Wheat Pentosans. I. Cultivar Variation and Relationship to Kernel Hardness[1]

B. H. HONG,[2] G. L. RUBENTHALER,[3] and R. E. ALLAN[3]

ABSTRACT

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A wide range of water-soluble, enzyme-extractable, and total pentosans was found among the hard, soft, and club wheat market classes. A significant correlation was observed between each of the three types for pentosans and kernel hardness. Hard wheats had significantly higher levels of water-soluble pentosans than soft or club wheats. Club wheats were consistently lower in all types of pentosans than the common soft wheats. In addition to water-soluble pentosans, protein content and vitreosity were correlated with grain hardness, suggesting that the differences in hardness from one environment to another could be due to the correlated variations of protein and water-soluble pentosans. However, genotype variance was 1.6 times greater than that of environment, which indicated significant proportions of heritability in the total variations of water-soluble pentosans.

Hardness of wheat endosperm is of considerable importance to milling quality and functional properties of dough. Hardness of endosperm can be explained as the degree of adhesion between the major endosperm components such as starch granules and proteins (Simmonds et al 1973). The amount of soluble materials from wheat flour prepared from a range of wheat classes was shown to be associated with endosperm hardness. The soluble materials comprise protein, xylose, arabinose, mannose, and glucose in a ratio of 2:1 carbohydrate/protein.

Fractionation of the water-soluble pentosans by diethylaminoethyl cellulose chromatography (Kuendig 1961) established that the major fraction was arabinoxylan and the minor constituents were xylose, arabinose, and protein-bound galactose. The minor constituents were responsible for oxidative gelation of aqueous extracts of wheat flour.

Simmonds (1974) referred to the buffer-soluble cementing material on the starch granule and protein matrix interface as a reinforcing space filler. The amount is both environmentally and genetically controlled.

Water-soluble pentosans in the different market classes of wheat were studied by Perlin (1951), who reported identical structures and functions in both bread and durum wheat varieties. Medcalf et al (1968) conducted detailed wheat pentosan studies by fractionating pentosans into water-soluble and water-insoluble pentosans; the latter are mainly associated with the starch tailings portion of flour. In the water-soluble fraction, Nugaines, a soft white winter wheat, had lower molecular weight pentosan and less branched arabinose than Thatcher, a hard red spring wheat.

Pomeranz et al (1985) used various hardness parameters and tests on wheat genotypes grown in diverse environments. Near-infrared reflectance (NIR) spectroscopy at 1,680 nm expressed consistent positive correlations with hardness parameters within cultivars across environments and among genotypes. Despite the theories that favor a continuous structure of protein matrix (Stenvert and Kingswood 1977) to explain hardness of wheat endosperm, most studies have demonstrated poor relationships between protein content and endosperm hardness. However, variances for hardness are greater for genotype than environment.

The objectives of this study were to evaluate the various market classes of wheat known to differ in grain hardness and related traits, and grown in diverse environments, and to measure the association of the three types of pentosans (total, water-soluble, and enzyme-extractable) to grain hardness.

MATERIALS AND METHODS

Three groups of wheat cultivars were selected from the yield performance nursery grown in 1986 at Pullman, WA (high precipitation, soft wheat area), and Lind, WA (low precipitation, hard wheat area). Cultivars Batum, Hatton, Wanser, McKay, Wampum, Spillman, and Burt represented the hard wheat group. Daws, Yamhill, Stephens, Nugaines, Dirkwin, Owens, and WA 7074 comprised the soft wheat group. Tyee, Paha, Crew, and Moro made-up the club wheat group. Samples were ground in a Udy cylone sample mill fitted with a 0.5-mm screen. Samples varied little in moisture content; all were within the range of 9–10%. These whole wheat ground samples were used in the respective tests.

Hardness score of the grain was estimated with a Technicon NIR-400 using a two-wavelength calibration standard AACC method (AACC 1983) with the following coefficients:

$$\text{Hardness score} = 243.03 - 1{,}098.9\ (\log 1/R \text{ at } 1{,}680 \text{ nm}) + 1{,}474.8\ (\log 1/R \text{ at } 2{,}230 \text{ nm})$$

Pentosan determinations were made for water-soluble, enzyme-extractable, and total pentosans and analyzed using the orcinol-HCl method of Hashimoto et al (1987). The percent pentosan was calculated using the following equation:

$$\text{Pentosan } (\%) = A \times 2 \times 0.88 \times m - 100,$$

where A is the absorbance of xylose in the solution at 670 nm and m is the slope of the standard curve. The dilution factor was increased to 8 for total pentosan calculations due to sequential dilution of the extract solution after yeast fermentation. The m values were 97.63, 89.01, and 91.52 for water-soluble, enzyme-extractable, and total pentosan, respectively.

In the analysis of enzyme-extractable pentosan, 200 μl of 0.5% Meicellase was added to a mixed solution consisting of 100 mg of whole wheat flour and 10 ml of 0.1M sodium acetate buffer, which was adjusted to pH 4.5 with acetic acid. Meicellase, a muticomponent enzyme system of *Trichoderma viride* origin included cellulases (I and II), β-glucosidase, xylanases (A and B), β-xylosidase, and α-L-arabinosidase (Hashimoto et al 1971, Hashimoto 1982).

Enzyme extraction was done for 18 hr in a shaking water bath maintained at 30°C. Four replications were made for water-soluble pentosan and two each for enzyme-extractable and total pentosans.

[1]College of Agriculture and Home Economics Research Center, Washington State University, Pullman, WA.
[2]Department of Agronomy, College of Agriculture, Korea University, Seoul, Korea. (Visiting scientist, USDA, ARS, Wheat Genetics, Quality, Physiology and Disease Research Unit.)
[3]USDA, ARS, Wheat Genetics, Quality, Physiology and Disease Research Unit, Pullman, WA.

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Particle size was measured with an ATM Sonic Sifter. At the top position, a 104-μm opening sieve screen was fitted, and 2 g of whole wheat flour was sifted in the pulse/sift function for 5 min. The sieve tray was rotated 180° and then operated for another 5 min. Samples remaining on the sieve were collected and weighed. Particle size was expressed as percent of flour weight left on the sieve from the original sample weight.

Protein content was obtained by NIR Technicon model 400 analyzer simultaneously with the hardness index. Grain vitreosity was classified into 10 categories ranging from 1.0 for 100% glassy to 0.1 for 100% mealy. Fifty randomly chosen grains were cut perpendicular to the crease with a razor blade, and the cut surface was read under a magnifying glass. The average rating of the 50 grains represented the degree of vitreosity of the given cultivar.

Statistical analyses of variance were made for various traits, and sample correlations were obtained among means of the traits using the SAS PC packages (SAS 1985a,b).

RESULTS AND DISCUSSION

Grain Hardness Characteristics and Their Relationship to Pentosan Levels

Cultivar means across the two locations for grain hardness and its related characteristics are given in Table I. As expected, the hard wheats exhibited harder endosperms than both the soft and club wheats. A hardness score above 50 characterized the hard wheat group. Among the hard wheats, Wanser and Burt had significantly lower hardness scores than the other hard wheat cultivars, and Spillman had the highest score. Soft wheat cultivars ranged from 34.10 to 19.34 in hardness scores, showing wider variation than found in the hard wheats and the clubs. Some of the club wheats exceeded most of the soft wheat cultivars in hardness score. As a group they were not statistically different in hardness. Pomeranz et al (1988) reported similar results in their NIR studies on wheat grain hardness. Means for the three types of pentosans are also presented in Table I. In general, the harder the grain, the higher the content of each type of pentosan. However, the soft white winter wheat Daws exhibited both the highest content of water-soluble and enzyme-extractable pentosans and the hardest grain texture in its class, making it uniquely different among the soft wheat cultivars. This type of inconsistency in expression of high pentosan levels suggested genotypical specificity and may be useful material for further studies in pentosan genetics and biochemistry. We found the enzyme-extractable and total pentosans generally followed the soluble pentosan (except in the soft white variety Daws). The hard white winter wheat Burt was significantly lower in total pentosan compared with those of the hard red winter and spring cultivars, whereas it expressed typical hard wheat content for soluble and enzyme-extractable pentosans.

Protein levels of the wheats tested were relatively high. Hard red spring cultivars had the highest protein content followed by hard red winter, soft white spring, soft white winter, and club wheat cultivars. Many studies of the relationship between protein content and grain hardness have reported either positive or negative relationships depending upon the genotypes and hardness parameters studied. Pomeranz et al (1985) showed a significant positive relationship between protein level and NIR hardness score within some cultivars. Particle size and vitreosity of the grain were also investigated as supplementary parameters to grain hardness. A higher proportion of large particles was strongly associated with higher vitreosity for hard wheat groups.

Correlation coefficients of the related grain hardness parameters are given in Table II and those estimated separately by location are presented in Table III.

Scatter plots of the hardness score and pentosan levels of the respective groups of material are shown in Figures 1, 2, and 3. Grain hardness was significantly correlated with three types of pentosans ($r = 0.6121$, 0.5877, and 0.4459) as well as with particle size ($r = 0.8235$), protein content ($r = 0.6672$), and vitreosity ($r = 0.9291$).

Kulp (1968) and Jelaca et al (1971) found that wheat flour pentosans, and particularly the water-soluble pentosans, usually absorb 10 times their weight in water. Accordingly, if water is added to flour on the basis of protein level alone during the dough development process, it will produce stiffer and drier dough in a flour high in pentosan content. Shogren et al (1987) reported that water-soluble pentosan was negatively correlated with hard

TABLE I
Varietal Differences of Grain Hardness and Related Characteristics

No.[a]	Cultivar	NIR[b] Grain Hardness	Pentosan (%) Water-Soluble	Enzyme-Extractable	Total	Protein (%)	Particle Size (%)	Vitreosity
Hard red and white								
1	Batum	64.89	0.683	1.038	5.396	13.56	32.83	0.872
2	Hatton	66.14	0.709	1.152	5.742	13.01	31.68	0.820
3	Wanser	59.88	0.648	1.164	5.773	11.85	34.06	0.715
4	McKay	66.29	0.559	0.994	5.651	14.60	32.73	0.888
5	Wampum	63.65	0.533	0.975	6.059	15.36	32.40	0.835
6	Spillman	69.66	0.633	0.925	5.762	15.89	31.05	0.840
7	Burt	60.47	0.568	1.055	4.839	11.13	33.44	0.808
Soft white								
8	Daws	34.10	0.755	1.112	5.565	12.62	27.46	0.358
9	Yamhill	26.06	0.475	0.866	5.303	11.01	28.14	0.247
10	Stephens	29.60	0.499	0.905	5.510	10.51	28.35	0.440
11	Nugaines	24.13	0.549	0.918	5.671	10.04	28.71	0.333
12	Dirkwin	30.84	0.535	0.943	5.536	13.01	29.27	0.418
13	Owens	24.90	0.375	0.896	5.112	12.28	21.75	0.215
14	WA7074	19.34	0.481	0.947	5.185	12.30	24.55	0.368
White club								
15	Tyee	27.56	0.386	0.866	4.862	9.57	25.35	0.247
16	Paha	32.33	0.306	0.824	4.066	9.30	26.72	0.160
17	Crew	21.83	0.411	0.847	4.772	9.58	28.39	0.175
18	Moro	31.61	0.408	0.863	4.579	10.24	28.88	0.213
LSD (0.05)		3.41	0.044	0.063	0.523	0.84	0.99	0.112

[a]Sample numbers 1–3, hard red winter; 4–6, hard red spring; 7, hard white winter; 8–11, soft white winter; 12–14, soft white spring; 15–18, club wheat.
[b]Near-infrared reflectance.

wheat flour protein and loaf volume, and partial correlations were positively significant in some hard wheat groups between water-soluble pentosan and loaf volume when protein level was held constant. Yet our results clearly demonstrated significant relationships between protein level and each of the three types of pentosans but weak correlations. Figures 1–3 show obvious differences between hardness score and the pentosan type between the market classes. They also indicate that grain hardness tends to be enhanced as water-soluble and total pentosan contents increase among cultivars of the same market class, even though that tendency is not quite so obvious with the change in enzyme-extractable pentosan. Particle size, an indirect parameter for grain hardness, gave highly significant correlation coefficients with the three types of pentosans but exhibited a poorer relationship with protein content, which indicates these cereal gums may have a significant role in the particle size phenomena.

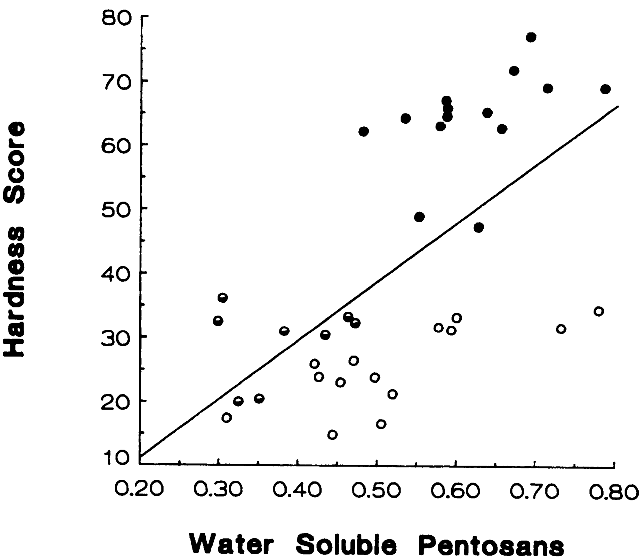

Fig. 1. Relationship between water-soluble pentosan and grain hardness in different market classes of wheat (● = hard wheat, ○ = soft wheat, ◒ = club wheat).

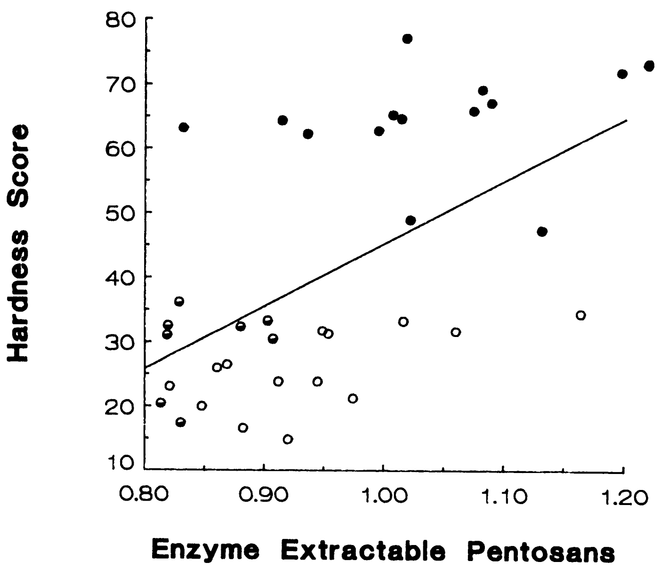

Fig. 2. Relationship between enzyme-extractable pentosan and grain hardness in different classes of wheat (● = hard wheat, ○ = soft wheat, ◒ = club wheat).

TABLE II
Correlation Coefficients Between Grain Hardness and Pentosans Parameters

Parameters	Water-Soluble Pentosan	Enzyme-Extractable Pentosan	Total Pentosan	Particle Size	Protein Content	Vitreosity
Grain (NIR)[a] hardness	0.6121***[b]	0.5877***	0.4459**	0.8235***	0.6672***	0.9291***
Water-soluble pentosan		0.8529***	0.7164***	0.6060***	0.5646***	0.6684***
Enzyme-extractable pentosan			0.6110***	0.5461***	0.4467**	0.6339***
Total pentosan				0.4184*	0.6064***	0.5750***
Particle size					0.4882*	0.7831***
Protein content						0.7383***

[a]Near-infrared reflectance.
[b]*, **, ***: Significant at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively ($n = 36$).

TABLE III
Correlation Coefficients Between Grain Hardness and Pentosan Parameters by Location (Pullman and Lind, WA)

	Lind						
Parameter	NIR[a] Hardness Score	Water-Soluble Pentosan	Enzyme-Extractable Pentosan	Total Pentosan	Particle Size	Protein Content	Vitreosity
Pullman							
Hardness score		0.589**[b]	0.660**	0.470*	0.864**	0.533*	0.884**
Water-soluble pentosan	0.578*[b]		0.886**	0.813**	0.578**	0.555*	0.761**
Enzyme-extractable pentosan	0.453*	0.809**		0.651**	0.612**	0.460*	0.745**
Total pentosan	0.320	0.632**	0.457*		0.504*	0.637**	0.683**
Particle size	0.795	0.614**	0.495*	0.353		0.382	0.836**
Protein content	0.655**	0.542*	0.320	0.523*	0.308		0.649**
Vitreosity	0.901**	0.587**	0.496*	0.517*	0.717**	0.785**	

[a]Near-infrared reflectance.
[b]*, **: Significant at $P = 0.05$, and $P = 0.01$, respectively ($n = 18$ each location).

Location Effects on Grain Hardness and Pentosan Level

Correlation coefficients estimated separately for Pullman and Lind also revealed that the three types of pentosans were significantly correlated with hardness score at both locations, except for total pentosan at Pullman. However, correlation coefficients were generally higher at Lind where higher pentosan and protein contents were realized regardless of the market class.

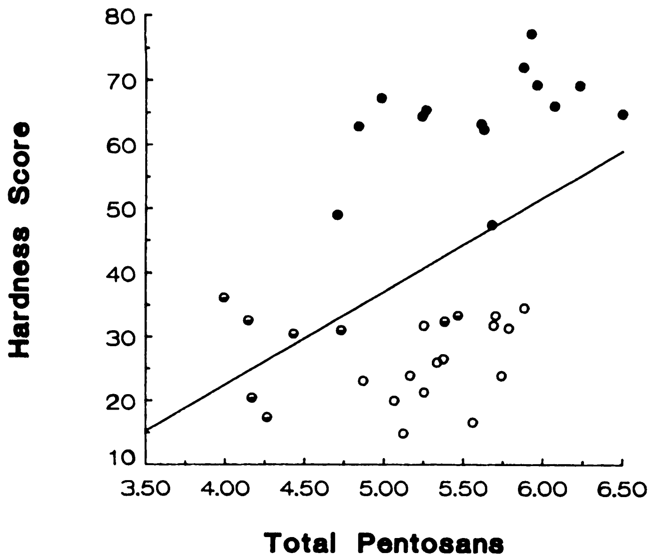

Fig. 3. Relationship between total pentosan and grain hardness in different classes of wheat (● = hard wheat, ○ = soft wheat, ◒ = club wheat).

The two distinctly different growing environments of Pullman and Lind (cool and moist vs. warm and dry) made a great difference in hardness score, pentosan levels, and protein content as seen in Table IV. As expected, Lind generally produced significantly higher values for hardness score, three types of pentosans and protein. These results indicate that environment influences grain protein content, pentosan levels, and ultimately grain hardness. Even though the soft wheats had wider genotype variability for hardness, club wheats were consistently higher in hardness values at both locations. This fact is not explained by pentosan level or protein content since club wheats had lower values for those parameters. Medcalf et al (1968) analyzed pentosans in hard red spring and durum wheat and found the very hard durums to have lower pentosan levels. Hardness characteristics of durum wheat may not be due to the quantity but rather quality of pentosans since they exhibited more arabinose residues as a side chain on the pentosan (arabinoxylan) molecule. In this regard, additional research is needed to establish the hardness nature of the club wheat endosperm.

The genotype differences between locations were correlated with the across-locations means for seven parameters. This estimation was made to clarify which parameters were more linearly responsive to environmental changes. Enzyme-extractable and total pentosans and particle size were not significantly correlated

TABLE IV
The Mean, Maximum and Minimum (Range), and Standard Deviation of Grain Hardness and Pentosan Parameters by Class and Location

Parameter	Class[a]	Lind Mean	Lind Range	Lind SD[b]	Pullman Mean	Pullman Range	Pullman SD
Grain hardness	H	68.63	75.1–64.3	3.12	59.65	61.2–46.1	7.06
	S	29.71	38.8–14.8	5.31	24.30	31.7–14.8	4.88
	C	31.20	33.3–30.7	0.49	26.54	31.7–17.4	4.48
Soluble pentosan, %	H	0.659	0.88–0.56	0.07	0.579	0.71–0.36	0.06
	S	0.570	0.83–0.49	0.11	0.478	0.74–0.58	0.12
	C	0.456	0.50–0.41	0.02	0.337	0.45–0.29	0.03
Enzyme-extractable pentosan, %	H	1.109	1.33–0.98	0.10	0.976	1.17–0.79	0.09
	S	0.987	1.19–0.87	0.08	0.894	1.07–0.80	0.08
	C	0.897	0.94–0.87	0.01	0.823	0.85–0.80	0.08
Total pentosan, %	H	5.929	6.86–4.85	0.47	5.276	5.81–4.60	0.39
	S	5.601	6.09–4.85	0.27	5.223	5.82–4.51	0.23
	C	5.091	5.73–4.15	0.57	4.286	4.78–3.69	0.32
Particle size, %	H	33.36	36.00–31.25	1.38	31.84	33.25–30.55	1.04
	S	27.46	29.45–22.10	2.47	26.32	29.40–20.70	3.11
	C	28.30	29.45–26.55	1.59	26.76	29.05–24.50	2.46
Protein, %	H	14.29	16.82–12.51	1.51	12.98	15.82–9.92	2.27
	S	12.63	14.62–11.39	1.05	10.73	12.87–8.39	1.65
	C	11.49	11.85–11.19	0.22	8.05	8.87–7.50	0.47
Vitreosity, %	H	88.07	96.0–80.0	4.50	74.43	90.0–59.0	9.39
	S	43.21	63.0–20.0	3.02	24.71	41.0–8.0	10.54
	C	33.00	45.0–25.0	0.82	8.75	15.0–4.0	8.75

[a] H = Hard wheats; S = soft wheats; C = club wheats.
[b] Standard deviation.

TABLE V
Variance Components and Ratio Estimates of Genotype Over Locations for Hardness, Pentosans, and Related Characteristics[a]

Source of Variation	Grain Hardness	Pentosans Water-Soluble	Pentosans Enzyme-Extractable	Pentosans Total	Protein	Particle Size	Vitreosity
Market class (c)	28,033.46	0.77	0.35	6.29	211.60	371.32	4.30
Cultivars (g)	6,506.29	0.93	0.36	6.15	160.79	372.99	0.50
Locations (e)	1,267.34	0.57	0.17	2.13	94.90	31.79	0.52
$\sigma c/\sigma e$	22.1	1.3	2.0	3.0	2.2	11.6	8.16
$\sigma g/\sigma e$	5.1	1.6	2.1	2.4	1.7	11.7	0.98
CV (%)	7.79	9.06	4.88	6.13	6.61	2.65	15.52

[a] All mean squares are significant at 0.0001 probability level, except mean squares for cultivars under Total Pentosan, which are significant at the 0.001 probability level.

with grain hardness ($r = 0.123$, 0.037, and 0.275, respectively). Water-soluble pentosan ($r = 0.701$, $P = 0.01$), protein contents ($r = 0.695$, $P = 0.01$), and vitreosity ($r = 0.514$, $P = 0.05$) were highly correlated with grain hardness, suggesting that the difference in hardness between locations was due to the correlated variations of protein content and water-soluble pentosan.

Variance components and ratio estimates are presented in Table V. Variances estimated for market class, cultivar, and location were all highly significant. Market class and cultivar variance were larger than location variance in all parameters studied. The lowest cultivar versus location variance ratio occurred for water-soluble pentosan, protein, and vitreosity among the parameters studied. More detailed research may help elucidate interactions of genotype and environment for those traits.

In summary, harder grains may be attributable to the effects of environment on responsive changes of water-soluble pentosan and endosperm protein levels. Pomeranz et al (1985) reported high genotype versus environment variance ratios in hardness scores and suggested grain hardness to be highly heritable. This experiment also revealed the high genotype versus environment variance ratio in hardness score. However, pentosan and protein levels seemed to be equally affected by environment. Because water-soluble pentosan had a lower cultivar × location ratio and higher coefficient of variation than either enzyme-extractable or total pentosans, its synthesis may have been influenced more by environment than the two other pentosans. Yet, genotype variance was 1.6 times higher than that of environment, indicating significant proportions of heritablility in the total variations of water-soluble pentosan.

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