Covariation for Microsatellite Marker Alleles Associated with *Rht*8 and Coleoptile Length in Winter Wheat

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ABSTRACT

Wheat (*Triticum aestivum* L.) cultivars with greater coleoptile elongation are preferred in low-precipitation dryland regions and in early-planted management systems of the Great Plains, but the presence of GA3 (gibberellin)-insensitive dwarfing genes tends to restrict coleoptile elongation. The agronomic value of *Rht*8 and the discovery of its diagnostic microsatellite marker, Xgwm 261, have accelerated breeders' interest in *Rht*8 as an alternative dwarfing gene. Our objectives were to determine allelic distributions at the marker locus in contemporary samples of hard winter and soft red winter wheat relative to samples of Chinese accessions from a *Rht*8-rich geographic region, and to compare coleoptile elongation in the presence or absence of *Rht*8 determined by the Xgwm 261 marker. The 165-bp (primarily hard winter wheats) and the 174-bp (primarily soft red winter wheats) alleles of Xgwm 261 were most frequent. About 8% of all U.S. accessions carried the 192-bp allele diagnostic for *Rht*8, compared with 64% of the Chinese accessions. Coleoptile length varied among accessions from 4.4 to 11.4 cm. Frequency distributions for 192- and non-192-bp genotypes showed no advantage of the 192-bp allele to coleoptile elongation. None of the 192-bp genotypes from the Great Plains showed greater coleoptile length than 'TAM 107', a hard red winter cultivar without *Rht*8 often chosen over contemporary cultivars for its greater emergence capacity with deeper seed placement. Since coleoptile elongation may be controlled by several quantitative trait loci, identifying only the presence of 192-bp allele of Xgwm 261 may be misleading if the primary motivation for its deployment is to increase coleoptile length in a semidwarf plant type.

I N ENVIRONMENTS where successful crop establishment is hindered by poor seedling emergence, wheat breeders are challenged by the need to improve coleoptile elongation in the presence of GA3-insensitive dwarfing genes, which tend to restrict it. Although coleoptile elongation is under polygenic control (Singhal et al., 1985; Rebetzke et al., 1999, 2001), a major QTL that maps directly to the *Rht* (Reduced height)-*B1* locus (formerly *Rht1*) and another QTL on chromosome arm 4BL may account for the majority of genotypic variation in coleoptile length measured at 11 to 19°C (Rebetzke et al., 2001). This restriction provides an incentive to winter wheat breeding programs to use alternative dwarfing genes in the low-precipitation dryland regions

of the Great Plains and Pacific Northwest, where deep seed placement is needed to reach moist soil to initiate germination (Budak et al., 1995; Schillinger et al., 1998).

In the southern and central Great Plains, there is further incentive for long coleoptile because winter wheat is seeded early as a dual-purpose crop for forage and grain production. Deep seed placement and reduced coleoptile elongation in the predominately hot soils can combine to have a potentially devastating impact on stand establishment (Stockton et al., 1996). Historically, earlier-planted wheat produces lower grain yield than later-planted grain-only wheat (Epplin et al., 2000). Hence, poor stand establishment translates, in part, to reduced profitability of both components of the dual-purpose system, estimated to account for the majority of the area seeded in Oklahoma (Epplin et al., 1998).

Two strategies may be followed to achieve adult-plant height reduction without the negative consequences of reduced coleoptile elongation. One might be to generate populations void of *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) and select phenotypically for minor height-suppressing genes. Removal of these genes in bread wheat near-isogenic lines produced only minor increases (less than 28%) in height, but substantially greater increases (up to 65%) in coleoptile length (Trethowan et al., 2001). Independent expression of plant height and coleoptile length in non-*Rht1* or non-*Rht2* populations should allow divergent selection responses for these traits, i.e., shorter height, longer-coleoptile genotypes, in the same population (Rebetzke et al., 1999; Trethowan et al., 2001). A second strategy might be to introduce GA-responsive dwarfing genes, such as *Rht8*, *Rht9*, and *Rht12*, that may not reduce coleoptile elongation, though their phenotypic detection may be more challenging (Worland et al., 1994; Worland and Snape, 2001).

Following its debut in the Japanese cultivar Akakomugi, a relatively weak height-reducing allele at the *Rht8* locus gained attention from southern European, Russian, and Chinese breeding programs targeting semidwarf stature in lieu of GA-insensitive *Rht* genes. In near-isogenic backgrounds, this allele has shown moderate reductions in plant height, and additional reductions when combined with the closely linked photoperiod-insensitive, height-reducing gene, *Ppd-D1* (Worland et al., 1998). Other GA-responsive genes, *Rht9* and *Rht12*, have not gained a similar level of popularity because of their negative associations with grain yield (Worland and Snape, 2001). The discovery of a microsatellite marker, Xgwm 261, 0.6 cM from the *Rht8* locus has made it possible to detect allelic variants that confer varying degrees of height reduction or promotion. The 192-bp allele of Xgwm 261 is indicative of the more

commercially favorable *Rht8* allele, while the other alleles of Xgwm 261 marker locus are considered associated with various levels of height promotion (Korzun et al., 1998).

The agronomic value of *Rht8* and the discovery of its diagnostic marker have ignited breeders' interest in *Rht8* as an alternative dwarfing gene. As expected, *Rht8* is concentrated in regions where it was first introduced and subsequently spread through Italy to additional countries: Bulgaria, Greece, Yugoslavia, Ukraine, and China (Worland et al., 1998, 2001). The distribution of *Rht8* in North American gene pools is not extensively characterized, though the gene has been found in a few cultivars (Ahmad and Sorrells, 2002). A more extensive survey of the Great Plains gene pool is justified given that cultivars featuring *Rht8* as the primary dwarfing gene might potentially have greater success in early-planted management systems or in High Plains dryland environments.

Breeding programs throughout the Great Plains occasionally introduce germplasms from Europe and Asia where *Rht8* is known to occur, if not predominate relative to other dwarfing genes. Thus, we hypothesized that the Xgwm 261 192-bp allele diagnostic of *Rht8* could be identified in advanced breeding lines and cultivars originating from European programs. Because soft red winter (SRW) wheat is sometimes used by hard winter wheat breeders in interclass hybridizations, this germplasm pool might serve as a more useful *Rht8* provider than germplasms from international programs. The objectives of this study were to (i) determine allelic distributions at the Xgwm 261 locus in contemporary samples of hard winter and SRW wheat, (ii) compare those distributions to a genotypic sample (Chinese landraces and cultivars) from a *Rht8*-rich region of the world, and (iii) compare coleoptile elongation in the presence and absence of the Xgwm 261 192-bp allele.

MATERIALS AND METHODS

Plant Materials

The study involved a primary set of 135 wheat accessions, mostly from the USA and China. The U.S. accessions included 80 hard winter wheat (primarily hard red winter, HRW) and 25 SRW experimental lines and cultivars. Selection of contemporary hard winter wheat cultivars was based on their commercial importance to the southern and central Great Plains. Additionally, we evaluated a historical set of 12 HRW cultivars previously assessed for adaptation to a dual-purpose system (Khalil et al., 2002), and all hard winter experimental lines and check cultivars tested in the 2001 Southern Regional Performance Nursery. The SRW genotypes included experimental lines from the University of Illinois and some entries submitted to the 1997 Uniform Eastern Soft Red Winter Wheat Nursery and the 1997 Five-State Advanced Nursery. Pedigree and origin of a total of 135 accessions are listed in Appendix 1.

Accessions from other sources were selected based on their putative *Rht8* genotype, including the Italian cultivars Mara and Funo, and the experimental lines ARS96329, ARS96339, and ARS96342 (Schillinger et al., 1998). Others were selected on the basis of their coleoptile elongation potential, including the Australian experimental lines PH179 and PH18 (G.J. Rebetzke, personal communication, 1997) and a selection from

'Sturdy', TX9129-962 (K. Porter, personal communication, 1998).

Molecular Marker Analysis

DNA was isolated from bulked leaves of two to three seedlings by the CTAB procedure (Saghai-Maroof et al., 1984). Microsatellite marker Xgwm 261 from chromosome 2DS was analyzed for all accessions in an IR^2–4200 DNA sequencer (LI-COR Inc., Lincoln, NE) by labeling one primer with an infrared (IR) fluorescence dye. Each 10 μL PCR sample contained 30 ng DNA, 1× PCR buffer, 0.25 m*M* dNTP, 2.5 m*M* $MgCl_2$, 0.5 pmol each of labeled and unlabeled SSR primers, and 1 unit of Taq polymerase. The following touchdown thermal profile was used for SSR amplification: 5 min at 95°C, 5 min at 68°C, and 1 min at 72°C for five cycles, in which the annealing temperature was lowered by 2°C per cycle; five more cycles with 2 min annealing time in which the temperature was lowered by 2°C per cycle; and 25 cycles in which the annealing temperature remained constant at 50°C. Five minutes at 72°C was used for the final extension. Molecular sizes of the SSR fragments were determined by comparison with the DNA size standard (LI-COR Inc., Lincoln, NE) by RFLPscan software (Scanalitics, Inc., Fairfax, VA).

Coleoptile Length Measurement

Seeds for the coleoptile length measurements were obtained from greenhouse-grown plants and germinated 60 d after harvesting. Coleoptile length was measured following the method of Hakizimana et al. (2000) with some modifications. Fifteen uniform seeds per accession were spaced 1 cm apart and about 7 cm from the bottom of a germination towel (no. 76 germination paper; Anchor Paper Co., St. Paul, MN). Each towel contained a different accession. The towel was folded at about 5 cm from the bottom, placed inside wax paper, rolled loosely, and secured with a rubber band. The wrapped towels were arranged vertically on a metal rack, set in distilled water to wet the germination towels thoroughly, and then drained of excessive water. The samples were covered with black plastic and placed in a cold room at 4°C for 2 d to interrupt any dormancy. The samples were incubated in a growth chamber at 100% relative humidity and 15°C for 7 d, followed by 6 d at 20°C. This procedure was conducted six times for all accessions, with re-randomization of entries in each replicate.

Means comparisons were performed for coleoptile length between allelic classes of Xgwm 261 using a *t* test. Frequency distributions for coleoptile length were compared among allelic classes of Xgwm 261 (192-bp, 165-bp, or all allelic classes excluding 192-bp allele) on the basis of the Kolmogorov-Smirnov test (Steel et al., 1997).

RESULTS AND DISCUSSION

Allelic Variation at Xgwm 261 Locus

Microsatellite Xgwm 261 was highly polymorphic among the 135 accessions examined in this study. The microsatellite primers amplified nine SSR fragments that varied in size from 165 bp to 212 bp (Table 1). One hundred sixteen accessions amplified a single fragment, 15 accessions (13 from the HWW class) amplified two SSR fragments of different sizes, and four accessions from the HRW class amplified three SSR fragments of different sizes. Among these microsatellite alleles, the 165-bp fragment occurred with greatest frequency (39%), followed by 174-bp (17%), 192-bp (16%), 210-bp (14%), and 197-bp (10%) fragments (Table 1). The 184-, 194-, 202-, and 212-bp fragments were most uncommon (<3%).

Table 1. Distribution of allelic variants for Xgwm 261 and coleoptile length among 135 wheat accessions.

Accession type†	Number of accessions‡		Size of Xgwm 261 fragments (bp)						Coleoptile length			
			165	174	192	197	210	Other§	Mean	Min	Max	SD
					number						cm	
USA	HWW	80	53	4	6	10	22	6	7.8	4.4	10.2	0.9
	SRW	25	5	18	2			1	8.3	6.1	10.9	1.0
China	cultivars	17	2	2	13			1	9.6	6.4	11.4	1.4
	landraces	5			1	1		3	10.6	9.8	11.2	0.7
Other genotypes	ARS lines¶	3		3					9.9	9.2	10.3	0.6
	PH lines#	2	1		1				7.4	5.9	8.8	
	Funo	1			1				8.2			
	Mara	1			1				7.9			
	TX9129	1	1						9.3			
Coleoptile length (cm)	mean		7.9	8.5	8.8	7.9	7.9	8.3				
	min		4.4	6.5	6.4	7.1	6.9	6.1				
	max		10.6	11.2	11.4	9.8	10.6	11.3				
	SD		1.1	1.3	1.5	0.7	0.7	1.6				

† HWW = Hard winter wheat, comprised of hard red winter and hard white winter classes; SRW = soft red winter class.
‡ Total no. of Xgwm 261 genotypes may exceed the number of accessions due to within-accession heterogeneity at the marker locus.
§ Among these 11 accessions, one showed 184-bp, three showed 194-bp, two showed 202-bp, and five showed 212-bp alleles.
¶ ARS lines include ARS96329, ARS96339 and ARS96342 (Schillinger et al., 1998).
# PH lines are PH 18 and PH 179 from Australia.

Among the nine SSR fragments, eight were detected in the hard winter accessions, and only four fragments were detected in each of the other accession types, indicating that the polymorphic level of microsatellite Xgwm 261 was highest among the hard winter accessions in this study.

Seven of the nine Xgwm 261 alleles were the same as those reported by Ahmad and Sorrells (2002) and Worland et al. (2001). The 165-, 174-, and 192-bp fragments were more common among the three surveys, including this one. The 210-bp allele only appeared in the HRW class and was not reported in previous studies. Seven other alleles (195-, 196-, 201-, 203-, 205-, and 215-bp fragments) reported by Worland et al. (2001) and three alleles (180-, 198-, and 200-bp fragments) reported by Ahmad and Sorrells (2002) were not found in our samples. The 184- and 212-bp fragments detected in four U.S. HRW accessions and two Chinese SRW accessions represent unreported alleles of Xgwm 261.

The majority of the 80 hard winter accessions contained the 165-bp allele, whereas the majority of the 25 SRW accessions contained the 174-bp allele. These two alleles prevailed in more limited samples of U.S. wheat cultivars (Worland et al., 2001; Ahmad and Sorrells, 2002), but differentiation of the predominant U.S. hard and soft wheat classes at Xgwm 261 was not possible in those surveys. These alleles were also found in the highest frequency among CIMMYT-derived semidwarf wheat accessions (165 bp) and in United Kingdom, German, and French wheat gene pools (174 bp) (Worland et al., 1998). Their worldwide prevalence in regions outside of southern Europe, Japan, and China is attributed to a possible compensatory effect on plant stature in the presence of GA-insensitive *Rht* genes and photoperiod-insensitive genes (Worland et al., 1998). Their hypothesis may also explain the dominance of the stronger height promoting 165-bp allele among predominately photoperiod-insensitive winter wheat cultivars adapted to the arid environment of the Great Plains, where extreme height reduction would be unacceptable. We found the 165-bp allele consistently among ancestors of modern Great Plains cultivars, such as 'Turkey', 'Khar-kof', 'Triumph 64', and 'Scout 66'. In addition, the 210-bp allele only appeared in modern Great Plains cultivars and formed the second largest genotypic group in the class, suggesting this allele may offer some selection advantage to modern cultivars in this region.

The diagnostic marker allele for *Rht*8 (192 bp) was found in only six HRW accessions and two SRW accessions, representing 6% of the total accessions in both classes. From the HRW class, accessions carrying the 192-bp allele included 'TX97D6377', 'G97380', and 'HG-9'. Cultivars 2163, Ok102, and 2137 (with 50% of its parentage from 2163) were heterogeneous for the 192-bp allele and either the 174- or 165-bp allele. Though present in low frequency, the germplasm with 192-bp allele appears to be scattered among hard winter wheat breeding programs in the Great Plains. In the SRW class, the 192-bp allele was limited to two highly related experimental lines from Illinois, IL 94-2426 and IL 95-2909 (also heterogeneous for the 165-bp allele). However, on the basis of the available pedigrees, we are neither able to determine the origin of *Rht*8 in these U.S. accessions carrying the 192-bp allele nor affirm the presence of *Rht*8 in these accessions because the 192-bp allele is a linked marker to *Rht*8, not part of the gene.

In contrast to the two U.S. gene pools, the majority (76%) of Chinese accessions contained the 192-bp allele (Table 1), which is consistent with Worland et al. (2001). Most of these accessions had Funo or a relative of Funo in their pedigrees, indicating a high possibility of *Rht*8 in these accessions. These results confirm the value of Chinese germplasm as a potential *Rht*8 donor. Of particular interest was the Chinese cultivar, Sumai 3, which we found to contain the 192-bp allele contributed from Funo. If not by design, then certainly by accident, *Rht*8 introgression has already commenced in many wheat breeding programs that targeted Sumai 3 as a source of Type II resistance to Fusarium head blight caused by *Fusarium graminearum* Schwake [teleomorph *Gibberella zeae* (Schwein.)] (Bai et al., 2003). We would expect this to be the case in U.S. wheat because of introduction of Sumai 3 as a scab resistant parent in winter or spring wheat breeding programs.

We evaluated several other accessions thought to have *Rht*8 or long coleoptile potential (Table 1, "Other genotypes"). Two Italian cultivars, Funo and Mara, and one Australian line having Mara as one of its parents, PH 18, contained the 192-bp allele. We could not confirm that three soft white winter experimental lines, ARS96329, ARS96339, and ARS96342, contained the 192-bp allele, which were previously claimed to have *Rht*8 (Schillinger et al., 1998). This could result either from the absence of *Rht*8 in these selections, or from recombination between *Rht*8 and the marker locus. Although TX9129 was selected from Sturdy for its greater coleoptile elongation, that characteristic is not attributable to *Rht*8.

Coleoptile Elongation and Xgwm 261 192-bp Allele

Coleoptile length was measured with moderate repeatability among the 135 accessions, as estimated by the intraclass correlation coefficient of 0.40 ± 0.04. Hakizimana et al. (2000) reported slightly higher repeatability of 0.6 to 0.7 among 15 HRW genotypes. A 7.0-cm range in coleoptile length was found among individual accessions, and their mean was 8.2 cm. The longest coleoptile was 11.4 cm for Chinese cultivars Wannian 2 and F 60096. The shortest was 4.4 cm for HRW cultivar '2180'. Chinese accessions tended to have longer coleoptiles than U.S. accessions, yet considerable overlap occurred among U.S. and Chinese cultivars (Table 1). Genotypes with the Xgwm 261 locus varied in mean coleoptile length from 7.9 cm (genotypes carrying 165-, 197-, and 210-bp alleles) to 8.8 cm (192-bp genotypes).

Frequency distributions for coleoptile length were generated for 192-bp genotypes, all genotypes lacking the 192-bp allele, and for the more common genotype with the 165-bp allele (Fig. 1). Only the non-192-bp distribution departed from normality ($P < 0.01$, Shapiro-Wilk test). However, these distributions did not differ significantly on the basis of the Kolmogorov-Smirnov statistic. An obvious association between greater coleoptile length and the presence of the 192-bp allele could not be detected from these results and visual examination of the distributions. Several accessions that contained the 192-bp allele had no greater coleoptile
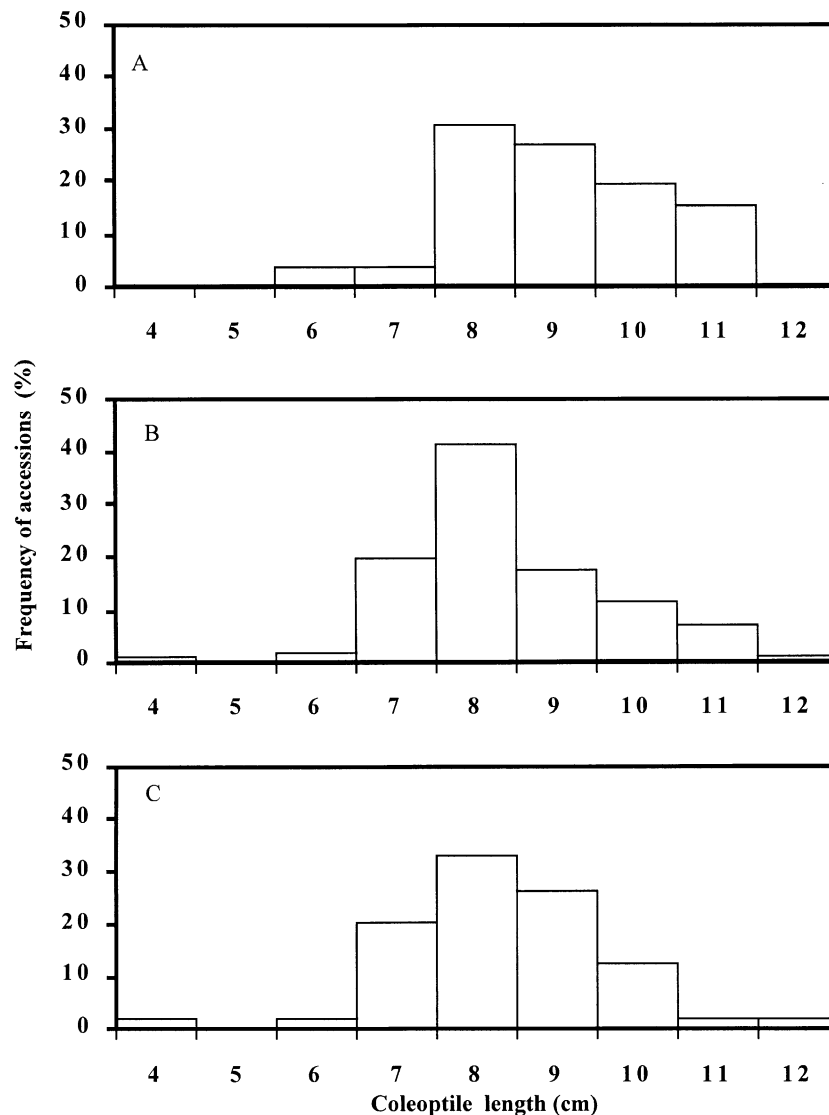

Fig. 1. Distributions for coleoptile length of wheat accessions containing only the 192-bp allele (A, *n* = 26), of all accessions not containing the 192-bp allele (B, *n* = 121), or of accessions strictly containing the 165-bp allele (C, *n* = 46) of the microsatellite marker Xgwm 261.

length than those that did not (Fig. 1), especially those in the HRW and other genotypes classes (Fig. 1).

Restricting marker-genotype comparisons to accessions of a common type revealed no significant benefit of *Rht*8 to coleoptile elongation for U.S. or Chinese modern cultivars (Table 2). Among the 25 accessions with the 192-bp allele, 16 had coleoptile lengths no greater than the value recorded for TAM 107 (9.6 cm), whereas 22 accessions had coleoptile lengths no greater than Scout 66 (10.6 cm). These non-*Rht*8 cultivars, in which Scout 66 is standard height and TAM 107 is semidwarf, are often chosen in the Great Plains over modern semidwarf cultivars for their greater coleoptile length and capacity for emergence with deeper seed placement. None of the 192-bp HRW genotypes exceeded Scout 66 or TAM 107 in coleoptile length.

A plausible argument for the lack of a detectable advantage in coleoptile elongation is that other height-reducing genes already present in modern *Rht*8 genotypes offset or mask any potential benefit of *Rht*8. For example, coleoptile length in Dwarf Sumai 3 (Sumai 3*2/'Tom Thumb') was reduced by >4 cm compared with Sumai 3 (6.4 vs. 10.6 cm), but both accessions contained the 192-bp allele. Identifying only the presence of the 192-bp allele may be misleading if the primary motivation for its deployment is to increase coleoptile elongation in a semidwarf plant type. It is recommended that selection for *Rht*8 using the 192-bp Xgwm 261 marker can be attempted in the absence of GA-insensitive *Rht* genes, followed by selection for minor genes

to achieve the desired level of plant height. Though *Rht*8 appears to be accessible in other gene pools, this study supports additional genotyping of Great Plains-adapted materials with special emphasis on detection of *Rht*8 in a standard-height genetic background. On the basis of the cultivars we screened, Midwestern SRW genotypes do not appear to offer any advantage over locally adapted materials as an *Rht*8 donor.

Table 2. Pairwise means comparisons for coleoptile length (cm) of wheat accessions possessing the Xgwm 261 192-bp allele (*Rht*8) versus all those without the 192-bp allele (non-*Rht*8) or strictly those possessing the 165-bp allele.

Accession type†	Xgwm 261 genotype (bp)‡			*t* Test
	192	Minus 192	165	
	cm			
All§	9.0	8.3		**
All	9.0		8.3	*
HWW	8.0	7.8		NS
HWW	8.0		7.9	NS
SRW	9.2#	8.2		NS
Chinese cultivars	9.5	10.4		NS
Chinese landraces	11.0	10.5		NS

* Indicates *t* test significant at *P* = 0.05.
** Indicates *t* test significant at *P* = 0.01.
NS = nonsignificant (*P* > 0.05).
† HWW = Hard winter wheat, comprised of HRW and hard white classes; SRW = soft red winter class.
‡ Only those accessions homogeneous for the 192-bp or 165-bp alleles were included in the genotype means; accessions heterogeneous for alleles other than 192-bp were included in the minus-192-bp mean.
§ All includes 80 of HWW accessions, 25 of SRW accessions, 22 of Chinese accessions, and 8 of other accessions as listed in Appendix Table 1.
\# Based on only one homogeneous accession.

Appendix 1. Allelic identity for microsatellite Xgwm 261 and mean coleoptile length of 135 wheat accessions.

Cultivar	Origin	Source†	Pedigree	Fragment size (bp)	Mean coleoptile length‡
			Hard winter wheat		
2137	USA	PI 592444 Pioneer, KSU	W2440/W9488A//2163	165, 192	6.4
2157	USA	Pioneer, KSU	Caprock/B 86//Sc 3212	165	7.4
2158	USA	Pioneer	Unknown	165	9.1
2163	USA	Pioneer, KSU	Pioneer line W558/5/Etoile de Choise//Thorne/Clarkan/3/CI15342/4/Pur 4946A4-18-2	174, 192	6.5
2174	USA	Pioneer, OSU	IL 71-5662/PL 145//2165	165	8.6
2180	USA	PI 532912, Pioneer	TAM W-101/Pioneer W603//Pioneer W558	165	4.4
Above	USA	CSU	TAM 110*4/FS2	165	8.4
AP502CL	USA	AgriPro, CSU	TXGH12588-26*4/FS2	165	9.7
Chisholm	USA	PI 486219, OSU	Sturdy sib/Nicoma	165	7.8
CO970498	USA	CSU	Ogallala/Halt	210	8.2
CO970531	USA	CSU	Ike/Halt	210	8.2
CO970547	USA	CSU	Ike/Halt	165, 210	6.9
CO970940	USA	CSU	Yuma/T-57//Lamar/3/4*Yuma/4/NEWS16	165	7.1
Coronado	USA	AgriPro	Mustang/W80-425//COMP76B-1-84-1/SW74-8A-47	212	6.8
Custer	USA	OSU	F29-76/TAM 105//Chisholm	165	7.6
Cutter	USA	AgriPro	KS84063-9-39-3//TAM-200/W81-296	210	7.7
Dumas	USA	AgriPro	F2SPS-102/TAMW-101//RPB/Mustang/W80-425/Comp. Sel.	165	6.9
Enhancer	USA	Goertzen Seed	HT43H-331-9 (Nebraska winter hardy selection)	165	9.2
G1878	USA	Goertzen Seed	Hawk//Sturdy/Plainsman V	165	9.2
G97209	USA	Goertzen Seed	Karl 92/G525/Arlin	165	7.7
G97380	USA	Goertzen Seed	GSR2500/Plainsman V//KARL92	192	7.4
HG-9	USA	Hardeman Grain & Seed	TAM 200 outcross selection	192	9.1
Ike	USA	PI 574488 KSU	Dular/Eagle//2* Cheney/Larned/3/Colt	212	7.7
Intrada	USA	PI 631402 OSU	Rio Blanco/TAM 200	197	8.1
Jagger	USA	PI 593688 KSU	KS82W418/Stephens	165, 212	7.5
Kalvesta	USA	Goertzen Seed	Oelson/Hamra//Australia215/3/Karl	165	7.8

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Appendix 1. Continued.

Cultivar	Origin	Source†	Pedigree	Fragment size (bp)	Mean coleoptile length‡
Karl 92	USA	PI 564245, KSU	Plainsman V/3/Kaw/Atlas 50//Parker *5/Agent	165, 210	7.1
Kharkof	Ukraine	PI 5641	Landrace from Ukraine	165	7.5
KS920709-B-5-2	USA	KSU	ABI 86*3414/X84W063-9-39-2//Karl 92	210	7.5
KS920946-B-15-2	USA	KSU	T67/X84W063-9-45//Karl 92	210	7.7
KS98HW151-6	USA	KSU	Arlin//TA2460/*3 TAM107	165	9.1
KS98HW220-5	USA	KSU	Arlin/Yuma	165	7.4
Lakin	USA	PI 617032 KSU	KS89H130/Arlin	165	7.8
Lockett	USA	PI 604245 TAM	TX86V1540/TX78V2430-4	165	8.2
NE97465	USA	UNL	SD3055/KS88H164//Colt*2/Patrizanka	210	10.2
NE97V121	USA	UNL	N87V106/OK88767	165, 210	7.7
NE98466	USA	UNL	KS89H50-4/3/Brl//Benn	210	7.0
NE98564	USA	UNL	Colt/Cody//Yuma	165, 210	7.3
NE98632	USA	UNL	Niobrara/5/Aiv/Nbr/Bolal//Hiplains/3/Lov6/4/Redland	165	7.3
NI98439	USA	UNL	Benn/BRL//X10927 592-1-5	165	7.7
NW97S218	USA	USDA-ARS Lincoln	KS85W663-1-1/Karl 92	210	7.6
NW97S278	USA	USDA-ARS Lincoln	Pronghorn/Arlin	197	7.2
Ogallala	USA	AgriPro	TX81V6187//OK711252/W76-1226	197, 210	7.6
Ok102	USA	OSU	2174/Cimarron	165, 192	7.5
OK93P656-RMH3299	USA	OSU	W0405D/HGF112//W7469C/HCF012	165	6.6
OK94P549-99-6704	USA	OSU	HBY756A/Siouxland//2180	202	7.6
OK96705-99-6745	USA	OSU	2180/OK88803//Abilene	165	7.0
OK96717-99-6756	USA	OSU	Abilene/2180//Chisholm	165	7.7
OK98680	USA	OSU	Odessa 06/Mesa	212	7.6
Onaga	USA	AGSECO		165	7.7
Scout 66	USA	CItr 13996 UNL	Composite of 85 selections from Scout, CItr 13546	165, 210	7.9
T001X	USA	Trio Seed	Hybrid	165, 174, 210	7.9
T002X	USA	Trio Seed	Hybrid	165, 174, 210	7.3
T003X	USA	Trio Seed	Hybrid	165, 174, 210	7.5
T122	USA	Trio Seed	Tecumseh/5627//T91	165	7.3
TAM 105	USA	CItr 17826, TAM	'short wheat' Sturdy composite bulk selection	165	7.8
TAM 107	USA	PI 495594 TAM	TAM 105*4/Amigo	165	9.6
TAM 110	USA	PI 595757 TAM	(TAM 105 *4/Amigo)*5/Largo	165	9.9
TAM 111	USA	TAM	TAM 107//TX78V3620/CTK78/3/TX87V1233	194	8.8
TAM 202	USA	PI 561933 TAM	Siouxland outcross	197	7.9
TAM 302	USA	PI 605910 TAM	Probrand 812/Caldwell//TX86D1310 (TAM 300 sib)	165	8.0
TAM W-101	USA	CItr 15324, TAM	Norin 10/3/Nebraska 60//Mediterranean/Hope/4/Bison	165, 210	7.3
Thunderbolt	USA	AgriPro	OK711252A/W76-1226//KS90WGRC10	165	8.2
Tomahawk	USA	AgriPro	Ironstraw S4	210	7.3
Tonkawa	USA	OSU	F29-76/TAM 105//Chisholm	165	7.1
Trego	USA	PI 612576 KSU	KS87H325/Rio Blanco	197	7.1
Triumph 64	USA	CItr 13679 OSU	Danne Beardless Blackhull/3/Kanred/Blackhull//Florence/4/ Kanred/Blackhull//Triumph	165	9.2
Turkey	Ukraine/Russia		Landrace	165	8.9
TX 95A1161	USA	TAM	TAM W-101//NE78488/Veery	165	7.5
TX97A0122	USA	TAM	TX88V4328/TX87V1613//TX87V1233-1	165, 197	7.5
TX97A0219	USA	TAM	TX71562-6*4/AMI*4//LGO/3/NE86582	165, 210	8.1
TX97A0244	USA	TAM	TAM 105*4/AMI*5//LGO/3/Sturdy	165	8.6
TX97D6377	USA	TAM	HBG026+NE78659*Arkan/2180	192	7.6
TX97V2838	USA	TAM	U1254-1-5-2-1/TX81V6582	197	7.8
TX98D1170	USA	TAM	TX89D1253*2/TTCC404	165	8.3
TX98V9315	USA	TAM	U1254-4-7-2/Dong Xie 4	197	8.1
TX98V9618	USA	TAM	U1254-1-8-1-1/TAM-202	197	8.0
TX98V9930	USA	TAM	U1254-7-9-2-1/TX86A5616	165, 197, 210	7.3
Venango	USA	Goertzen Seed	HBE 1066-105/HBF0551-131	210	8.1
Vona	USA	CItr 17441, CSU	II 21183/CO 652363//Lancer/KS 62136	165	6.6
			Soft red winter wheat		
Bacup	USA	PI 596533	Nuy Bay/Pioneer 2375//Marshall	165	9.3
Cardinal	USA	Ohio State Univ.	Logan*2/3/Va63-5-12/Logan//Blueboy	165	8.8
Clark	USA	PI 512337	Beau Caldwell sib/67137B5-16/4/Sullivan/3/Beau// 5517B8-5-3-3/Logan	174	8.1
Ernie	USA	PI 584525 U M	Pike/MO9965/3/Stoddard/Blueboy//Stoddard/D1707	174	8.6
Foster	USA	PI 593689 UK	Coker65-20/Arthur/4/Chul* 8CC//VA68-22-7/Abe/3/VA 72-54-14/Tyler//Suwon 92/Arthur//Arthur/VA 70-52-2	174	7.6
Freedom	USA	Ohio State Univ.	GR876/OH217	165	8.5
IL 93-2283	USA	UI	Tyler/Caldwell//Auburn/Wheeler	174	8.1
IL 94-1549	USA	UI	Auburn/Ark38-1/Arthur/Blueboy	174	8.6
IL 94-1909	USA	UI	Fillmore/Amigo//Tyler/Howell	174	7.9
IL 94-2426	USA	UI	Roland/4/Coker 68-15/3/IL69-1751/5/IL70-2227-1/McNair 1003/2/Howell	192	9.2
IL 94-6280	USA	UI	Tyler/Caldwell//Auburn/Wheeler	174	8.7
IL 95-1966	USA	UI	Tyler/Howell/3/Howell//Oasis/Arkansas38-1/4/Auburn/3/ Rosen//Arthur/Blueboy	174	7.0
IL 95-2066	USA	UI	IN7688G1/3/Caldwell//Spritzer Agrotriticum/LRC40/4/P 79424H1-20-2-74	174	7.9

Continued next page.

Appendix 1. Continued.

Cultivar	Origin	Source†	Pedigree	Fragment size (bp)	Mean coleoptile length‡
IL 95-2909	USA	UI	Freedom/6/Roland/4/Coker 68-15/3/IL69-1751/5/Roy/4/ Coker 68-15/3/IL69-1751	165, 192	8.8
IL 9634-24851	USA	I	P76788G2-5-494/5/Caldwell/4/Coker68-15/3/IL69-1751/6/ Caldwell/Tyler//Auburn/7/Ning 7840	174	7.5
Kaskaskia	USA	PI 602969 UI	(IL70-2255/CI13855//McNair48-23)/(Arthur/Blueboy// TN1571)//Pike/Caldwell	174	7.0
MO 94-193	USA	97FSAN	MO 11728/Becker	174	7.6
MO 94-312	USA	97FSAN	Pioneer brand 2551/Caldwell	174	8.9
OH 552	USA	97UESRWN	Pur71761A4-31-5-33/VA68-26-331/6/Thorne*5/199-4/5/ Thorne/4Taylor*2/2/Norin 10/Brevor/3/unknown parent	174	10.5
OH 569	USA	97FSAN	Pur71761A4-31-5-33/VA68-26-331/6/Thorne*5/199-4/5/ Thorne/4 Taylor*2/2/Norin10/Brevor/3/unknown parent	174	10.9
P91193D1-10-2	USA	97UESRWN	851423/INW9853	174	7.7
PA8769-158	USA	97UESRWN	Titan/Caldwell	174	7.8
PB 2555	USA	Pioneer	Coker 68-16/MoW 7140//Pioneer Brand W521	165	8.7
Pontiac	USA	PI 573038 AgriPro	Magnum/Auburn	174	7.8
Roane	USA	PI612958 VA Tech	VA 71-54-147/Coker 68-15//IN6 5309C1-18-2-3-2	202	6.1
			Chinese accessions		
			Cultivars		
Chuanyu 35050	China		Chuanyu 5/Chuanyu 9461	192, 212	7.9
Dwarf Sumai 3	China	JAAS	Sumai 3/Tom Thumb//Tom Thumb	192	6.4
F 5114	China	JAAS	LongXi 18/(Avrora/Anhui 11//Sumai 3)	165	9.9
F 5125	China	JAAS	Fufan 904/(Avrora/Anhui 11//Sumai 3)	165	10.4
F 60096	China	JAAS	Jinzhou 1/Sumai 2	192	11.4
Fumai 3	China	PI 447405	Orofen/Funo	192	9.4
JG 1	China	PI 531193	Mayo/Armadillo//Yangmai 3/Avrora/Ningmai 3	192	9.7
Ning 7840	China	PI 531188	Aurora/Anhui 11//Sumai 3	192	10.4
Ning 8026	China	PI 531189	Avrora/Sumai 3//Yangmai 2	192	8.1
Ning 8331	China	PI 53119	Yangmai 4/(Avrora/Anhui 11//Sumai 3)	192	8.7
PC-2	China	CIMMYT	Unknown	174	10.2
Sumai 3	China	JAAS	Funo/Taiwan Wheat	192	10.6
Sumai 49	China	JAAS	N7922/(Aurora/Anhui 11//Sumai 3)	192	9.9
Wannian 2	China	PI 447403	Selection of Mentana	192	11.4
Wuhan 3	China	CIMMYT	Unknown	192	9.9
Xianmai 1	China	PI 481544	Ardito/Tevere//Wannian 2	174	11.2
Yangmai 1	China	PI 447404	Selection of Funo	192	8.2
			Landraces		
CaiZiHuang	China	PI 447402	Landrace from Jiangsu	197	9.8
FSW	China	JAAS	Landrace from Fujian Province	184	9.8
NTDHP	China	PI 462149	Landrace from Jiangsu	194	11.1
Wangshuibai	China	PI 462141	Landrace from Jiangsu	194	11.3
WZHHS	China	JAAS	Landrace from Zhejiang Province	192	11.0
			Other genotypes		
ARS96329	USA			174	9.2
ARS96339	USA			174	10.1
ARS96342	USA			174	10.3
Funo	Italy	PI 213833	Duecentodieci/Demiano	192	8.2
Mara	Italy	PI 244854	Autonomia A/Aquila sib I	192	7.9
PH179	Australia		Skua/Shortim	165	5.9
PH18	Australia		Insignia/Skua//Shortim/Mara	192	8.8
TX 9129	USA		Selection from Sturdy	165	9.3

† 97UESRWN = 1997 Uniform Eastern Soft Red Winter Wheat Nursery; 97FSAN = 1997 Five State Advanced Nursery; UK = Univ. of Kentucky; UI = Univ. of Illinois; OSU = Oklahoma State Univ.; KSU = Kansas State Univ.; TAM = Texas A&M Univ.; CSU = Colorado State Univ.; UNL = Univ. of Nebraska, Lincoln; CIMMYT = International Maize and Wheat Improvement Center; JAAS = Jiangsu Academy of Agricultural Sciences, Nanjing, China.
‡ Mean of six replicates.

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