



## Milling and Chinese raw white noodle qualities of common wheat near-isogenic lines differing in puroindoline b alleles

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### ABSTRACT

Understanding the effects of different alleles at the puroindoline b (*Pinb*) locus on processing quality will provide crucial information for quality improvement. Seven near-isogenic lines (NILs) planted at two locations in the 2008 cropping season were used to determine the effect of puroindoline b alleles on milling performance and Chinese raw white noodle (CRWN) quality. The *Pina-D1b/Pinb-D1a* genotype possessed significantly higher values in grain hardness, protein content and starch damage than other genotypes, whereas the *Pina-D1a/Pinb-D1d* genotype had the lowest grain hardness and starch damage, with higher break flour yield, and less reduction flour yield, higher flour colour L\*, and lower flour colour b\*, than other genotypes. Farinograph parameters, except for water absorption, were not significantly affected by variation of puroindoline b alleles. *Pina-D1a/Pinb-D1e* had the highest peak viscosity, whereas the lowest value was observed in a *Pina-D1b/Pinb-D1a* genotype. For CRWN quality, higher noodle viscoelasticity was obtained in the genotype *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g*, whereas *Pina-D1a/Pinb-D1d* had a lower smoothness score. Genotypes with *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* produced the best total noodle score. It was concluded that genotype *Pina-D1a/Pinb-D1d* had better milling qualities, whereas *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* had slightly superior CRWN qualities in comparison with other genotypes.

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### 1. Introduction

Grain hardness in common wheat (*Triticum aestivum* L.) is one of the most important parameters affecting milling and end-use qualities. Common wheat is classified and traded as either hard or soft based on endosperm texture. Soft-textured wheat typically has increased break flour yield, a smaller flour particle size, and less starch damage compared with hard-textured wheat. Therefore, flours from hard wheat are usually used for making bread, whereas flours from soft wheats are more suitable for producing biscuits, cookies and cakes.

**Abbreviations:** CRWN, Chinese raw white noodle; DH, double haploid; NIL, near-isogenic line; *Pina*, gene coding for puroindoline-a protein; *Pinb*, gene coding for puroindoline-b protein; RIL, recombinant inbred line; RVA, rapid visco analyzer; SKCS, Single Kernel Characterization System.

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Greenwell and Schofield (1986) reported the occurrence of 'friabilin', a small group of 15-kDa proteins that are abundant on the surface of water-washed starch granules from soft wheat and suggested to act as a non-stick surface between starch granules and protein matrix, allowing their easier separation. N-terminal sequencing has shown that friabilin is composed primarily of two proteins, PINA and PINB (Gautier et al., 1994; Rahman et al., 1994). The tightly linked genes *Pina* and *Pinb* code for the PINA and PINB proteins, respectively, which together function as the hardness (*Ha*) locus (Giroux and Morris, 1997, 1998). The *Ha* locus includes puroindoline a, puroindoline b and *GSP-1* genes (Giroux and Morris, 1998; Jolly et al., 1993, 1996). The wild type *Pina-D1a/Pinb-D1a* determines soft endosperm texture, and mutations in either the *Pina* or *Pinb* genes lead to hard endosperm (Giroux and Morris, 1997; Lillemo and Morris, 2000). Both *Pina* and *Pinb* genes are represented by many alleles in common wheat and related species, and different wheat cultivars contain different combinations of *Pina* and *Pinb* alleles (Bhave and Morris, 2008; Morris and Bhave, 2007). To date, 17 *Pina* (designated as *Pina-D1a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q*) and 25 *Pinb* (designated as *Pinb-D1a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, aa, ab*) alleles have been reported (Morris and

Bhave, 2007). *Pina-D1b* is a dominant genotype in CIMMYT, Latin American, and Indian wheat germplasm (Lillemo et al., 2006; Lillemo and Morris, 2000; Ram et al., 2002). *Pinb-D1b* was the most frequent allele in cultivars from north America, northern Europe, and southern Australia (Cane et al., 2004; Lillemo and Morris, 2000; Morris et al., 2001; Pickering and Bhave, 2007). The *Pinb-D1c* and *Pinb-D1d* alleles were frequently found in northern Europe (Lillemo and Morris, 2000). *Pinb-D1b*, *Pina-D1b* and *Pinb-D1p* were the main genotypes in Chinese wheats, and the frequency of *Pinb-D1b* increased in hard wheats over time from the landraces to historical cultivars and current wheats, whereas *Pina-D1b* and *Pinb-D1p* declined correspondingly (Chen et al., 2006, 2007; Xia et al., 2005). The distribution of puroindoline alleles in different countries could be closely associated with the core germplasm used in breeding programs and the end-use products consumed in the regions.

Different puroindoline alleles could have diverse effects on milling and the quality of end-use products. *Pinb* was a major quantitative trait locus for milling and cookie baking traits in a recombinant inbred population (RIL) segregating for the *Pinb-D1a* (soft) and *Pinb-D1b* (hard) alleles (Campbell et al., 2001). Storlie et al. (2006) reported that *Pinb* alleles in a doubled haploid (DH) population had significant effects on noodle texture, explaining 30% of the variation. Martin et al. (2001, 2008) showed that lines with *Pinb-D1b* in a RIL population had significantly softer grain, higher break flour yield and milling score, and lower flour ash content and crumb grain score than the *Pina-D1b* group. Nagamine et al. (2003) found that *Pinb-D1b* genotypes in a DH population had larger flour particle size, higher  $L^*$  and  $a^*$  flour colour scores, and smaller breakdown viscosity than the *Pinb-D1a* soft lines. Eagles et al. (2006) indicated that the *Pina-D1a/Pinb-D1b* genotype increased extensibility, dough development time and milling yield in comparison with the *Pina-D1b/Pinb-D1a*. For Chinese raw white noodle (CRWN) quality, *Pinb-D1b* genotypes were significantly higher in noodle  $a^*$  colour score, viscoelasticity and total score than *Pina-D1b* and wild types (Chen et al., 2007). Therefore, it is important to manipulate the allelic combinations of *Pina* and *Pinb* genes in wheat breeding programs targeting for end-use product improvement. However, much more effort is needed to understand the effects of the individual *Pina* and *Pinb* alleles on milling and end-use qualities. Most of the previous reports used wheats with different genetic backgrounds and therefore had much less precision in determining the exact effects of puroindoline alleles compared with near-isogenic lines (NILs).

China is the largest producer and consumer of wheat in the world. Noodles are the most widely consumed wheat products in China, representing about 40% of the national wheat consumption. Chinese white noodle quality attributes are related to grain hardness, gluten quality, flour whiteness, and starch pasting properties (He et al., 2004; Liu et al., 2003; Zhang et al., 2005). Therefore, genetic improvement of grain hardness is an important breeding objective for Chinese noodle quality. NILs differing in allelic status provide a unique opportunity to investigate the effects of puroindoline genes and alleles on end-use product quality. The objectives of this study were to determine the influence of allelic variation in *Pinb* loci on milling performance and CRWN quality using seven NILs differing in puroindoline b alleles. This study provides useful information for quality improvement in China and other countries with an interest in grain hardness manipulation.

## 2. Experimental

### 2.1. Experiment

Seven near-isogenic lines of spring wheat differing in puroindoline b alleles were used. They included *Pina-D1b/Pinb-D1a*,

*Pina-D1a/Pinb-D1b*, *Pina-D1a/Pinb-D1c*, *Pina-D1a/Pinb-D1d*, *Pina-D1a/Pinb-D1e*, *Pina-D1a/Pinb-D1f*, and *Pina-D1a/Pinb-D1g*. These lines were developed at the USDA-ARS Western Wheat Quality Laboratory, Pullman, Washington. The NILs were developed by crossing donor parents possessing unique puroindoline a and puroindoline b gene haplotypes as male to the soft white spring wheat cultivar Alpowa. Seven backcrosses were conducted such that the general pedigree of each NIL is: Alpowa/donor parent//7\* Alpowa (Morris and King, 2008). Field trials were conducted in a completely randomized block design with two and three replications in the experimental stations of Xinjiang Academy of Agri-Recalamation Sciences in Shihezi and Xinjiang Academy of Agricultural Sciences in Urumqi, respectively, during the 2008 growing season. Each plot was 4.8 m<sup>2</sup>, consisting of six 4 m rows spaced 0.2 m apart and planted at the rate of 27 g of seeds/m<sup>2</sup>. Field trials were managed according to local cropping practices.

### 2.2. Flour preparation and characterization of grain and flour properties

No rain was recorded over the 3 weeks prior to harvest, thus pre-harvest sprouting was not observed. Harvested samples were cleaned and tempered to a moisture content of 16.5% (all are hard wheat). Milling was performed on a Buhler laboratory mill based on Approved Method 26-21A (AACC, 1995). Grain hardness was measured on 300-kernel samples with a Perten SKCS 4100 (Perten Instruments, Springfield, IL), following the manufacturer's instructions. Grain and flour protein contents were determined with a near infrared transmittance (NIT) analyzer (Foss-Tecator 1241, Foss, Höganäs, Sweden). Flour ash content and Farinograph parameters were recorded according to AACC Approved Methods 08-01 and 54-21, respectively (AACC, 1995). Starch pasting properties were measured in flour with the Rapid Visco Analyzer (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia) according to AACC Approved Method 76-21 (AACC, 1995). Flour colour was measured with a Minolta Chroma Meter 310 (Minolta Camera Co. Ltd., Tokyo, Japan) using the CIE 1976  $L^*$ ,  $a^*$ , and  $b^*$  colour scale equipped with a D65 illuminant. Damaged starch content was determined with a damaged starch analyzer SDmatic (Chopin, France) (Approved Method AACC 76-31).

### 2.3. Flour particle size distribution

Flour particle size distribution was obtained from a laser beam particle size analyzer HELOS and RODOS (SYMPATEC GmbH). Distributions were expressed as proportions in volumes for different classes of particle size.

### 2.4. Preparation of Chinese raw white noodles

Chinese raw white noodles were prepared and evaluated as described by Zhang et al. (2005). Noodle score included colour score (weighting 15), appearance (10), firmness (20), viscoelasticity (30), smoothness (15) and taste/flavour (10). The experiment was performed at room temperature 20–25 °C and relative humidity 50–60%. For sensory evaluation of noodle quality, commercial flour (Hetao Xuehua flour) was used as a control.

### 2.5. Statistical analysis

Data analysis was performed by SAS software 9.0 (SAS institute, Cary, NC) using analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

### 3. Results and discussions

#### 3.1. Grain and milling characteristics

As shown in Table 1, analysis of variance indicated that both cultivar and location contributed significantly to variation in grain hardness, protein content, break flour yield, ash content, starch damage, flour colour, water absorption, peak viscosity, break down viscosity, noodle viscoelasticity and smoothness. Puroindoline b alleles had a significant effect on hardness index, milling quality and CRWN quality, but not on grain weight, flour particle size distribution >150 µm, development time, stability, set back viscosity, noodle colour score and noodle taste/flavour. L × G had no significant effect on any of the tested quality parameters of these NILs.

As listed in Table 2, the genotype *Pina-D1b/Pinb-D1a* had the highest grain hardness, and *Pina-D1a/Pinb-D1d* had the lowest value. Significant differences were observed among the *Pina-D1b/Pinb-D1a*, *Pina-D1a/Pinb-D1b*, *Pina-D1a/Pinb-D1c*, and *Pina-D1a/Pinb-D1d* genotypes, but no significant difference was observed among the *Pina-D1a/Pinb-D1e*, *Pina-D1a/Pinb-D1f* and *Pina-D1a/Pinb-D1g* genotypes. The *Pina-D1b/Pinb-D1a* genotype showed the highest grain protein content, whereas *Pina-D1a/Pina-D1d* showed the lowest protein content. The *Pina-D1a/Pinb-D1d* genotype showed the highest break flour yield and lowest reduction flour yield, whereas *Pina-D1b/Pinb-D1a* showed the lowest break flour yield and highest reduction flour yield. These results generally agreed with Greffeuille et al. (2006); for hard NILs the greatest flour yield was obtained at the reduction stage, whereas soft NILs produced as much flour in the second break stage as in the reduction stage. In Table 2, the *Pina-D1b/Pinb-D1a* genotype had significantly higher starch damage than *Pina-D1a/Pinb-D1d* and *Pina-D1a/Pinb-D1b*, consistent with the change of grain hardness. For ash content, a significant difference was observed only between the *Pina-D1a/Pinb-D1d* and *Pina-D1b/Pinb-D1a* genotypes.

#### 3.2. Flour colour and particle size distribution

The *Pina-D1a/Pinb-D1d* genotype showed the highest L\*, whereas the *Pina-D1b/Pinb-D1a* genotype showed the lowest L\* (Table 2). However, significant differences occurred only between *Pina-D1b/Pinb-D1a* and all other genotypes. The *Pina-D1b/Pinb-D1a* genotype showed the highest flour b\* colour and *Pina-D1a/Pinb-D1d* had the lowest value. The NILs in this experiment showed typical particle size distribution characteristics of hard wheat (detailed data not shown), in agreement with Greffeuille et al. (2006). The *Pina-D1a/Pinb-D1d* genotype had more particles <50 µm and less particles in the range of 50–150 µm. Campbell et al. (2007) reported that the proportion of particles from 74 to 149 µm was highly and positively correlated with increasing hardness and the proportion of particles <53 µm decreased with increasing hardness. This study also showed that hardness was significantly positively correlated with the proportion of particles from 72 to 150 µm and negatively correlated with the proportion of particles <50 µm (detailed data not shown). Results indicated that harder wheat produced larger particle sizes. The differences in flour ash content, starch damage, flour colour and flour particle size distribution among the NILs indicated that the genotype *Pina-D1a/Pinb-D1d* had superior milling qualities compared with other genotypes.

#### 3.3. Dough and starch pasting properties

No significant difference was found for Farinograph stability and development time among the tested genotypes (Table 1). As shown in Table 2, the *Pina-D1a/Pinb-D1d* genotype had the lowest water absorption. A significant difference was observed between *Pina-D1a/Pinb-D1d* and all other genotypes except for the *Pina-D1a/Pinb-D1e* genotype. Eagles et al. (2006) reported that the *Pina-D1a/Pinb-D1b* genotype increased extensibility and dough development time relative to *Pina-D1b/Pinb-D1a*, but this was not observed in the present study using NILs. Thus higher water absorption might

**Table 1**  
Mean square values from ANOVA for grain and milling characteristics, flour quality traits and CRWN qualities of seven NILs.

Source of variation	DF	Hardness index	Grain weight	Grain protein	Break flour yield	Reduction flour yield	Ash content	Starch damage
Locations (L)	1	543.7**	5.5	28.5**	24.2**	2.0	0.4	3.4**
Genotypes (G)	6	27.5**	1.2	0.6*	4.4**	2.4**	0.2*	0.2**
L × G	6	2.5	2.9	0.1	0.3	0.2	0.1	0.1
Error	21	1.4	0.4	0.2	0.1	0.6	0.1	0.1
Source of variation	DF	Flour L* colour	Flour a* colour	Flour b* colour	Flour particle size distribution			
					<50 µm	50–72 µm	72–150 µm	>150 µm
Locations (L)	1	2.4**	0.6**	1.4**	11.6*	0.1	8.0*	0.6
Genotypes (G)	6	0.2**	0.2**	0.2**	23.6**	0.5**	16.0**	0.3
L × G	6	0.1	0.1	0.1	1.8	0.1	1.1	0.9
Error	21	0.1	0.1	0.1	2.0	0.1	1.1	0.6
Source of variation	DF	Water absorption	Stability	Development time	Peak viscosity	Break down viscosity	Set back viscosity	Pasting temperature
Locations (L)	1	23.6**	0.4	0.8	645089**	54472**	38926**	0.8
Genotypes (G)	6	0.8*	0.7	0.2	17708**	9535*	3716	18.3*
L × G	6	0.1	0.1	1.6	2277	1522	3065	12.8
Error	21	0.2	0.6	0.5	2040	3136	3102	6.8
Source of variation	DF	Colour	Appearance	Firmness	Viscoelasticity	Smoothness	Flavour	Total score
Locations (L)	1	3.6*	0.1	0.4	20.5**	1.3**	0.1	70.1**
Genotypes (G)	6	0.1	0.2*	0.6**	0.9**	0.2**	0.1	4.0**
L × G	6	0.1	0.1	0.1	0.3	0.1	0.1	0.8
Error	21	0.3	0.1	0.1	0.2	0.1	0.1	0.7

\* and \*\* are significant at  $p = 0.05$  and  $p = 0.01$ , respectively.

**Table 2**  
Comparison of characterization of grain and flour properties among seven NILs.

Genotype	Hardness index	Grain protein (14%)	Break flour yield (%)	Reduction flour yield (%)	Starch damage (%)	Ash content (%)	Flour L* colour	Flour b* colour	Flour a* colour	Flour particle size distribution (%)			Water absorption (%)	Peak viscosity (cp)	Break down viscosity (cp)	Pasting temperature (°C)
										<50 µm	50–72 µm	72–150 µm				
<i>Pina-D1b/Pinb-D1a</i>	75.5a	12.6a	12.4d	63.2a	6.6a	0.53a	91.4b	7.14a	-0.63a	37.1d	18.8a	40.8a	63.5a	2468c	606c	68b
<i>Pina-D1a/Pinb-D1b</i>	69.6d	12.0bc	14.7b	61.6bc	6.4b	0.52ab	91.8a	6.86bc	-0.68bc	42.1ab	18.4a	36.4de	63.0a	2563b	667abc	70b
<i>Pina-D1a/Pinb-D1c</i>	72.7b	12.2abc	14.4bc	62.2ab	6.5ab	0.52ab	91.7a	6.76cd	-0.62a	39.4c	18.8a	38.5b	63.2a	2623ab	701ab	67b
<i>Pina-D1a/Pinb-D1d</i>	67.9e	11.6c	15.5a	61.0c	6.1c	0.49b	91.9a	6.60d	-0.64a	43.7a	17.9b	35.4e	62.2b	2561b	642bc	74a
<i>Pina-D1a/Pinb-D1e</i>	71.5bc	11.9bc	14.4bc	62.3ab	6.4ab	0.53a	91.8a	6.99abc	-0.70c	41.6b	18.7a	36.7cde	62.8ab	2657a	696ab	69b
<i>Pina-D1a/Pinb-D1f</i>	70.6cd	11.9bc	14.5bc	62.5ab	6.5ab	0.51ab	91.7a	7.05ab	-0.70c	40.2bc	18.9a	38.1bc	63.1a	2601ab	680abc	68b
<i>Pina-D1a/Pinb-D1g</i>	71.7bc	12.2ab	14.9b	61.9bc	6.5ab	0.52ab	91.7a	6.84bc	-0.65ab	41.3bc	18.8a	37.0cd	63.2a	2632a	742a	67b

\* The data are averages from two locations. Different letters indicate significant difference at  $p = 0.05$ .**Table 3**  
Comparison of noodle parameters among seven NILs.

Genotype	Appearance	Firmness score	Viscoelasticity	Smoothness	Total score*
<i>Pina-D1b/Pinb-D1a</i>	7.8ab	15.2c	22.3b	11.7cd	75.7c
<i>Pina-D1a/Pinb-D1b</i>	7.5b	15.2c	22.7b	11.8bc	76.0bc
<i>Pina-D1a/Pinb-D1c</i>	8.0a	15.7abc	22.7b	12.0ab	77.1ab
<i>Pina-D1a/Pinb-D1d</i>	7.9a	15.9ab	22.8ab	11.5d	76.7bc
<i>Pina-D1a/Pinb-D1e</i>	7.7ab	16.2a	23.4a	11.9abc	77.9a
<i>Pina-D1a/Pinb-D1f</i>	7.8ab	15.4bc	23.0ab	11.7cd	76.6bc
<i>Pina-D1a/Pinb-D1g</i>	7.7ab	15.9ab	23.5a	12.1a	78.0a

The data are averages from two locations. Different letters indicate significant difference at  $p = 0.05$ . \* indicate that the total score including colour score and taste/flavour score.

mainly result from increased starch damage. The *Pina-D1a/Pinb-D1e* genotype showed the highest peak viscosity, whereas *Pina-D1b/Pinb-D1a* showed the lowest peak viscosity and breakdown viscosity. A significant difference was also observed between the *Pina-D1a/Pinb-D1e* and *Pina-D1b/Pinb-D1a* genotypes. The *Pina-D1a/Pinb-D1d* genotype showed the highest pasting temperature and was significantly different from all other genotypes.

### 3.4. Chinese raw white noodle quality

Noodle parameters among the different NILs are presented in Table 3. Noodles made from *Pina-D1a/Pinb-D1c* and *Pina-D1a/Pinb-D1d* genotypes had the best appearance, but were significantly different only from *Pina-D1a/Pinb-D1b*. The *Pina-D1a/Pinb-D1e* genotype had the highest firmness score, which was not significantly different from *Pina-D1a/Pinb-D1c*, *Pina-D1a/Pinb-D1d* and *Pina-D1a/Pinb-D1g*. The *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* genotypes had higher viscoelasticities than other genotypes, but were not significantly different from *Pina-D1a/Pinb-D1d* and *Pina-D1a/Pinb-D1f*. The *Pina-D1a/Pinb-D1c* and *Pina-D1a/Pinb-D1g* genotypes had better smoothness, whereas *Pina-D1a/Pinb-D1d* had an inferior smoothness score. Chen et al. (2007) reported that noodle quality of the *Pinb-D1b* genotype was superior to *Pina-D1b/Pinb-D1a* and wild type. In the present study, the total score of *Pina-D1a/Pinb-D1b* was slightly higher than the *Pina-D1b/Pinb-D1a* genotype, but no significant difference was observed. Higher total noodle scores were observed in genotypes *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* with higher scores in firmness, viscoelasticity and smoothness, but not significantly different from *Pina-D1a/Pinb-D1c*. Overall, the noodle qualities of the seven NILs were ranked: *Pina-D1a/Pinb-D1e* = *Pina-D1a/Pinb-D1g* > *Pina-D1a/Pinb-D1c* > *Pina-D1a/Pinb-D1d* = *Pina-D1a/Pinb-D1f* = *Pina-D1a/Pinb-D1b* > *Pina-D1b/Pinb-D1a*. The superiority of *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* allele for noodle qualities would provide useful information for wheat breeding programs targeting for noodle quality improvement. However, 17 *Pina* and 25 *Pinb* alleles have been reported, thus new near-isogenic lines need to be developed to investigate the effect of puroindoline alleles on milling performance, and qualities of pan bread, steamed bread, and noodles.

## 4. Conclusions

The genotype *Pina-D1b/Pinb-D1a* possessed significantly higher values in grain hardness, starch damage, and lower flour colour L\* than other genotypes, whereas the *Pina-D1a/Pinb-D1d* genotype had the lowest grain hardness and starch damage, a higher break flour yield, less reduction flour yield, higher flour colour L\* and lower flour colour b\* than other genotypes. Water absorption was significantly affected by variation in puroindoline b alleles.

*Pina-D1a/Pinb-D1e* had the highest peak viscosity. Higher noodle sensory scores were obtained for the *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* genotypes. Thus the overall results indicated that genotype *Pina-D1a/Pinb-D1d* had better milling qualities, whereas *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* had slightly superior CRWN qualities in comparison with other genotypes.

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