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ORIGINAL PAPER

K. R. Gedye · C. F. Morris · A. D. Bettge

Determination and evaluation of the sequence and textural effects of the puroindoline a and puroindoline b genes in a population of synthetic hexaploid wheat

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Abstract *Aegilops tauschii* (2*n*=2 *x*=14, DD) is a rich source of genetic variability for hexaploid wheat (*Triticum aestivum*, 2*n*=6 *x*=42, AABBDD) improvement. This variability can be accessed through utilizing synthetic hexaploid wheat lines, which contain genomes from *Ae. tauschii* and *T. turgidum* (2*n*=4 *x*=28, AABB). Numerous desirable characteristics can and have been introgressed into common hexaploid wheat with this germplasm. In this work, the genetic variability in the two puroindoline genes (a and b) contained on the D genome, and the relationship that sequence polymorphisms in these genes have on endosperm texture among a population of 75 CIMMYT synthetic hexaploid accessions is described. Kernel texture was evaluated using the single kernel characterization system (SKCS). Kernel texture differed significantly ($P \leq 0.0001$) among the synthetic hexaploid accessions (range 2.6–40.9) and the parent types, durum or *Ae. tauschii*. The interaction term between parent types was also a significant effect ($P \leq 0.0001$). In addition to the 'wild-type' protein sequences of the puroindoline genes (those present in 'Chinese Spring' and all other soft wheats), three other translated sequences were identified in puroindoline a and two others in puroindoline b. These protein sequences were associated with significantly ($P \leq 0.0001$) softer endosperm textures than the wild-type protein sequences. As the softer alleles are expressed in a hexaploid background, they are immediately available to wheat breeding programs.

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K. R. Gedye
Department of Food Science and Human Nutrition, Washington State University,
Pullman, WA, 99164-6376, USA

C. F. Morris (✉) · A. D. Bettge
USDA-ARS Western Wheat Quality Laboratory,
Pullman, WA, 99164-6394, USA
e-mail: morrisc@wsu.edu
Tel.: +1-509-3354062
Fax: +1-509-3358573

Introduction

Hexaploid wheat (*Triticum aestivum* L., 2*n*=6*x*=42, AABBDD) is an allo-hexaploid which evolved from the hybridization of a tetraploid wheat (AABB) with *Aegilops tauschii* (2*n* =2*x* =14, DD), syn. *T. tauschii*; *Ae. squarrosa* (McFadden and Sears 1946). This hybridization event is thought to have occurred more than once, but the exact number is unclear (Zohary et al. 1969; Talbert et al. 1998). The small number of hybridization events and the restricted geographic origin of these events has resulted in a narrow genetic diversity for hexaploid wheat (Lagudah et al. 1991; Talbert et al. 1998). The D genome of wheat has remained largely unchanged from that of its wild relative *Ae. tauschii*, so much so that *Ae. tauschii* can be considered as an extension of the wheat gene pool (Kimber et al. 1981; Pflüger et al. 2001). The close relationship between the D genome of wheat and of *Ae. tauschii* has greatly reduced the genetic recombination barriers, allowing the introgression of several new genes into cultivated wheat, including a number of disease and pest resistances, agronomic traits and quality traits (Eastwood et al. 1991; Villareal et al. 1995, 2001; Peña et al. 1995, 1996; Pflüger et al. 2001).

Two methods can be employed to incorporate *Ae. tauschii* genes into a hexaploid wheat breeding population. The direct cross method, which involves direct hybridization between *Ae. tauschii* and hexaploid wheat (Gill and Raupp 1987; Cox et al. 1995), or an indirect method that relies on the intermediate step of producing synthetic hexaploids (Mujeeb-Kazi et al. 1996). Synthetic hexaploid production mimics *T. aestivum* hexaploid wheat evolution by crossing tetraploid wheat *T. turgidum* ssp. *durum* and *Ae. tauschii*. Genes from *Ae. tauschii* are then available via direct crossing of synthetic hexaploids to *T. aestivum* (Mujeeb-Kazi et al. 1996). The CIMMYT (International Maize and Wheat Improvement Center, Mexico) has produced numerous synthetic hexaploids facilitating the exploitation of genetic variability in *Ae. tauschii*.

Investigations into variation in quality traits in *Ae. tauschii* have been limited predominantly to the study of

high and low molecular weight subunits of glutenins and of gliadins (Lagudah and Halloran, 1988; Peña et al. 1995, 1996; Pflüger et al. 2001). The study of these proteins in both *Ae. tauschii* and synthetic hexaploids has identified many polymorphisms, allowing assumptions to be drawn regarding the phylogenetic relationships between *Ae. tauschii* and hexaploid wheat (Lagudah and Halloran 1988). Evaluation of the physical and chemical properties, rheological characteristics and bread baking tests has also identified potentially useful variation in the D genome of synthetic hexaploids (Peña et al. 1996; Pflüger et al. 2001). *Ae. tauschii* was found to carry novel glutenin proteins with positive effects for improving the bread making quality of wheat (Peña et al. 1996; Pflüger et al. 2001).

Another important quality characteristic in hexaploid wheat is kernel texture. Depending on the texture of the kernel, wheat is classified as either hard or soft. Variation in kernel texture is controlled predominantly by the *Hardness* (*Ha*) locus, located on the extreme distal end of chromosome 5DS. Tightly linked to the *Ha* locus are three genes, puroindoline a (*Pina-D1*), puroindoline b (*Pinb-D1*) and the grain softness protein (*Gsp-1*) (Giroux and Morris 1997; Tranquilli et al. 2002; Igrejas et al. 2002). The wild-type puroindoline genes (*Pina-D1a* and *Pinb-D1a*) have been found to be associated with soft kernel texture in Chinese Spring and all other 'soft' hexaploid wheat varieties (Morris 2002). Mutations in either of the two puroindoline genes have been shown to cause the 'hard' phenotype in hexaploid wheat (Giroux and Morris 1997; Lillemo and Morris 2000; Morris et al. 2001). Durum wheat, lacking the D genome, is considered to be 'very hard' (Morris 2002). Variation in puroindoline gene sequence has also been investigated in *Ae. tauschii* (Lillemo et al. 2002; Massa et al. 2004). Lillemo et al. (2002) found that puroindoline a in *Ae. tauschii* contained 99.3% amino acid sequence homology to the wheat cultivar 'Penawawa' and 90.5% amino acid sequence homology in puroindoline b. Among 50 *Ae. tauschii* accessions, four alleles of puroindoline a and four alleles of puroindoline b were identified, encoding two and three different proteins, respectively (Massa et al. 2004). However, the effect that these sequence polymorphisms may have on kernel texture is unknown, as no test of texture has been developed for *Ae. tauschii*. The incorporation of *Ae. tauschii* into synthetic hexaploids facilitates the analysis of puroindoline sequence polymorphism and other genetic effects on kernel texture, using testing methods designed for hexaploid wheat, such as the Perten single kernel characterization system (SKCS) or NIR (Igrejas et al. 2002). Analysis of synthetic hexaploids can also facilitate the analysis of the effect of the A and B genomes, contributed by the durum parent on kernel texture.

In addition to the puroindolines, other genes contribute to variation in kernel texture. Previous analysis has identified quantitative trait loci (QTL) on chromosomes 2A, 2DL and 6B for kernel texture. However, the effects of the QTL were small relative to the puroindoline genes which had the greater influence on kernel texture, with R^2 values approximating 0.6 in five of the six environments investigated (Campbell et al. 1999). Transgressive segregation has also been observed for kernel texture, indicating the presence of additional genes associated with the trait (Campbell et al. 1999; Igrejas et al. 2002).

In this work the sequence polymorphism of the puroindoline genes in a collection of synthetic hexaploids, produced at the CIMMYT, was determined. Kernel texture was measured by the SKCS and variation was analyzed based upon puroindoline protein sequence, the durum parent and the *Ae. tauschii* parent. Analysis of variance (ANOVA) of these groups was used to assess the effect that the puroindolines, the A and B genomes, and other factors on the D genome have on kernel texture.

Materials and methods

Plant material

A population of 75 synthetic hexaploid lines produced from the hybridization of *T. turgidum* spp. *durum* and *Ae. tauschii* was obtained from the CIMMYT. The synthetic hexaploids were derived from crosses between seven durum cultivars: 'Doy 1', 'Croc 1', 'Altar 84', 'Dverd D', 'Laru', 'Rok/KML'and 'Arlin 1' and a total of 66 different *Ae. tauschii* accessions. One plant of each synthetic hexaploid line was grown to maturity in a greenhouse. No cytological assessments were made. However, the 75 lines reported here represent a sub-set of a larger group, some of which showed low vigor or low fertility and were dropped from further analysis.

Kernel texture

Kernel texture of all 75 lines was determined using the Perten SKCS (AACC Method 55-31) on clean, unbroken wheat kernels. SKCS hardness values were produced from crushing a sample of 100 kernels from each line.

Protein content

Nitrogen content of seed of all 75 lines was determined using a Leco FP-528 (AACC Method 46-30), converted to protein by multiplying by 5.7, and expressed on a constant 12% moisture basis. Two replications of approximately 0.25 g of whole seed per line were analyzed for nitrogen content.

Puroindoline gene sequence analysis

DNA extraction was carried out as described by Guidet et al. (1991). Amplification of the two puroindoline genes was performed using sequence-specific primers (Gautier et al. 2000; Massa et al. 2004). Puroindoline gene amplifi-

cation and sequencing was carried out as described by Massa et al. (2004). Sequences were aligned and compared to published puroindoline sequences of the cultivar Penawawa, accessions AJ302091 and AJ302100 for *Pina-D1a* and *Pinb-D1a*, respectively (Corpet 1988; Lillemo et al. 2002).

Statistical analysis

Single kernel characterization system (SKCS) hardness, kernel weight, kernel diameter and protein data were analyzed using simple correlation to investigate any association between these variables. SKCS kernel texture was analyzed using the general linear model (GLM) approach to ANOVA (SAS v 8.2, Cary, N.C., USA) using a model incorporating firstly all 75 accessions, secondly the accessions classified by the durum and *Ae. tauschii* parent types, and thirdly the puroindoline genes and the different translated puroindoline protein sequences. Fully-balanced designs were tested using Type I sums of squares (SS), unbalanced designs Type III SS, and designs with missing cell means (i.e., ANOVA of synthetic lines classified by durum and *Ae. taushcii* parent) Type IV SS.

Results and discussion

Kernel texture, protein content and growth characteristics

Although not all of the synthetic lines obtained from CIMMYT produced sufficient seed for this study, the subset of 75 accessions included here generally exhibited a high level of vigor and fertility. Kernels of all the synthetic hexaploid accessions were large and plump, as indicated by their average weight and diameter, 45.7–75.9 mg and 2.9–3.8 mm, respectively. The kernel protein content of the synthetic hexaploid accessions ranged from 11.5 to 23.2%. The correlation between weight and diameter was high (r=0.91), as would be expected (Table 1). Correlations between kernel weight and diameter with protein were negative and moderate in magnitude (approximately $r \approx -0.4$).

Significant variation in the average kernel texture among the 75 synthetic hexaploid accessions was observed (Fig. 1). Kernel texture, as measured by SKCS, ranged from 2.6 to 40.9, with a corresponding range in standard deviation from 8.2 to 14.8. Standard deviation values in this range are indicative of homogeneous samples. The median SKCS hardness value for this population of synthetic hexaploids was 25.3. ANOVA using Duncan's multiple range test (DMRT) produced a critical value (α=0.05) of 3.2 for "adjoining" accession means ($P \leq 0.0001$). At the extremes (the highest and lowest accession means) the critical value was 4.2. The correlation coefficients between SKCS hardness and the other kernel traits indicated that the variation in kernel texture of the synthetic hexaploid accessions was not primarily related to their diameter, weight or protein content (Table 1) (non-linear relationships were also examined, data not shown). Although only one environment was utilized here, previous research (Morris et al. 2004) indicates that although kernel texture will vary due to environment, that genotype-by-environment interaction is relatively small and genotype rankings remain relatively constant.

Table 1 Correlation coefficients among SKCS kernel texture, diameter, weight and protein content of 75 synthetic hexaploid accessions. *NS* Not significant

	SKCS	Diameter	Weight
Diameter	0.32*		
Weight	0.16 *NS*	0.91*	
Protein content	−0.22, *NS*	−0.42*	−0.38*

*P<0.05

Classification based on synthetic hexaploid parents

From the foregoing analysis, the synthetic hexaploid accessions clearly differed for kernel texture. To attempt to identify the source of this variation, kernel texture was analyzed based on a classification of accessions by durum and *Ae. tauschii* parent. Both parent types, durum and *Ae. tauschii*, and the interaction between them had a significant effect ($P \leq 0.0001$) on SKCS kernel texture (Table 2). The synthetic hexaploid accessions, when grouped according to their durum parent, fell into three distinct, non-overlapping groups according to DMRT (Table 3). Accessions derived from the durum parent Rok/KML produced the highest mean SKCS kernel texture (32.2) and were in a DMRT group by themselves. A second DMRT group was comprised of four durum parents and ranged from 27.6 to 29.3. The final DMRT group consisted of accessions with two durum parents, Croc_1

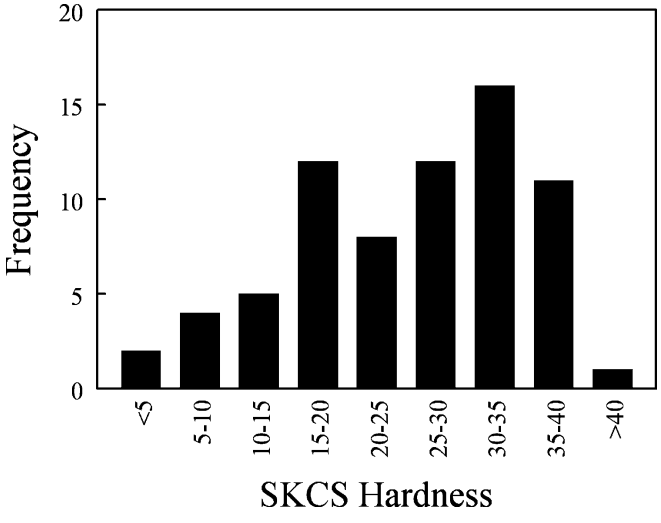

Fig. 1 Frequency distribution of SKCS kernel texture of the 75 synthetic hexaploid wheat accessions

Table 2 Analysis of variance (GLM) for the effect of the parental type on the kernel texture of 75 synthetic hexaploid accessions. The degrees of freedom for this model were derived from individual tests of the model components as generated by the SAS GLM procedure

Parental type	*Df*	Type IV SS	Mean square	*F*-value	*P*>*F*
Whole model	68	636,088	9,354	78.6	<0.0001
Durum	4	12,891	3,222	27.1	<0.0001
Ae. tauschii	59	414,742	7,029	59.1	<0.0001
Durum × *Ae. tauschii*	3	16,451	5,483	46.1	<0.0001
Error	7,095	844,190	118	–	–

and Dverd_2, and produced the softest lines (18.7 and 18.1, respectively).

While the effect of the *Ae. tauschii* parent was also significant, results from this analysis should be interpreted with caution. Although the analysis was statistically sound and Type IV SS were used to assess significance, this set of synthetic hexaploid accessions was necessarily unbalanced relative to the frequency of specific durum and *Ae. tauschii* parents. The majority of the accessions were created from unique *Ae. tauschii* parents. Similarly, the unbalanced nature of the preceding analysis, which produced three DMRT groups based on the durum parent, suggested that these results should be further validated. To provide this independent validation, a fully balanced factorial sample sub-set was drawn from the total population of 75 synthetic hexaploid accessions. The six accessions in this sub-set consisted of lines with two *Ae. tauschii* parents and three separate durum parents in common. The analysis found that again the durum and *Ae. tauschii* parents, and their interaction had a significant effect ($P \leq 0.0001$) on kernel texture (Table 4). Using DMRT and the *Ae. tauschii* parent classification, these accessions were separated into two distinct groups with means of 17.3 (parent WX507) and 33.2 (parent TA2482) (critical value, α=0.05, of 2.0). The difference (33.2 −17.3), which was assignable to the *Ae. tauschii* parents, was notably greater than that assignable to the durum parents (means of 19.9, 26.6 and 29.7). These results indicate that as yet unknown traits independent of the puroindoline genes play a critical role in kernel texture. These unknown traits may include QTL and *Gsp-1*. QTL associated with kernel texture have been identified in regions of the hexaploid wheat genome other than 5DS, the location of the puroindoline genes (Campbell et al. 1999, 2001; Igrejas et al. 2002). *Gsp-1* is tightly linked to the puroindoline genes, and is also found on the homoeologous chromosome 5 of the A and B genomes. However, *Gsp-1* does not appear to be associated with kernel texture (Tranquilli et al. 2002). Although as yet not fully characterized, this genetic variability from both the durum and *Ae. tauschii* parents potentially offers diversity and variation in kernel texture which is exploitable by wheat breeders.

Table 3 Mean SKCS kernel texture of the 75 synthetic hexaploid accessions based on classification by the durum parent. Means followed by the same letter are not significantly different according to DMRT (α=0.05)

Durum parent	Mean	No. of accessions
Rok/KML	32.2a	3
Doy_1	29.3b	29
Altar 84	28.7bc	11
Arlin_1	27.8bc	1
Laru	27.6c	3
Croc_1	18.7d	26
Dverd_2	18.1d	2

Classification based on puroindoline allelic composition

The gene-specific primers amplified the coding regions of both puroindoline genes in all 75 accessions. Amplification of the puroindoline a gene (*Pina-D1*) identified eight unique sequences one of which was identical to the soft wild-type allele found in *T. aestivum*. Of the non-wild-type alleles, four were identical to alleles previously identified and designated as *Pina-D1c*, *Pina-D1d*, *Pina-D1e* and *Pina-D1f* (Massa et al. 2004). The additional three novel alleles have been submitted to the NCBI data base and are catalogued as accession numbers: AY573898, AY573899 and AY573900. These three alleles have been designated *Pina-D1h*, *Pina-D1i* and *Pina-D1j*, respectively. The translation of the *Pina-D1* alleles *c*, *d*, *e*, *f*, and *h* produce identical proteins. The translation of the *Pina-D1* alleles *i* and *j* produced unique proteins. The translation of the wild-type puroindoline a allele (*Pina-D1a*) will be designated here as PINAa. The translation of the alleles *Pina-D1c*, *d*, *e*, *f* and *h* will be designated here as protein PINAc, and the translation of alleles *Pina-D1i* and *Pina-D1j* will be designated PINAi and PINAj, respectively. The total number of accessions containing each of the eight alleles is provided in Table 5.

Amplification of the *Pinb-D1* identified seven alleles among this population of synthetic hexaploid accessions, one of which was the soft wild-type allele. Of these alleles, three were identical to alleles previously identified and designated as *Pinb-D1h*, *Pinb-D1i* and *Pinb-D1j* (Massa et al. 2004). The additional three alleles have been submitted to the NCBI data base and are catalogued as accession numbers: AY573901, AY573902 and AY573903. These three alleles were designated *Pinb-D1m*, *Pinb-D1n* and *Pinb-D1o*, respectively. The translation of *Pinb-D1h, i, m, n* and *o* were identical and are designated as PINBh in this

Table 4 Analysis of variance (GLM) for the effect of the parental type on the kernel texture of a balanced sub-population of synthetic hexaploid accessions

Parental type	*Df*	Type I SS	Mean square	*F*-value	*P*>*F*
Model	5	53,433	10,687	90.54	<0.0001
Durum	2	9,138	4,569	38.7	<0.0001
Ae. tauschii	1	40,647	40,647	344.4	<0.0001
Durum × *Ae. tauschii*	2	3,646	1,823	15.4	<0.0001
Error	610	72,002	118	–	–

Table 5 Classification of a population of 75 CIMMYT synthetic hexaploid accessions based on puroindoline a and b gene sequences, and puroindoline a and b translated proteins. *Wild-type* indicates the puroindoline a and b protein sequence present in Chinese Spring and other soft *Triticum aestivum* varieties

Puroindoline a		Puroindoline b		No. of accessions
Allele	Polypeptide	Allele	Polypeptide	
Pina-D1a	Wild-type	*Pinb-D1a*	Wild-type	2
Pina-D1a	Wild-type	*Pinb-D1h*	PINBh	1
Pina-D1a	Wild-type	*Pinb-D1i*	PINBh	8
Pina-D1a	Wild-type	*Pinb-D1j*	PINBj	2
Pina-D1a	Wild-type	*Pinb-D1m*	PINBh	1
Pina-D1a	Wild-type	*Pinb-D1n*	PINBh	2
Pina-D1c	PINAc	*Pinb-D1h*	PINBh	24
Pina-D1c	PINAc	*Pinb-D1i*	PINBh	6
Pina-D1c	PINAc	*Pinb-D1m*	PINBh	6
Pina-D1c	PINAc	*Pinb-D1n*	PINBh	2
Pina-D1d	PINAc	*Pinb-D1i*	PINBh	8
Pina-D1d	PINAc	*Pinb-D1n*	PINBh	3
Pina-D1d	PINAc	*Pinb-D1o*	PINBh	1
Pina-D1e	PINAc	*Pinb-D1i*	PINBh	4
Pina-D1e	PINAc	*Pinb-D1o*	PINBh	1
Pina-D1f	PINAc	*Pinb-D1i*	PINBh	1
Pina-D1h	PINAc	*Pinb-D1i*	PINBh	1
Pina-D1i	PINAi	*Pinb-D1m*	PINBh	1
Pina-D1j	PINAj	*Pinb-D1i*	PINBh	1
Total				75

paper. *Pinb-D1j* translated into a unique protein and is designated as protein PINBj. The translation of the wild-type puroindoline b allele (*Pinb-D1a*) will be designated here as PINBa. The total number of accessions containing each of the seven *Pinb-D1* alleles is presented in Table 5.

Both puroindoline genes had a significant effect ($P \leq 0.0001$) on kernel texture (Table 6). The low R^2 value (0.07) obtained with this analysis was lower than other studies (Martin et al. 2001; Lillemo and Ringlund 2002). However, none of these prior studies utilized synthetic hexaploids and, perhaps more importantly, the individual kernel texture phenotype data from the SKCS. The results observed by Martin et al. (2001) and Lillemo and Ringlund (2002), when compared to the results observed in this research suggest that the effect of the 'hard' puroindoline sequences found in *T. aestivum* are profoundly different than the puroindoline sequences observed here, i.e., none of the present puroindoline sequences produced a 'hard' kernel texture phenotype. Of all the *Ae. tauschii* accessions examined to date, either as synthetic hexaploids or in their diploid form, none of the 'hard' mutations present in *T. aestivum* have been identified. While more accessions should be examined, this result suggests that the 'hard' mutations observed in *T. aestivum* occurred after the allopolyploidisation event.

Due to the limited number of accessions containing either the PINAi and PINAj translated proteins (one each), or the PINBj translated protein (two), these four accessions were dropped from the following analysis. Only accessions containing the wild-type (PINAa or PINBa) and either the PINAc or PINBh translated proteins (n=71) were analyzed to determine the association of these sequence polymorphisms with kernel texture. Accessions carrying the novel puroindolines, PINAc and PINBh, were significantly softer than their wild-type PINAa and PINBa counterparts respectively, based on DMRT (Table 7). Allele *Pina-D1c* varies from the wild-type allele (*Pina-D1a*) by one nucleotide at position 257; the wild-type G is replaced by an A. In frame, this mutation alters the amino acid sequence, changing the position 58 arginine (R) to glutamine (Q) (data not shown). The increased softness associated with the PINAc allele may be due to this single substitution at position 58. The precedent for single amino acid substitutions causing changes in kernel texture was first described by the substitution of glycine to serine at position 46 of the puroindoline b gene (Giroux and Morris 1997). It is not implausible for the increased softness of the PINAc protein to be due to this single substitution.

Thirteen amino acids differ between the puroindoline b wild-type, PINBa, and the PINBh proteins (Fig. 2). Potentially one or more of these amino acid changes could cause the increased softness observed in the accessions possessing the PINBh protein. The amino acid substitution which could possibly be most influential in causing the softer kernel texture variation is the position 28 change from arginine (R) to tryptophan (W). This change places another tryptophan in close proximity to the tryptophan-rich domain of puroindoline b. The amphiphilic tryptophan rich domains of the puroindolines are

Table 6 General linear model for the effect of the translated puroindoline protein variants on the endosperm texture of the synthetic hexaploid accessions

Source	*Df*	Type III SS	Mean square	*F*-value	*P*>*F*
Model	2	95,021	47,510	242	<0.0001
Puroindoline a	1	90,853	90,853	462	<0.0001
Puroindoline b	1	2,042	2,042	10	<0.001
Error	6,794	1,333,285	196	–	–

Table 7 Duncan's multiple range test for the translated protein of the allelic variants of the puroindoline a and b genes. Mean values with different Duncan grouping letters are significantly different (α=0.05)

Puroindoline gene	Translated protein	Mean	Number of accessions
a	PINAa	32.4a	14
	PINAc	23.1b	57
b	PINBa	29.4a	2
	PINBh	24.8b	69

relatively unique among proteins and are thought to be associated with lipid binding and anti-microbial activities (Blochet et al. 1993; Dubriel et al. 1998). The addition of the tryptophan at position 28 could increase the amphiphilic nature of the protein. Three of the six 'hard' puroindoline b alleles (*Pinb-D1d, Pinb-D1e* and *Pinb-d1f*) previously identified (Morris et al. 2000) contain mutations within the tryptophan-rich domain (Fig. 2), indicating the importance of this region for the "softening" function of the gene. Further research will be required to confirm this hypothesis.

Among the six synthetic hexaploid accessions having accession WX224 as the *Ae. tauschii* parent, four haplotypes were encountered. These accessions were re-amplified and re-sequenced four times each to confirm these results. The haplotypes (*Pina-D1a/Pinb-D1i*, *Pina-D1a/Pinb-D1n*, *Pina-D1c/Pinb-D1 h*, and *Pina-D1c/Pinb-D1i*) indicate that either WX224 is heterogeneous for both *Puroindoline* loci or that some other error in producing, recording, etc. these synthetics occurred. Two durum parents were used to produce these six synthetic hexaploids; the nucleotide polymorphisms do not appear to be associated with the durum parent. While changes to the phenotypic expression of traits when compared to their parental types has been observed previously in synthetic hexaploids, no specific changes to genes or DNA have been identified (Kema et al. 1995; Ma et al. 1995).

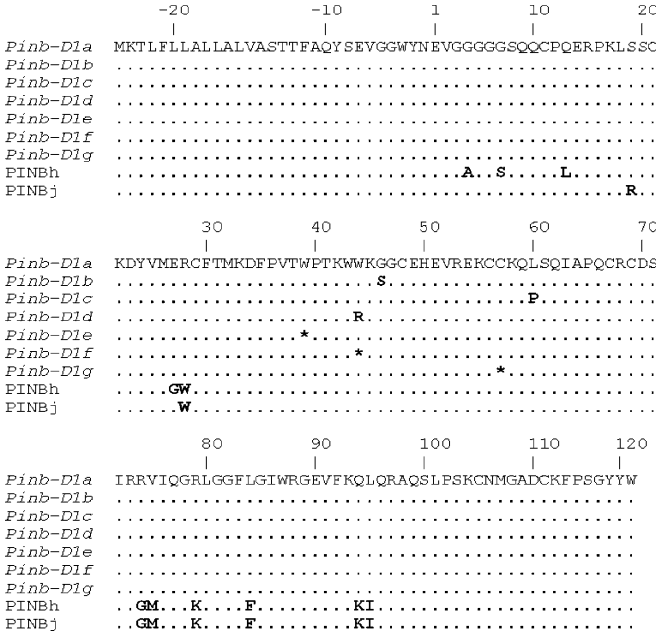

Fig. 2 Deduced amino acid sequence of puroindoline b alleles, where PINBh is encoded by alleles *Pinb-D1h*, *i*, *m*, *n* and *o*, and PINBj is encoded by *Pinb-D1j*. *Asterisks* in the amino acid sequences designates a stop codon. Amino acid changes causing a hard endosperm texture are in *bold*. The tryptophan-rich domain spans the amino acids between positions 39 and 44

Conclusions

The results of this experimentation indicate that synthetic hexaploid wheat accessions vary in kernel texture and that a significant amount of that variation is assignable to both the *Ae. tauschii* and durum parents. Furthermore, some of this variation is directly attributable to the puroindoline genes. A definitive association between specific sequence polymorphisms (the PINAc and PINBh translated proteins) and a phenotype significantly softer than the wild-type alleles (PINAa and PINBa) present in 'soft' varieties of *T. aestivum* was found. Some variation in kernel texture, however, was independent of the puroindoline genes, and not directly related to the gross morphological features and protein content of the kernels. This research has demonstrated that all puroindoline alleles found to date in *Ae. tauschii* (Lillemo et al. 2002; Massa et al. 2004) encode 'soft' phenotypes. No 'hard' alleles identical to those found in *T. aestivum* have been identified. The absence of 'hard' alleles in *Ae. tauschii* suggests that there may be a selection pressure on this species to maintain the 'soft' alleles, a selection pressure that either the allopolyploidisation event or human intervention and cultivation of hexaploid wheat has removed. The location of the amino acid changes and their effect on kernel texture may assist in future modeling of the structure and function of the puroindoline proteins.

Aegilops tauschii is an extension of the common wheat gene pool and as such genes identified in *Ae. tauschii* are available to wheat breeding programs for germ plasm enhancement. As the softer puroindoline alleles have been characterized in a synthetic hexaploid, they are immediately available for transfer into hexaploid wheat breeding programs. *Ae. tauschii* is a rich source of genetic variability for end-use quality genes, the additional unknown genes which caused some of the variation observed in this population could also be of use for developing softer wheat varieties.

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