ute M-2; **C**, Ute M-3; **D**, Ute M-4; **E**, Ute with hard red spring wheat morphological characteristics (Ute HRS-1); **F**, Ute HRS-2; **G**, Ute HRS-3; **H**, Ute HRS-4.

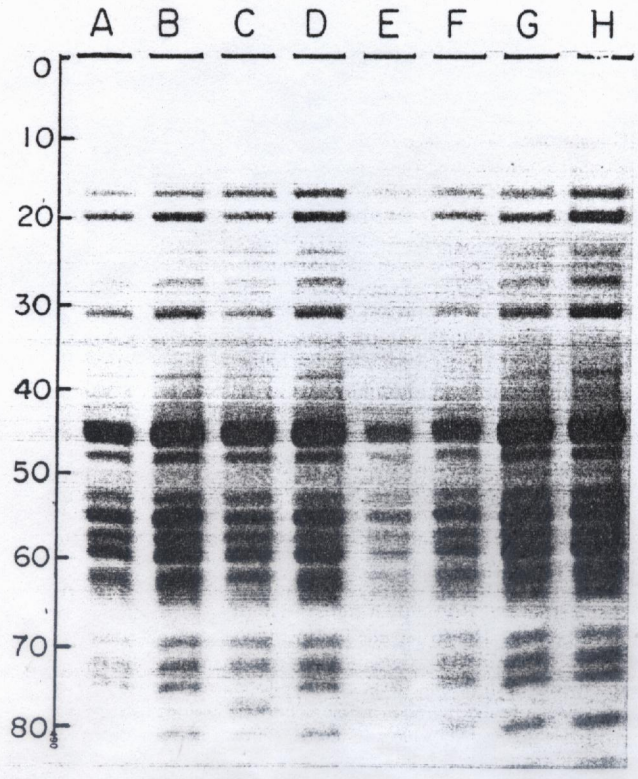

Fig. 5. Gliadin electrophoregrams of A, Guard major fraction (G M-1); B, G M-2; C, G M-3; D, G M-4; E, Guard with hard red winter wheat morphological characteristics (G HRW-1); F, G HRW-2; G, G HRW-3; H, G HRW-4.

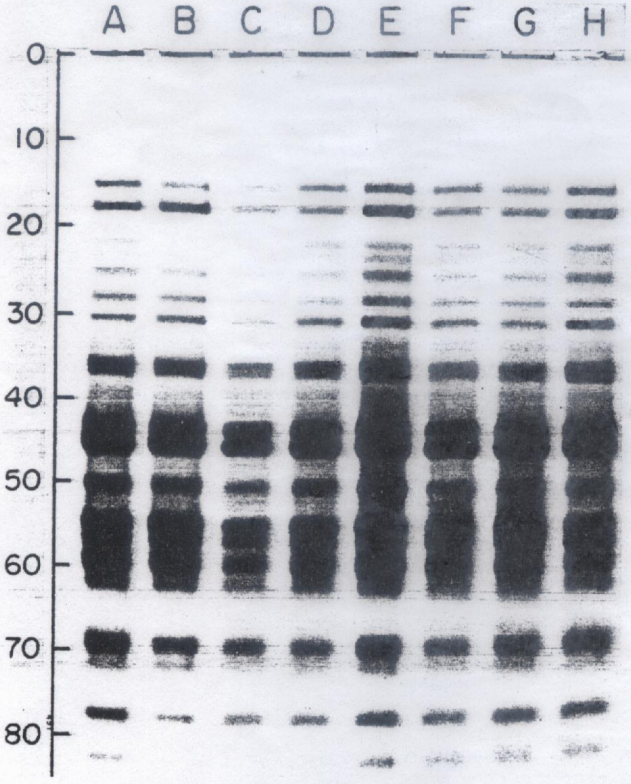

Fig. 6. Gliadin electrophoregrams of A, Stoa major fraction (Stoa M-1); B, Stoa M-2; C, Stoa M-3; D, Stoa M-4; E, Stoa with hard red winter wheat morphological characteristics (Stoa HRW-1); F, Stoa HRW-2; G, Stoa HRW-3; H, Stoa HRW-4.

kernel 1 were higher than those of the other Arthur kernels, which implies a higher protein content. Thus, in Arthur 1 and Sage 1, higher protein contents may have affected the ability of the hardness test to accurately predict hardness in individual kernels, even though in bulk samples protein content does not have a consistent effect on hardness characteristics (Miller et al 1982).

The PHV for Newton segregates the kernels into soft types (1, 4) and hard types (2, 3). The soft types (1, 4) were previously found to exhibit electrophoretic band patterns in the top fourth of the gel (Fig. 4) similar to patterns of Arthur (Fig. 3), a soft wheat. Thus, in the Newton samples examined, a correlation exists between PHV and electrophoretic pattern.

Eagle kernel 1 was predicted as intermediate and kernel 4 was soft by the PHV. PAGE, however, showed all Eagle kernels to be identical.

All Ute and Ute HRS kernels were predicted hard except for HRS-3, which was intermediate. All Ute and Ute HRS kernels appeared identical upon PAGE.

All Guard samples were predicted hard except Guard M-3 and Guard HRW-1, which were intermediate in hardness. The electrophoretic pattern of Guard M-3 (Fig. 6), which showed a minor band change, and the light gliadin band pattern of Guard HRW-1 (Fig. 6) are the only electrophoretic pattern differences found with the kernels of intermediate hardness. Therefore no consistent interaction of electrophoretic pattern with intermediate hardness was found.

The PHVs for Stoa kernels (major HRS fraction) and those of its HRW subset are all hard, consistent with their identical electrophoretic patterns (Fig. 7). Because the intensities of the bands in the Stoa kernels varied from dark in M-2 to light in M-3 and no difference in hardness value was found, hardness is probably not directly related to intensity of gliadin patterns.

Ram major kernels were predicted hard. Ram HRS-1 was predicted intermediate and Ram HRS-2 was soft; Ram HRS-3 and Ram HRS-4 were predicted hard. The electrophoretic bands of HRS-1 are very intense, implying a high protein content. The effect of possibly higher protein content affecting the PHV of individual kernels was noted previously for Arthur 1, Sage 1, and Guard HRW-1. The PHV of Ram SRW-1 was soft, of SRW-3 intermediate, and of SRW-2 and SRW-4 hard. PAGE patterns, however, appeared nearly identical for all 12 Ram kernels; only in Ram HRW-1, HRS-1, HRS-4, and SRW-1 were minor band

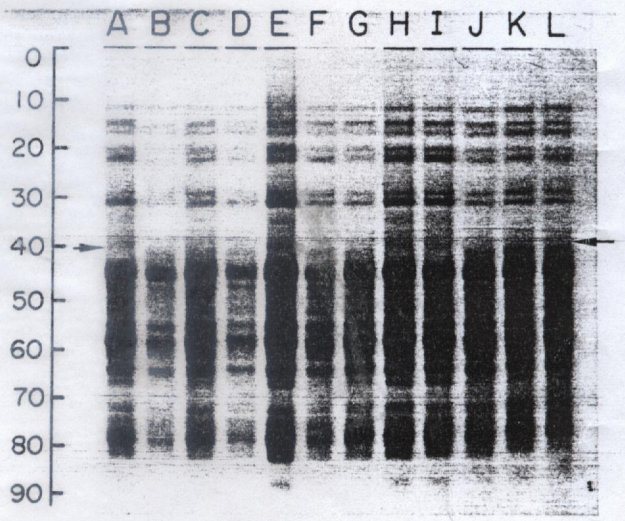

Fig. 7. Gliadin electrophoregrams of A, Ram major fraction (Ram M-1); B, Ram M-2; C, Ram M-3; D, Ram M-4; E, Ram with hard red spring wheat morphological characteristics (Ram HRS-1); F, Ram HRS-2; G, Ram HRS-3; H, Ram HRS-4; I, Ram with soft red winter wheat morphological characteristics. Ram SRW-1; J, Ram SRW-2; K, Ram SRW-3; L, Ram SRW-4. Arrows indicate light bands not easily seen.

of morphological characteristics and apparent texture from expected typical kernels of the certified wheat cultivars. Seven kernels appeared to have intermediate (soft-hard) kernel characteristics as predicted from a hardness test. In seven kernels, hardness characteristics were different from that expected of the cultivar. Thus, significant differences in both hardness and PAGE characteristics were found among kernels identified as morphologically pure and among kernels identified as morphologically different.

CONCLUSIONS

This study established how morphologically equal and morphologically different kernels (as evaluated by FGIS) of foundation seed (as provided by plant breeders) compare in hardness characteristics and electrophoretic patterns. The number of kernels analyzed was not sufficient to allow a statistically significant determination of the percentage of biotypes in certified seed of pure cultivars; that would be a comprehensive project of its own and should be done in the future. However, the manner in which the samples were selected and graded lends appreciable credence to our results.

The gliadin patterns of Arkan seed with Sage morphological characteristics were more similar to Sage than to Arkan, which implies that gliadin patterns are related to grain morphology as well as to genetic background or that considerable genotypic heterogeneity also exists.

Four of the Ram kernels exhibited gliadin patterns different from the other eight kernels and the typical bulk pattern. Because those four kernels included members of each class, no clear-cut conclusions can be derived. No differences in gliadin patterns of the other cultivars of mixed morphological characteristics were found. The data from Ute, Guard, and Stoa also imply no correlation of kernel type to electrophoretic pattern. Finally, the lack of consistent electrophoretic difference between the kernels of Ram with HRW, HRS, or SRW morphological characteristics is of interest and implies again that kernel morphology is not necessarily related to grain hardness or gliadin electrophoretic patterns. A possible effect of high protein content on hardness characteristics of individual kernels (unlike bulk samples) was also noted.

The relative protein content seems to be related to the energy required to crush a single seed (first peak height) and the intensity of the electrophoretic bands. Moreover, a high protein content, as indicated by intense electrophoregram bands, seems to affect borderline (intermediate) hardness values.

Our results seem to demonstrate that gliadin patterns are not necessarily related to hardness, that hardness measurements are not necessarily related to phenotype, that many cultivars are significantly heterogeneous, and that standards for grading on the basis of morphological characteristics alone may relate to the genetic background of some varieties but were of limited value for most of the varieties included in this study.

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The Effects of *Fusarium graminearum* Infection on Wheat Kernels[1]

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ABSTRACT

A number of tests were conducted on scabby wheat to determine what damage was caused by fungal infection. Hard red winter wheat infected with *Fusarium graminearum* was graded into three categories based on appearance: 1) normal kernels appearing sound, of good color and weight; 2) lightly infected kernels of normal size but of light weight and color; and 3) heavily infected kernels that were shriveled and light colored. These wheat classes were then analyzed to detect fungal presence using light and electron microscopy, histochemistry, polyacrylamide gel electrophoresis of storage proteins, germination tests, and plating. The results of these tests revealed that the fungus is an aggressive invader destroying starch granules, storage proteins, and cell walls. The fungus was most prevalent in aleurone and pericarp tissues, but hyphae were found throughout the starchy endosperm. The germ seemed to be spared infection except in heavily invaded kernels, however, the lightly infected kernels with apparently uninfected germs exhibited reduced germination and vigor. Microscopic examination of lightly infected kernels germinating revealed extensive invasion of the scutellum and embryonic axis, indicating renewed fungal growth during imbibition. The results of this study suggest that a visual inspection of scabby wheat kernels can be used to discern gross differences in infection.

Wheat scab, or head blight, is a fungal disease caused by *Fusarium graminearum* Schwabe. The fungus infects developing wheat panicles and results in kernels with varying degrees of infection. During the 1982 growing season much greater than normal amounts of wheat scab were reported for eastern Kansas and southeast Nebraska. Because of several local severe outbreaks of the disease and reports of high levels of the toxin deoxynivalenol (DON), there was a great deal of public concern and a reluctance of buyers to accept Kansas or Nebraska wheat. According to USDA estimates, less then 3.5% of the total hard red winter wheat crop was affected, however. We undertook a multifaceted research project to investigate the wheat scab problem. This report describes the structural and biochemical changes in wheat caused by the fungus.

MATERIALS AND METHODS

Samples

Wheat samples were from the 1982 growing season. The wheat was divided by hand into three categories based on gross morphological characteristics. Group 1 (normal) contained kernels normal in appearance, group 2 (light to moderately infected) kernels were normal in shape and size but light in color and weight, and group 3 (shriveled) kernels were small and shriveled. Wheat appearing normal had the highest protein, lowest ash, highest 1,000-kernel weight, and lowest levels of DON and ergosterol (a measure of fungal infection, Seitz et al 1979) (Table I). DON was measured by the methods of Pollmann et al (1985). Group 2 wheat had lower protein, higher ash, lower 1,000-kernel weight, and higher levels of DON and ergosterol than the normal wheat. Shriveled kernels had near normal protein and ash, very low 1,000-kernel weight, and extremely high DON and ergosterol levels (Table I).

Light Microscopy

Wheat samples for histochemistry were fixed in 3% paraformaldehyde (w/v) and 3% glutaraldehyde (v/v) in 0.1*M* sodium dibasic and potassium monobasic phosphate buffer (Lillie 1954) for 1 hr at 21° C and 16 hr at 4° C. Samples were washed four times in buffer at 21° C for a total of 2 hr and then dehydrated, infiltrated, and embedded as for electron microscopy (see next paragraph). Plastic thick sections (1-μm thick) were cut with glass knives and affixed to slides at 80° C for 30 min. Protein was stained using the Coomassie Brilliant Blue method (Bechtel and Pomeranz 1978). Carbohydrates were stained by the periodic acid/Schiff's (PAS) reaction (Jensen 1962) with appropriate controls (Bechtel and Pomeranz 1981).

Electron Microscopy

Wheat samples were fixed and washed in the same solutions as for light microscopy and postfixed in phosphate-buffered 1% osmium tetroxide for 2 hr at 21° C. Then the tissue was washed in water three times for a total of 30 min, dehydrated in a graded acetone series, and infiltrated and embedded in a low viscosity epoxy resin. Plastic sections 1-μm thick were cut with glass knives; then thin sections were cut with a diamond knife, stained in 2% aqueous uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963), and viewed in a Philips EM201 electron microscope at 60 kV.

Germination and Culture Conditions

Whole kernels (25 grains in each group) and dissected germs (10 in each group) were placed on sterile 2% agar in petri dishes at 27° C, and germination was monitored for one week. Germs were removed from surface disinfected kernels using the corner of a razor blade to pop the germ free from the endosperm. Microscopy revealed that the germ was removed between the scutellar epithelium and endosperm. Whole kernels and dissected germs were fixed after 72 hr according to the electron microscopy procedure. Germination vigor was considered low if seedling length was less than half and very low if less than one quarter that of uninfected kernels. *F. graminearum* that grew out of kernels was subcultured onto either potato-dextrose agar or 2% water agar with 1% (w/v) wheat flour added. Cultures were maintained at 25° C.

TABLE I
Scabby Wheat Characteristics

Wheat	Protein[a] (%)	Ash[b] (%)	1,000-Kernel Weight (g)	DON[c] (ppm)	Ergosterol[d] (ppm)
Normal	13.7	1.7	29.9	0.43	2.8
Lightly to moderately infected	11.8	2.0	25.6	22.7	29.0
Shriveled	12.3	1.8	13.1	68.7	103.0

[a] Determined by AACC method 46-11.
[b] Determined by AACC method 08-01.
[c] DON = deoxynivalenol, by the method of Pollmann et al 1985.
[d] Determined by the method of Seitz et al 1979.

[1] Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.
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