Puroindoline Genotype of the U.S. National Institute of Standards & Technology Reference Material 8441, Wheat Hardness

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ABSTRACT

Grain hardness (kernel texture) is of central importance in the quality and utilization of wheat (*Triticum aestivum* L.) grain. Two major classes, soft and hard, are delineated in commerce and in the Official U.S. Standards for Grain. However, measures of grain hardness are empirical and require reference materials for instrument standardization. For AACC Approved Methods employing near-infrared reflectance (NIR) and the Single Kernel Characterization System (39-70A and 55-31, respectively), such reference materials were prepared by the U.S. Dept. of Agriculture Federal Grain Inspection Service. The material was comprised of genetically pure commercial grain lots of five soft and five hard wheat cultivars and was made available through the National Institute of Standards and Technology (SRM 8441, Wheat Hardness). However, since their establishment, the molecular-genetic basis of wheat grain hardness has been shown to result from puroindoline a and b. Consequently, we sought to define the puroindoline genotype of these 10 wheat cultivars and more fully characterize their kernel texture through Particle Size Index (PSI, Method 55-30) and Quadrumat flour milling. NIR, SKCS, and Quadrumat break flour yield grouped the hard and soft cultivars into discrete texture classes; PSI did not separate completely the two classes. Although all four of these methods of texture measurement were highly intercorrelated, each was variably influenced by some minor, secondary factors. Among the hard wheats, the two hard red spring wheat cultivars that possess the *Pina-D1b* (a-null) hardness allele were harder than the hard red winter wheat cultivars that possess the *Pinb-D1b* allele based on NIR, PSI, and break flour yield. Among the soft wheat samples, SKCS grouped the Eastern soft red winter cultivars separate from the Western soft white. A more complete understanding of texture-related properties of these and future wheat samples is vital to the use and calibration of kernel texture-measuring instruments.

Grain hardness or texture of wheat (*Triticum aestivum* L.) is a fundamental quality determinant and serves as a basis for classifying much of the world trade in this important cereal. Although the measurement of grain texture has been studied and characterized at a material property level (Glenn et al 1991; Delwiche 2000) it is still predominantly assessed empirically using either the granularity (particle size distribution) of the meal produced by grinding or the force/fracture characteristics of individual kernels observed while crushing. Methods that exploit differences in granularity characteristics of ground meals date back to Cutler and Brinson (1935). Currently, two methods predominate: one based on the proportion of meal separated by sieving or particle size index (PSI) (Approved Method 55-30, AACC 2000) (Williams and Sobering 1986) (see also, ICC Recommendation No. 207, Determination of the Particle Size of Milling Products using Sieve Analysis, http://www.icc.or.at/ methods3. php#ICC 207), the other based on near-infrared reflectance spectroscopy (NIR) (Approved Method 39-70A, AACC 2000) (Norris et al 1989). The crushing strategy was commercialized by the USDA and Perten Instruments (Springfield, IL) in the form of the Single Kernel Characterization System 4100 (SKCS) (Martin et al 1993). The SKCS gained Approved Method status with the AACC in 1998 (Method 55-31, AACC 2000) (Gaines et al 1996). PSI is simply the proportion (×100, percentage) of material passing through a defined sieve. The NIR method generates a unitless number based on a regression equation using predetermined hardness values from five soft wheat samples and five hard wheat samples that are maintained at the U.S. National Institute of Standards & Technology (NIST) as Reference Material 8441 (Certificate issued 28 October 1997) (http://patapsco.nist.gov/srmcatalog/common/view_detail.cfm?srm =8441). Because the SKCS method was designed to emulate the same NIR hardness readings, its calibration also relies on these NIST samples.

Variation in wheat kernel hardness has undoubtedly been observed since prehistoric times. In the 1960s and 1970s, the role of the *Hardness* gene was documented and the relationship between kernel hardness and *Hardness* alleles was established (reviewed in Morris 2002). As such, soft wheats possessed the *Ha* allele and hard wheats the *ha* allele. Durum wheats, which lack the D-genome where the *Hardness* locus resides, represent an additional, yet harder class. In the last 15 years, the molecular-genetic basis for these three major hardness classes was resolved and shown to result from an association with the proteins/genes, puroindoline a and b. Furthermore, it was established that hard grain texture could result from any one of seven mutant variants in puroindoline a or b (Giroux and Morris 1997, 1998; Lillemo and Morris 2000; Morris et al 2001, Morris 2002). Recent studies indicate that not all hardness mutations affect kernel texture equally (Giroux et al 2000; Martin et al 2001). Data tend to support the concept that those hard wheats that lack puroindoline a protein (genotype = *Pina-D1b Pinb-D1a*) are somewhat harder than those hard wheats that possess a normal soft puroindoline a but have an altered, mutant form of puroindoline b (genotype = *Pina-D1a Pinb-D1b*).

Clearly, due to the empirical nature of the hardness values obtained from the NIR and SKCS methods, the specific characteristics of these and future NIST reference samples are of interest. The aim of this study was to define the puroindoline genotype of the wheat cultivars comprising the NIST Reference Material 8441 and assess the possible relationship to hardness phenotype.

MATERIALS AND METHODS

Plant Material

The 10 wheat varietal samples of Reference Material 8441 were obtained from the U.S. National Institute of Standards & Technology, and were used throughout this study. The Certificate of this Standard Reference Material states that, *"One unit of RM 8441 consists of fifty pouches, five pouches each of five hard wheats and five soft wheats. Each pouch contains 20 g of material."* Furthermore, *"Ten separate lots of wheat (three pure varieties of hard red winter, two pure varieties of hard red spring, two pure varieties of soft red winter, and three varieties of soft white wheat) were purchased from commercial sources."* The hardness of RM

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8441 was characterized by the U.S. Dept. of Agriculture Federal Grain Inspection Service under the direction of A. C. Johnson. In the RM 8441 Certificate, the samples are simply identified as 'Hard-x' and 'Soft-x' where x = the cultivar sample, 1 through 5 (Table I). GIPSA FGIS provided us the varietal identities of the samples (A. C. Johnson, *personal communication*). The samples were leading cultivars in commercial production at the time of their selection. All but 'Len' and 'Malcolm' were registered with the Crop Science Society of America ('TAM 105', CItr 17826 [Porter et al 1980]; 'Arapahoe', PI 518591 [Baenziger et al 1980]; 'Newton', CItr 17715 [Heyne and Niblett 1978]; 'Yecora Rojo', CItr 17414 [Qualset et al 1985];'Cardinal', PI 502973 [Lafever 1988]; 'Titan', CItr 17762 [Lafever 1979]; 'Madsen', PI 511673 [Allan et al 1989]; and 'Tres', CItr 17917 [Allan et al 1986]). Len (CItr 17790) was released in 1979 by North Dakota State Univ. and Malcolm (PI 497672) was released in 1985 by Oregon State University.

Particle Size Index

Untempered wheat samples were ground with a Tecator Cemotec 1090 burr mill (Foss-Tecator, Eden Prairie, MN), set to 1, the lowest setting available on the mill. The resulting meal was sifted (Ro-Tap, W.S. Tyler Co., Mentor, OH) using 210-μm opening, 20.3-cm dia sieves, for 5 min/sample. The weight of the meal recovered from the bottom pan was used to calculate the PSI (weight of throughs divided by total sample weight, × 100) (Approved Method 55-30, AACC 2000).

Quadrumat Flour Milling

Samples were milled on a Quadrumat flour mill (C. W. Brabender Instruments, South Hackensack, NJ) following the modified procedure of Jeffers and Rubenthaler (1977). Only the first grinding head was used due to the limited sample. Two pouches of each sample were combined to provide 40 g of each. Soft wheats were tempered to 13% and hard wheats to 14.5% moisture contents, fresh weight basis (fwb). Product was sieved on 30.5-cm dia round U.S. No. 35 and U.S. No. 100 sieves (W. S. Tyler Co., Mentor, OH); break flour was the amount of material passing through the No. 100 sieve after 1 min of sieving (Great Western Manufacturing, Leavenworth, KS), 10-cm throw, and expressed as a percentage of total products.

Protein Analysis

Grain nitrogen was determined by the Dumas combustion method (Approved Method 46-30, AACC 2000) (model FP-428, Leco Corp., St. Joseph, MI). Protein was calculated as nitrogen × 5.7 and reported on an as-is moisture basis (≈10–12%).

DNA Analysis

Genomic DNA was isolated from the embryo-half of single wheat kernels using the Nucleon PhytoPure kit (Amersham Biosciences, Piscataway, NJ), which includes a polysaccharide binding resin. Full-length puroindoline a and b genes were amplified with primers described by Gautier et al (1994). Reactions were performed in 25 μL containing 50 ng of genomic DNA, 10 pmol of each primer, 250 m*M* of each dNTP (deoxynucleotide tri-phosphate), 1X *Taq* DNA polymerase reaction buffer, 0.5 unit of *Taq* DNA polymerase, and 1.5 m*M* of $MgCl_2$. Annealing temperature for both sets of primers was maintained at 58°C. Polymerase chain reaction (PCR) reactions were analyzed on 1.5% (w/v) agarose gel, stained with ethidium bromide, and visualized using UV light. PCR products were purified from dNTP and oligonucleotide primers with Exonuclease I and shrimp alkaline phosphatase (ExoSAP-IT, UBS, Cleveland, OH), and sequenced directly with amplification 5' primers. Sequence alignments were performed using MultAlin program (INRA, France) that utilizes the multiple alignment algorithm of Corpet (1988).

PAGE of Puroindoline Proteins

Wheat kernels were deembryonated with a razor and crushed between sheets of weighing paper. The crushed kernel was placed in a 2-mL microcentrifuge tube to which was added 1 mL of Tris-buffered saline (TBS), pH 7.5, and 150 μL of 12% (v/v) Triton X-114 detergent (precondensed) (Doering et al 1993). The crushed kernel was extracted for 0.5 hr at 4°C with frequent vortexing. The sample was centrifuged for 5 min at 12,000 × *g* and the supernatant transferred to a 1.7-mL microcentrifuge tube without warming. The supernatant was then warmed for 0.5 hr at 37°C and centrifuged for 5 min at 12,000 × *g*. The upper, aqueous phase was aspirated, and the lower, detergent-rich phase was retained. The detergent-rich phase was transferred to a new 1.7-mL microcentrifuge tube avoiding any residual pellet. An additional 1 mL of cold (4°C) TBS was added and the tube vortexed. The tube and its contents were incubated at 37°C for 0.5 hr, after which centrifugation and aspiration of the upper phase were conducted again as described above. To the detergent-rich phase was added 1 mL of cold (–20°C) acetone. After vortexing, the tube and its contents were kept at –20°C overnight. Precipitated protein was pelleted by 10 min of centrifugation at 12,000 × *g*.

After the pellet was dried for ≈10 min, 250 μL of electrophoresis sample buffer (62.5 m*M* Tris, pH 6.8; 10% [v/v] glycerol; 2% [w/v] SDS and 12.5 mg/L of bromophenol blue) was added. No reducing agent was incorporated in either the sample buffer or in the electrophoresis reagents. The sample was vortexed, incubated in a water bath at 70°C for 5 min and vortexed again. Before

TABLE I
Cultivar, Class, NIR, and SKCS Kernel Hardness, Protein, Particle Size Index, Quadrumat Milling Fraction Yields, and Puroindoline Genotype of the U.S. National Institute of Standards & Technology Reference Material 8441, Wheat Hardness

NIST Sample Identification	Cultivar	Class[a]	Kernel Hardness		Protein (%)	Particle Size Index (%)	Quadrumat[b] (%)			Puroindoline Genotype	
			NIR	SKCS			Bran	Midd's	Br.Flr.	*Pina-D1*	*Pinb-D1*
Hard-1	TAM 105	HRW	74.7	79.0	15.4	16.1	28.7	31.1	40.3	*a*	*b*
Hard-2	Arapahoe	HRW	75.8	66.3	12.8	17.1	29.2	28.8	42.1	*a*	*b*
Hard-3	Newton	HRW	63.7	68.5	14.1	18.2	30.2	26.4	43.5	*a*	*b*
Hard-4	Yecora Rojo	HRS	77.5	63.5	15.1	15.6	24.3	41.7	34.1	*b*	*a*
Hard-5	Len	HRS	91.8	85.5	15.9	15.4	24.8	37.1	38.1	*b*	*a*
Soft-1	Cardinal	SRW	30.0	24.7	11.4	21.4	24.9	25.8	49.3	*a*	*a*
Soft-2	Titan	SRW	29.9	26.1	11.1	23.0	27.2	23.2	49.6	*a*	*a*
Soft-3	Madsen	SWH	31.1	34.4	11.9	20.6	22.4	29.8	47.9	*a*	*a*
Soft-4	Malcolm	SWH	29.8	34.2	11.4	18.3	23.8	29.1	47.2	*a*	*a*
Soft-5	Tres	Club	31.5	36.6	11.6	24.8	24.3	26.3	49.4	*a*	*a*
LSD (0.05)	–	–	–	–	–	–	0.3	1.3	1.4	–	–

[a] U.S. market classes: HRW, Hard Red Winter; HRS, Hard Red Spring; SRW, Soft Red Winter; and SWH, Soft White; Club, Club Soft White wheat subclass.
[b] Milling fractions: Midd's = middlings stock, Br.Flr. = break flour.

loading the electrophoresis gel, the sample was centrifuged at 12,000 × *g* for 2 min to precipitate any remaining solids.

Electrophoresis was conducted using a 13.5% T, 2.67% C resolving gel (Laemmli 1970). The stacking gel was 4% T and 2.67% C. The cross-linker was piperazine diacrylamide. The resolving gel included 10% (v/v) glycerol to reduce diffusion of the lower molecular weight proteins. A 10-well, 0.75-mm comb was used in a Bio-Rad Protean II Xi, 16- × 20-cm gel format. Samples were loaded at 15 µL/lane, corresponding to ≈1 mg of endosperm equivalent per lane.

The gel was run at a constant 50 mA for ≈3.5 hr or until the dye front was judged to have migrated sufficiently far down the gel. For silver staining, each gel was fixed in a combination of 12.5% (v/v) trichloroacetic acid (TCA) and 45% (v/v) methanol for 20 min. All solutions were 200 mL and applied to gels on an orbital shaker. After decanting, a second fixing solution consisting of 7% (v/v) TCA and 5% (v/v) methanol was used for 10 min. After decanting, glutaraldehyde (5% v/v) was applied for 10 min. This step was followed by three water washes, 10 min each. The gel was bathed in dithiothreitol (10 mg/L) for 10 min, followed by a 15-min incubation with 0.1% (w/v) silver nitrate. Two quick water washes followed. The gel was then developed for visualization with 3% (w/v) sodium carbonate with 500 µL/L of formaldehyde (37% stock solution). The gel was incubated in this solution until the gels were judged to be sufficiently developed (≈5 min). The development reaction was stopped by adding 10 mL of 2.3*M* citric acid.

Standards representing wild-type (soft wheat), puroindoline a-null (*Pina-D1b* hard wheat) and puroindoline a- and b-null (tetraploid durum) were used with each gel. Puroindolines a and b were resolved as a doublet band near M_r 14,000. Puroindoline a was the upper, larger band and puroindoline b was immediately below it.

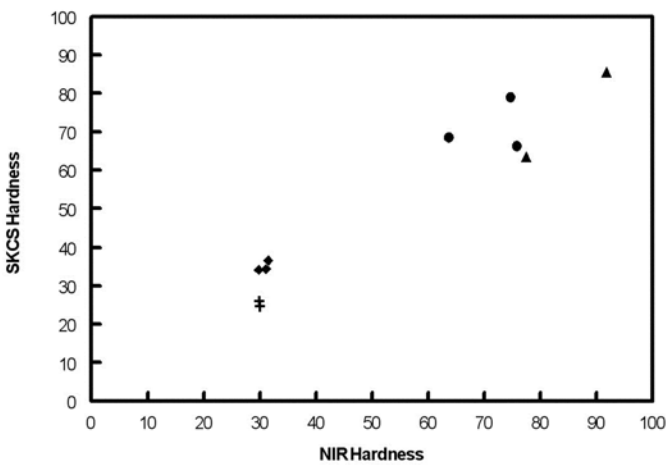

Fig. 1. Near-infrared reflectance (NIR) vs. Single Kernel Characterization System (SKCS) wheat kernel hardness of the 10 cultivars comprising the U.S. National Institute of Standards & Technology Reference Material 8441, Wheat Hardness. Wheat classes: hard red winter (●), hard red spring (▲), soft red winter (✚), and soft white (◆).

RESULTS AND DISCUSSION

NIR and SKCS hardness values were supplied with the samples, so these hardness measures were not repeated (Table I). NIR hardness clearly separated the soft and hard wheats as did the SKCS (Fig. 1). Len exhibited an especially high NIR hardness value of 91.8, followed by the other HRS wheat cultivar Yecora Rojo. The HRW sample range was 63.7–75.8. Len also exhibited the highest SKCS value, but for this measure, Yecora Rojo exhibited the lowest value among the hard wheat samples. For these specific soft wheat samples, NIR values were nearly identical (29.8–31.5), whereas SKCS values differed more (24.7–36.6). Although it may be an artifact, it is interesting to note that based on SKCS, the two eastern SRW cultivars were similar and in the mid-20s, whereas the western soft white cultivars were similar and in the mid-30s. Unpublished results of Morris and Campbell (data not shown) indicate that even when grown under identical environments, the Eastern and Western soft wheats exhibit a significant difference in texture character.

Dumas combustion nitrogen analysis was conducted and showed that protein content (N × 5.7) was relatively high among the hard wheat samples (12.8–15.9%) and lower, and more consistent among the soft wheat samples (11.4–11.9%).

To provide additional grain texture characterization of these samples, Particle Size Index (PSI) and modified Quadrumat milling were conducted (Table I). PSI ranged from 15.4% for Len to

TABLE II
Simple Correlation Coefficients (*r*) Among Hardness Measures and Protein of the U.S. National Institute of Standards & Technology Reference Material 8441, Wheat Hardness[a]

			Quadrumat Milling			
	NIR Hardness	Particle Size Index	Bran	Midd's	Br.Flr.	Protein
SKCS[b]	0.96***	–0.82**	0.47	0.60	–0.85**	0.94***
Hard	0.63	–0.45	–0.19	0.07	0.02	0.67
Soft	0.68	–0.03	–0.69	0.67	–0.50	0.64
NIR		–0.85**	0.42	0.69*	–0.92***	0.93***
Hard		–0.84	–0.75	0.65	–0.55	0.54
Soft		0.54	–0.50	0.28	0.14	0.77
PSI			–0.24	–0.74*	0.88***	–0.82**
Hard			0.87	–0.87	0.84	–0.72
Soft			0.44	–0.65	0.88	–0.06
Bran				–0.30	–0.18	0.29
Hard				–0.98**	0.94*	–0.64
Soft				–0.96*	0.69	–0.88*
Midd's					–0.89***	0.75*
Hard					–0.99**	0.62
Soft					–0.87	0.75
Br.Flr.						–0.91***
Hard						–0.59
Soft						–0.36

[a] Coefficients calculated using cultivar means (n = 10); * P = 0.01–0.05, ** P = 0.001–0.01, *** P < 0.001 for correlations for Hard (n = 5) and Soft (n = 5) classes.
[b] SKCS = Single Kernel Characterization System, NIR = near infrared reflectance, PSI = Particle Size Index, Midd's = middlings, and Br.Flr. = break flour.

24.5% for Tres. Although PSI values grouped the soft and hard wheats correctly based on their market classification, the softest hard wheat (Newton) and the hardest soft wheat (Malcolm) did not differ significantly. Again, the HRS cultivar Len exhibited the hardest texture by this measure, followed by Yecora Rojo. Of the Quadrumat milling fractions, bran yield appeared to have little relationship with the hard and soft market classes. And although middlings stock tended to be higher for the hard wheats, the two texture classes overlapped. Among the hard wheat samples, the two HRS samples exhibited the lowest bran and the highest middlings yields. Among the soft wheats, the eastern SRW samples were lower in middlings stock yield. Break flour yield, on the other hand, provided a clear separation of the two classes: hard wheat sample range of 34.1–43.5%, and soft wheat sample range of 47.2–49.6%. Within texture class, the two HRS cultivars produced the lowest break flour yields; among the soft wheats it was the two soft white common cultivars.

As expected for a group of hard and soft wheats, correlations among the various hardness measures were highly significant (Table II). NIR, SKCS, PSI and Quadrumat break flour yield were all intercorrelated from 0.82 to 0.96 (absolute values). The SKCS was developed after the NIR method and, by design, attempted to produce the same hardness scores; the two methods were the most highly correlated (Fig. 1). Because the hard and soft wheat groups were substantially different in protein content, texture correlations with wheat protein content were similarly high. About 72–85% of the variation (r^2) in Quadrumat break flour yield, which could be considered of primary interest due to its direct relationship to milling performance, was explained by the other hardness measures NIR, SKCS, and PSI. Yield of Quadrumat middlings stock was much less well correlated with hardness measures; bran was not significantly correlated with any parameter.

Within a hardness class, correlations were much lower, supporting the facts that 1) hardness tests capture primarily the effects of the *Hardness* gene; 2) within a hardness class, other secondary factors affect kernel hardness; and 3) the reduced data range influences the correlations themselves. Regarding secondary factors, these are presumably variably expressed depending on the method of texture measure. For example, each of the hardness class groupings tended to follow the geographic origin of the respective cultivars, hard red spring vs. winter, and eastern vs. western soft (Fig. 1 and data not shown). Within the hard wheat group, NIR, PSI, and all three Quadrumat milling fractions grouped the hard red spring cultivars, whereas SKCS did not. Within the soft wheat group, SKCS and Quadrumat bran and middlings yields grouped the SRW cultivars whereas NIR, PSI, and break flour yield did not. Within each hardness class, all correlations with protein content became essentially nonsignificant. Within the hard class, PSI was the most highly correlated with other parameters: correlations with NIR hardness and all three Quadrumat milling fractions were significant at $P \leq 0.08$ (Table II). Higher PSI, which is associated with softer texture, was correlated with higher break flour and bran yields. Softer texture was associated with the hard red winter wheat cultivars. Among the soft wheat samples, the correlation between PSI and break flour yield was significant at $P = 0.052$.

PCR and SDS-PAGE were used to ascertain the puroindoline hardness genotype of each cultivar. From the puroindoline a-primed PCR reaction, either a wild-type *Pina-D1a* allele (sequence) was obtained or no product was produced, which indicated the null *Pina-D1b* allele (Giroux an Morris 1997). All but the two HRS wheat samples produced the *Pina-D1a* puroindoline gene sequence. Upon sequencing the puroindoline b-primed PCR product, all 10 samples matched one of the known puroindoline b genes. The two HRS wheat samples and the five soft wheat samples all possessed the wild-type puroindoline *Pinb-D1a* allele sequence. All three HRW samples possessed the G to A nucleotide substitution that confers a Gly to Ser change in the protein at position 46. This allele is designated *Pinb-D1b* (Giroux and Morris 1997). As we commonly observe, the *Pina-D1b* hard wheats do not produce a puroindoline a PCR product. Consequently this negative result is corroborated by the absence of puroindoline a protein in SDS-PAGE gels (Giroux and Morris 1997) (data not shown).

As reported by Morris et al (2001), essentially all hard red winter wheat cultivars grown in the U.S. Great Plains possess the *Pinb-D1b* allele which originated from the Turkey Red and similar Crimean introductions to Kansas by Mennonite settlers. The RM 8441 hard red winter wheat cultivars TAM 105, Arapahoe, and Newton all possess this allele. Both of the hard red spring wheats, Yecora Rojo and Len, possess the puroindoline-a null allele (*Pina-D1b*). Although essentially absent among the winter wheat cultivars of the U.S. Great Plains, this allele is quite common among hard spring wheats of Canada and the United States (Morris et al 2001).

Results of this study are consistent with those that found that the classical hard and soft phenotype classes of the *Hardness* locus (*ha* and *Ha*) (Law et al 1978) are the major contributors to kernel texture variation, but that other, generally unknown factors influence further the variation within texture class (reviewed in Morris 2002; Perretant et al 2000; Lillemo and Ringlund 2002). Among soft wheats, at least one of these factors appears to be pentosans (Bettge and Morris 2000). Among hard wheats, one clear factor is the contribution of different *hardness* alleles of the puroindolines (Giroux et al 2000;, Lillemo and Morris 2000; Martin et al 2001; Lillemo and Ringlund 2002). In this regard, a-null (*Pina-D1b*) cultivars are usually harder than those possessing the *Pinb-D1b* allele.

It is well recognized that the environment can markedly affect the expression of the hardness genes in wheat. Consequently, the phenotypic hardness of individual grain lots may vary considerably depending on their origin. Although the present research was not aimed at investigating this aspect of wheat kernel texture, it is noteworthy that all 10 of these varietal samples were of commercial origin and representative of what would be encountered by end-users. The study of Gaines et al (1996) included six of these 10 cultivars. SKCS values were similar to those assigned by the NIST (Titan, 25.2, Tres, 33.5, TAM 105, 79.6, Newton, 66.7, Yecora Rojo, 78.4, and Len, 74.3).

CONCLUSIONS

NIR, SKCS, and Quadrumat break flour yield grouped the five hard and five soft wheat varietal samples of the NIST into discrete texture classes. PSI did not significantly delineate the softest hard wheat from the hardest soft wheat. Although all four of these methods of texture measurement were highly intercorrelated, each was variably influenced by some minor, secondary factors. Among the hard wheats, the two hard red spring wheat cultivars that possess the *Pina-D1b* (a-null) hardness allele were harder than the hard red winter wheat cultivars that possess the *Pinb-D1b* allele based on NIR, PSI, and break flour yield. Among the soft wheat samples, SKCS grouped the Eastern soft red winter cultivars separate from the Western soft white. A more complete understanding of texture-related properties of these and future wheat samples is vital to the use and calibration of kernel texture-measuring instruments.

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