



## Association of *Puroindoline b-B2* variants with grain traits, yield components and flag leaf size in bread wheat (*Triticum aestivum* L.) varieties of the Yellow and Huai Valleys of China<sup>☆</sup>

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### ABSTRACT

A total of 169 wheat (*Triticum aestivum* L.) varieties (landraces and cultivars) were used to assess the relationship between *Puroindoline D1* alleles and *Puroindoline b-B2* variants and grain hardness, other grain traits, yield components, and flag leaf size. Results indicated that the average SKCS hardness of *Pinb-B2v3* varieties was significantly greater than that of *Pinb-B2v2* varieties within the soft *Puroindoline D1* haplotype sub-group. Conversely, no statistically significant difference was obtained for SKCS hardness between varieties with the *Pinb-B2v3* vs. *Pinb-B2v2* alleles within the two hard *Puroindoline D1* haplotypes (*Pinb-D1b* and *Pinb-D1p* sub-groups). Therefore, the *Puroindoline b-B2* gene may have a bigger impact on soft wheat varieties than hard. Across all varieties, thousand-kernel weight, grain weight per spike, grain diameter, grain number per spike, flag leaf width and area of *Pinb-B2v3* varieties were significantly greater than those possessing *Pinb-B2v2*. These results indicated that the *Pinb-B2v3* allele was associated with preferable grain yield traits compared to the *Pinb-B2v2* allele in bread wheat. This study provides evocative information for better understanding the molecular and genetic basis of wheat grain yield.

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### 1. Introduction

*Puroindoline a* and *b* genes, located on the short arm of chromosome 5D in bread wheat (*Triticum aestivum* L.), have a dramatic impact on grain hardness (kernel texture), a significant effect on many quality characteristics, and may play a role in plant disease defense (Bhave and Morris, 2008a, b; Morris, 2002; Morris and Bhave, 2008). So far, a number of *puroindoline* alleles have been documented in different varieties from around the world (Chen et al., 2005, 2006, 2007b; Giroux and Morris, 1997, 1998; Li et al., 2008; Morris et al., 2001; Xia et al., 2005) and variation in *puroindoline* alleles has been associated with differences

in wheat grain quality (Chen et al., 2007a; Ma et al., 2009; Martin et al., 2001).

With the in-depth understanding of the molecular genetic basis of grain texture in wheat, additional *puroindoline*-like genes have been considered for a role in modulating grain texture. *Gsp-1* (*Grains Softness Protein*) was the first *puroindoline*-like gene identified and resides at the same *Hardness* locus with the *puroindolines*. In bread wheat, *Gsp-1* exists as a homoeologous series with copies on the 5A, 5B and 5D chromosomes (Blochet et al., 1993). However, no obvious impact of the *Gsp-1* genes on grain texture has been demonstrated (Gollan et al., 2007; Massa et al., 2004). A definitive function for GSP-1 proteins is still unknown, but they may possibly play a role in plant defense (Gollan et al., 2007).

Recently, Wilkinson et al. (2008) reported three *puroindoline*-like genes in bread wheat with more than 70% identity compared to *puroindoline b* (*Pinb-D1a*) at the DNA level. These three genes were named *Pinb-A1* (*puroindoline b* variant 1), *Pinb-A2* (*puroindoline b* variant 2) and *Pinb-A3* (*puroindoline b* variant 3). Genetic mapping results of *Pinb-A2* by Wilkinson et al. (2008) indicated that *Pinb-A2* was located on the long arm of chromosome 7A. These authors further suggested that all three variants in bread wheat were possibly encoded by the same *Pinb-2* locus on 7AL. More

Abbreviations: MAS, marker assisted selection; PCR, polymerase chain reaction; *Pinb-B2v*, *Puroindoline b-B2* variant; QTL, quantitative trait locus; SKCS, single kernel characterization system; RIL, recombinant inbred line.

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**Table 1**  
PCR primers used in generating *Puroindoline b-2* variant gene sequences in wheat.

Gene	Forward primer	Reverse primer	PCR annealing temperature	PCR size (bp)
<i>Pinb-D1b1</i>	ATGAAGGCCCTTCCTCA	CTCATGCTCACAGCCGCT	58 °C	250 bp
<i>Pinb-D1b2</i>	ATGAAGGCCCTTCCTCA	CTCATGCTCACAGCCGCC	58 °C	250 bp
<i>Pina-D1b</i>	AATACCACATGGTTCTAGATACTG	GCAATACAAAGGACCTCTAGATT	60 °C	776 bp
<i>Pina-D1</i>	CATCTATTTCATCTCCACCTGC	GTGACAGTTTATTAGCTAGTC	58 °C	524 bp
<i>Pinb-D1</i>	GAGCCTCAACCATCTATTTCATC	CAAGGGTGATTTTATTTCATAG	58 °C	597 bp
<i>Pinb-D2v1</i>	GGTTCTCAAACCTGCCCAT	ACTTGACAGTTGGAATCCAG	57 °C	319 bp
<i>Pinb-B2v2</i>	CTTGATGTGAGCACAACTTTGCA	GTATGGACGAACCTGCAGCTGGAG	65 °C	401 bp
<i>Pinb-B2v3</i>	GAGCACAACTTTGCGCAATG	CATTAGTAGGGACGAACCTGCAGCTA	65 °C	398 bp
<i>Pinb-A2v4</i>	CCITTTCTTGTAGTGAGCACACCA	GACGAACTTGACGTTGGAATCCAA	65 °C	403 bp

recently, a new puroindoline b variant was identified in genomic DNA of bread wheat by Chen et al. (2010a), and this and the previous three variants were renamed *Pinb-2v1* (puroindoline b variant 1), *Pinb-2v2* (puroindoline b variant 2), *Pinb-2v3* (puroindoline b variant 3) and *Pinb-2v4* (puroindoline b variant 4). Physical mapping of these four *Puroindoline b* variants using several types of aneuploid bread wheat stocks indicated that *Pinb-2v1* was located on 7DL (hence, *Pinb-D2v1*), *Pinb-2v2* on 7BL (*Pinb-B2v2*), *Pinb-2v3* on 7B (*Pinb-B2v3*) and *Pinb-2v4* on 7AL (*Pinb-A2v4*) (Chen et al., 2010a). Moreover, all of a small number of surveyed varieties were shown to possess both *Pinb-2v1* and *Pinb-2v4*, whereas some possessed *Pinb-2v2* and others *Pinb-2v3*. The function of these new *Puroindoline b* variants is still unknown, although Wilkinson et al. (2008) described linkage of polymorphism at the *Pinb-A2* locus with differences in kernel texture in two of three mapping populations.

Grain yield is usually the most important trait in breeding programs for wheat breeders over the long-term improvement of bread wheat and reflects the culmination of all the processes of vegetative and reproductive growth, and their interactions with the edaphic and aerial environments (Quarrie et al., 2006). With the development of molecular genetic techniques, combinations of MAS (marker assisted selection) and traditional breeding techniques are gradually becoming the preferred strategy for wheat improvement. A number of QTL related to grain yield on various chromosomes, including group 7 (1B, 1D, 4AL, 5AL, 4D, 7AL, 7BL and 7D) have been reported in bread wheat (Kirigwi et al., 2007; Kuchel et al., 2007; McIntyre et al., 2010; Quarrie et al., 2005, 2006).

In China, the wheat producing region of the Yellow and Huai Valleys, covering all of Henan and parts of Hebei, Shanxi, Shaanxi,

Gansu, Anhui, Jiangsu, and Shandong Provinces, is the largest and most important wheat production zone, with 60–70% of the total harvested area and the total wheat production. Improvement of grain yield through better adaptation to target environments has historically been the major focus of most breeding programs in this area, whereas only recently have detailed molecular genetic strategies been considered for increasing grain yield. Especially, the successful utilization of dwarfing genes (*Rht1*, *Rht2*, and *Rht8*) and the 1B/1R translocation have made significant contributions to yield improvement in the wheat producing region of the Yellow and Huai Valleys as indicated by Zhou et al. (2007).

In this study, we report on associations between *Puroindoline D1* alleles and *Puroindoline b-2* variants and grain hardness, other grain traits, grain yield components, and flag leaf size. Varieties with the *Pinb-B2v3* allele possessed more preferable grain yield traits than those of varieties with the *Pinb-B2v2* allele. These results identify additional avenues for gaining a better understanding of the molecular genetic basis of wheat grain yield and yield improvement.

## 2. Material and method

### 2.1. Wheat germplasm

A total of 169 bread wheat varieties from the wheat producing area of the Yellow and Huai Valleys of China included 38 landraces and 131 currently popular varieties, and were mainly collected from Henan, Hebei, Shaanxi, Shanxi and Shandong Provinces. Varieties were grown at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University during the 2006–2007 and 2007–2008 cropping seasons according to local

**Table 2**  
Mean square values from ANOVA for grain hardness, grain morphology, yield components and flag leaf size of genotypes surveyed.

Genotype	Source of variation	Df	SKCS hardness	1000-Kernel weight	Grain diameter	Grain number per spike	Grain weight per spike	Spikelet number per spike	Grain weight per plant	Length of flag leaf	Width of flag leaf	Area of flag leaf
<i>Pina-D1a</i> / <i>Pinb-D1a</i>	Year (Y)	1	90.1	321.5*	0.004	0.32	0.037	14.1	1.90	0.306	0.001	0.001
	<i>Pinb-B2</i> (P)	1	444.1**	353.7*	0.67*	748.8*	1.98**	26.3	245.4*	5.64	0.36**	134.6*
	Y × P	1	1.60	23.9	0.04	29.4	0.11	7.21	1.77	1.41	0.002	0.47
	Error	108	53.4	67.6	0.18	77.4	0.22	22.4	41.9	11.2	0.03	37.4
<i>Pina-D1a</i> / <i>Pinb-D1b</i>	Year (Y)	1	45.1	246.2	0.043	0.82	0.001	119.1*	0.015	75.1*	0.01	0.084
	<i>Pinb-B2</i> (P)	1	94.8	481.2*	1.68**	722.4*	4.89**	317.7	4.81	85.2	0.56*	574.1**
	Y × P	1	1.52	0.65	0.026	15.3	0.066	150.1	27.6	21.6	0.003	0.77
	Error	158	33.4	55.7	0.15	115.3	0.35	388.8	53.8	50.3	0.085	46.9
<i>Pina-D1a</i> / <i>Pinb-D1p</i>	Year (Y)	1	280	52.2	0.023	7.43	0.002	0	0.18	0.02	0	0.09
	<i>Pinb-B2</i> (P)	1	14.3	770.1**	1.56**	38.7*	0.78*	1.30	129.6*	0.39	0.099	20.1
	Y × P	1	2.3	0.02	0.006	10.1	0.013	0.15	3.31	0.058	0	0.023
	Error	24	36.6	63.6	0.13	12.4	0.18	1.41	37.1	6.12	0.075	23.87
Total	Year (Y)	1	166.6	606.3**	0.053	78.7	0.02	66.9	7.55	16.7*	0.05	7.58
	<i>Pinb-B2</i> (P)	1	1298.5	1309.1**	3.25**	1113.8*	8.73*	254.5	29.8	36.7	1.28**	721.9**
	Y × P	1	3.88	2.16	0.086	8.37	0.29	38.8	10.96	14.03	0.008	0.68
	Error	298	341.9	60.3	0.16	95.8	0.28	145.1	48.1	6.2	0.063	41.3

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

management practices, and evaluated for agronomic and morphological traits, SKCS grain hardness, and *Puroindoline D1* and *Puroindoline b-2* variant haplotypes. An augmented design was used in the field trial in order to reduce the negative influence of the possible difference of fertilization in plots and each variety surveyed randomly presented in different plots two times. Each plot was comprised of four 200-cm long rows with 23 cm between neighboring rows and 10 cm between neighboring plants. All surveyed varieties grew well with the supporting nets and no lodging occurred. After harvest, grain samples were cleaned. Falling number tests indicated that they were sound and free of sprouting damage (data not shown).

## 2.2. Measurement of agronomic traits and SKCS hardness index

Before harvesting, ten plants of each variety were randomly selected in each plot and used to determine spikelet number per spike, flag leaf length, flag leaf width, and flag leaf area (calculated from the product of leaf length and maximum leaf width  $\times$  0.75). At harvest, grain number per spike, grain weight per spike, and grain weight per plant were determined.

Kernel hardness, thousand-kernel weight and grain diameter were measured on a 300-kernel sample using the Perten Single Kernel Characterization System (SKCS) 4100, following the manufacturer's operation procedure (Perten Instruments North America Inc., Springfield, IL). Mean, standard deviation, and distribution of SKCS hardness data were used to classify the varieties into soft, mixed, and hard types.

## 2.3. Identification of *Puroindoline a* and *Puroindoline b* alleles and *Puroindoline b-2* variants

Genomic DNA of each surveyed hard grain variety based on SKCS classification was extracted from pulverized kernels, following the method of Chen et al. (2006). Two pairs of allele-specific primers (Table 1) were used for the detection of *Pinb-D1b* (Chen et al., 2006). An STS marker recently developed by Chen et al. (2010b) was used for identifying the *Pina-D1b* allele. *Pinb-D1p* was identified by amplifying the full-length *Pinb-D1* coding sequence (using *Pinb-D1* Forward and *Pinb-D1* Reverse, Table 1), and then restricting the product with *Pf1MI* (Li et al., 2008). For identifying other *puroindoline* alleles in varieties with hard grain, full-length *Pina-D1* and *Pinb-D1* coding regions were amplified using primers *Pina-D1* Forward and *Pina-D1* Reverse or *Pinb-D1* Forward and *Pinb-D1* Reverse (Table 1), and the product was directly sequenced from both directions by SinoGenoMax Co., Ltd. *Puroindoline b-2* variants were identified by using variant-specific primers (Table 1) according to the methods reported by Chen et al. (2010a).

PCR amplifications were performed in an MJ Research PTC-200 or ABI 9700 thermocycler; reactions were conducted in a 25- $\mu$ l volume containing 100 ng of genomic DNA, 10 pmol of each primer, 250  $\mu$ mol of each dNTP, 1  $\times$  reaction buffer (50 mmol of KCl, 10 mmol of Tris-HCl, 1.5 mmol of MgCl<sub>2</sub>, pH 8.3), and 2.0 units of Taq DNA polymerase (Promega). The PCR program was 94 °C for 5 min followed by 35 cycles of 94 °C for 50 s, 57–65 °C (specific annealing temperature of each primer pair is presented in Table 1) for 45 s, 72 °C for 1 min, with a final extension for 10 min at 72 °C. PCR products were separated and analyzed on 1.5% (w/v) agarose gels, stained with ethidium bromide, and visualized with UV light.

Analysis and multiple alignments of sequences were performed by DNAMAN Version 6.0. Reliability of sequencing results was checked by examining the sequence chromatograms using Chromas Version 1.4.5.

**Table 3**  
Association of *Puroindoline D1* alleles and *Puroindoline b-2* variants with grain hardness, grain morphology, yield components and flag leaf size.

Puroindoline	<i>Pina-D1a/Pinb-D1a</i>				<i>Pina-D1a/Pinb-D1b</i>				<i>Pinb-D1a/Pinb-D1p</i>				Total <sup>a</sup>				
	<i>Pinb-2v2</i>	<i>Pinb-2v3</i>	Mean	SD <sup>b</sup>	Range	<i>Pinb-2v2</i>	<i>Pinb-2v3</i>	Mean	SD	Range	<i>Pinb-2v2</i>	<i>Pinb-2v3</i>	Mean	SD	Range	<i>Pinb-2v2</i>	<i>Pinb-2v3</i>
Sample no.	10	47	—	—	—	18	60	—	—	—	6	6	—	—	37	— <sup>a</sup>	117
SKCS hardness	22.2a	27.2b	26.5	7.9	0–42	61.4a	63.6a	61.9	6.5	51–78	62.4a	64a	62.8	6.5	53–71	— <sup>a</sup>	— <sup>a</sup>
1000-Kernel weight (g)	41.6a	45.2b	44.7	8.3	23.8–61.4	39.9a	44.8b	43.9	7.6	24.1–56.9	41.0a	49.4b	45.1	7.2	28.4–56.4	40.3a	44.9b
Grain diameter (mm)	2.37a	2.54b	2.51	0.45	1.55–3.58	2.36a	2.62b	2.57	0.39	1.72–3.19	2.40a	2.76b	2.58	0.35	1.75–3.09	2.36a	2.59b
Grain number per spike	45.7a	51.5b	50.5	9.6	35–83	45.0a	49.4b	48.3	11.4	28–100	44.8a	47.9b	46.0	6.0	36–51	45.5a	50.3b
Grain weight per spike (g)	1.96a	2.24b	2.19	0.49	1.20–3.78	1.81a	2.18b	2.08	0.63	0.86–3.37	1.80a	2.10b	1.92	0.44	1.12–2.48	1.86a	2.18b
Spikelet number per spike	21.8a	20.6a	21.6	5.7	11–39	20.6a	21.7a	21.4	5.6	10–38	20.8a	20.5a	20.7	3.6	14–25	20.6a	21.5a
Grain weight per plant (g)	21.3a	24.8b	24.2	6.9	9.4–40.6	25.3a	24.5a	24.9	7.4	9.0–44.3	25.5a	29.8b	27.2	6.4	15.1–36.1	24.2a	24.7a
Length of flag leaf (cm)	19.7a	20.6a	20.4	3.3	14.5–27.5	19.1a	20.8a	20.3	3.2	14.3–34.3	19.9a	19.0a	19.5	2.7	15.2–23.1	19.5a	20.7a
Width of flag leaf (cm)	1.74a	1.88b	1.86	0.18	1.37–2.23	1.70a	1.83b	1.80	0.29	1.06–2.79	1.68a	1.74a	1.71	0.25	1.32–2.1	1.72a	1.85b
Area of flag leaf (cm <sup>2</sup> )	25.9a	29.1b	28.6	6.2	15.1–45.1	24.3a	28.7b	27.6	6.9	14.8–56.7	25.0a	24.9a	25.0	4.9	16.2–32.2	25.2a	28.8b

<sup>a</sup> SKCS hardness index of *Pinb-B2v2* and *Pinb-B2v3* was not compared because of the large effect of *Puroindoline D1* on grain hardness and the unbalanced number of varieties included in the study.

<sup>b</sup> SD indicated standard deviation.

## 2.4. Statistical analysis

SAS version 8.0 software GLM ANOVA and LSD multiple comparisons were used to calculate averages of the surveyed ten characteristics including SKCS hardness index, thousand-kernel weight, grain diameter, grain number per spike, grain weight per spike, spikelet number per spike, grain weight per plant, and the width, length and area of the flag leaf. Variety sub-groupings were made based on the various *Puroindoline D1* alleles and *Puroindoline b-2* variants.

## 3. Results

### 3.1. Distribution of *Puroindoline D1* alleles in the Yellow and Huai Valleys of China

Based on SKCS hardness index and distribution, 57, 15 and 97 of the surveyed 169 wheat varieties were classified as soft, mixed and hard, respectively. The 57 soft wheat varieties were assumed to possess the wild type *Puroindoline D1* haplotype (*Pina-D1a*/*Pinb-D1a*) (Bhave and Morris, 2008a, b; Morris, 2002; Morris and Bhave, 2008). No haplotype analysis of the mixed hardness wheat varieties was attempted. Based on the results of PCR amplification with allele-specific primers, digestion with restriction enzyme *Pf1MI* and sequencing results, 81, 12, 3 and 1 of 97 hard wheat varieties possessed the *Pinb-D1b*, *Pinb-D1p*, *Pina-D1b* and *Pina-D1l* alleles, respectively.

Based on results with *Puroindoline b-2* variant-specific primers, all of the surveyed varieties possessed *Pinb-D2v1* and *Pinb-A2v4* variants, which is consistent with the results of Chen et al. (2010a); 37 varieties possessed the *Pinb-B2v2* variant (haplotype = *Pinb-A2v4*/*Pinb-B2v2*/*Pinb-D2v1*), whereas the other 117 varieties possessed the *Pinb-B2v3* variant (haplotype = *Pinb-A2v4*/*Pinb-B2v3*/*Pinb-D2v1*). This result suggests that *Pinb-A2v4*/*Pinb-B2v3*/*Pinb-D2v1* was the dominant haplotype of *Puroindoline b-2* variants considering this set of Chinese wheat varieties of the Yellow and Huai Valleys. The results further support a conclusion that *Pinb-B2v2* and *Pinb-B2v3* are probably allelic.

### 3.2. Association of *Puroindoline b-2* variants with SKCS grain hardness, other grain traits, grain yield components, and flag leaf size

As noted above, 15 wheat varieties had a 'mixed' SKCS hardness classification and therefore were eliminated from further analysis

involving examining associations among grain hardness, grain traits, yield components and flag leaf size with *Puroindoline D1* and *Puroindoline b-2* variants (Varieties with mixed hardness classification generally contain multiple *Puroindoline D1* haplotypes [Chen et al., 2005; Lillemo et al., 2006; Morris et al., 2001], whereas 'medium' hardness phenotype varieties may be monogenic [Li et al., 2008].).

Analysis of variance indicated that the allelic variants at *puroindoline b-2* locus contributed significantly to variation in thousand-kernel weight, grain diameter, grain number per spike, grain weight per spike, width of flag leaf and area of flag leaf among traits surveyed ( $P < 0.05$ ) (Table 2). It suggested that *puroindoline b-2* had a significant effect on yield-related components and flag leaf size in Chinese wheat varieties.

In order to investigate the association of *Puroindoline b-2* variants with grain hardness independent of the *Puroindoline a* and *Puroindoline b* alleles, the average SKCS hardness index of the two different *Puroindoline b-2* variant variety groups (*Pinb-B2v2* vs. *Pinb-B2v3*) were compared for each of the *Puroindoline D1* allele sub-groups. The *Puroindoline D1* alleles exert a large effect on grain hardness (Bhave and Morris, 2008a, b; Morris, 2002; Morris and Bhave, 2008) that would otherwise occlude the association of *Puroindoline b-2*. Results indicated that in soft wheat varieties (*Pina-D1a*/*Pinb-D1a*), the average SKCS hardness index of varieties with *Pinb-B2v3* (27.2) was significantly higher than that of varieties with *Pinb-B2v2* (22.2), whereas no significant difference between the *Pinb-B2v2* and *Pinb-B2v3* groups was obtained among sub-groups of varieties with either the *Pinb-D1b* or the *Pinb-D1p* allele (Table 3). However, for both of these *Pinb-D1* allelic groups (*Pinb-D1b* and *Pinb-D1p*) the average SKCS hardness of varieties with *Pinb-B2v3* was slightly greater than those of varieties with *Pinb-B2v2*, but the significance level was above the  $P = 0.05$  threshold (Table 3). These results suggest that *Puroindoline b-2* variants have a greater association with grain hardness in soft wheat varieties than in hard wheat. Genotypes and phenotypes of some varieties surveyed are shown in Table 4.

Moreover, no significant association of *Puroindoline D1* alleles with the nine agronomic traits related to yield were observed in the surveyed Chinese wheat varieties of the Yellow and Huai Valleys, suggesting that the *puroindoline* genes would have little relationship or association with wheat grain yield. However, when the traits were examined across all varieties surveyed ( $n = 154$ ) by contrasting varieties based on the two different *Puroindoline b-2* variants, thousand-kernel weight, grain diameter, grain number per spike, grain weight per spike, and flag leaf width and area of

**Table 4**  
Genotype and phenotype of *puroindoline* and *puroindoline b-2* in some Chinese varieties surveyed.

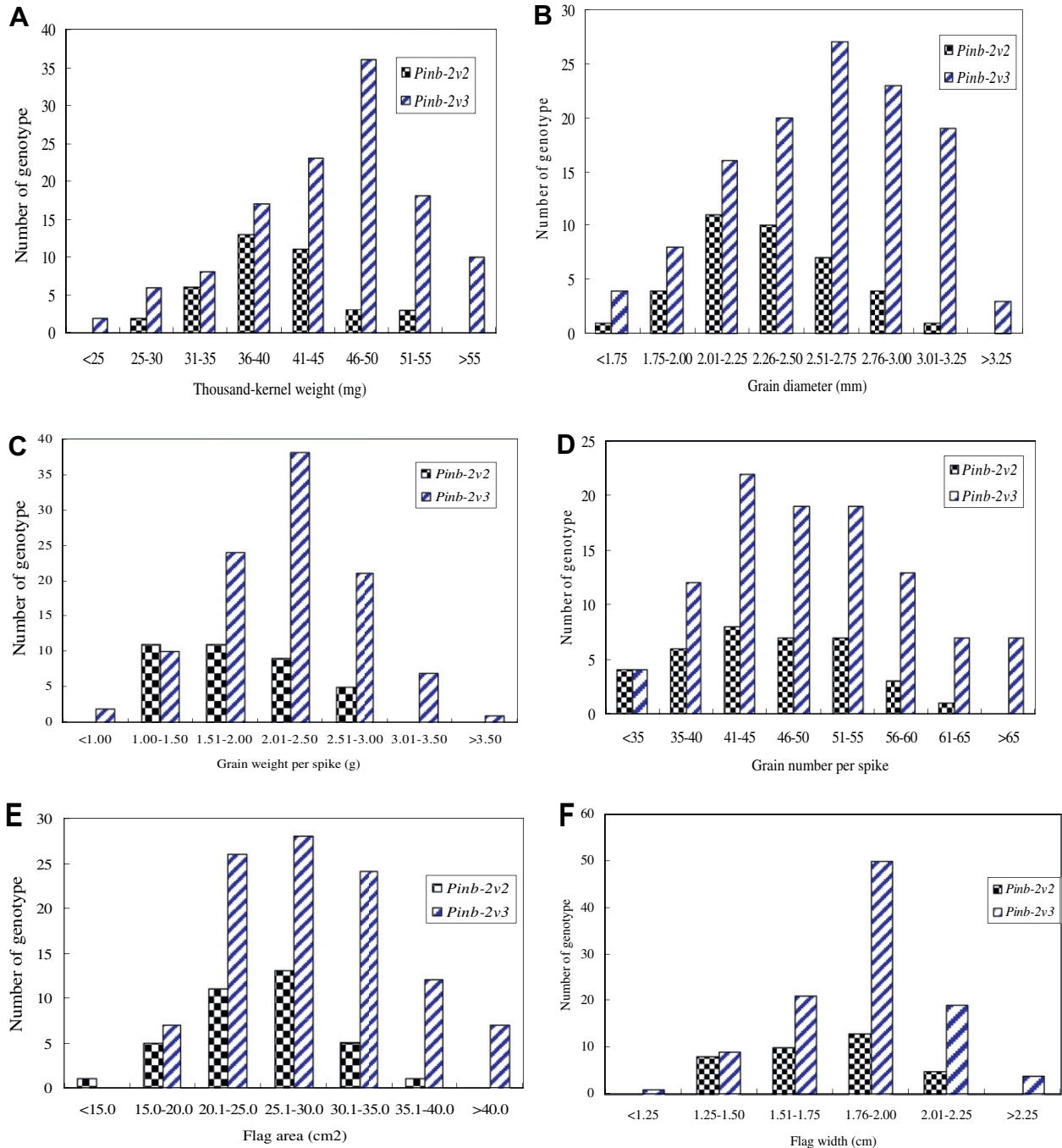
Variety	<i>Puroindoline</i>	<i>Puroindoline b-2</i>	Phenotype	SKCS hardness
Zaosui 30	<i>Pina-D1a</i> / <i>Pinb-D1a</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Soft	0
Yunong 949	<i>Pina-D1a</i> / <i>Pinb-D1a</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Soft	13
Zhenghua 3563	<i>Pina-D1a</i> / <i>Pinb-D1a</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Soft	26
Yumai 56	<i>Pina-D1a</i> / <i>Pinb-D1a</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Soft	30
Yumai 57	<i>Pina-D1a</i> / <i>Pinb-D1a</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Soft	18
Yumai 9	<i>Pina-D1a</i> / <i>Pinb-D1a</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Soft	31
Yumai 55	<i>Pina-D1a</i> / <i>Pinb-D1b</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Hard	69
Jimai 20	<i>Pina-D1a</i> / <i>Pinb-D1b</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Hard	60
Shanhe 6	<i>Pina-D1a</i> / <i>Pinb-D1b</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Hard	61
Yumai 53	<i>Pina-D1a</i> / <i>Pinb-D1b</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Hard	57
Zhengmai 9023	<i>Pina-D1a</i> / <i>Pinb-D1b</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Hard	71
Yumai 51	<i>Pina-D1a</i> / <i>Pinb-D1b</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Hard	60
Mazamai	<i>Pina-D1a</i> / <i>Pinb-D1p</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Hard	59
Fengkang 13	<i>Pina-D1a</i> / <i>Pinb-D1p</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Hard	69
Jimai 32	<i>Pina-D1a</i> / <i>Pinb-D1p</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v2</i> / <i>Pinb-D2v1</i>	Hard	62
Yumai 13	<i>Pina-D1a</i> / <i>Pinb-D1p</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Hard	70
Jimai 41	<i>Pina-D1a</i> / <i>Pinb-D1p</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Hard	69
Luohan 2	<i>Pina-D1a</i> / <i>Pinb-D1p</i>	<i>Pinb-A2v4</i> / <i>Pinb-B2v3</i> / <i>Pinb-D2v1</i>	Hard	62

varieties with *Pinb-B2v3* were significantly higher than those of varieties with *Pinb-B2v2* (Table 3).

Results were also examined within each of the *Puroindoline D1* haplotype groups (*Pina-D1a/Pinb-D1a*, *Pina-D1a/Pinb-D1b* and *Pina-D1a/Pinb-D1p*). Differences between the two *Puroindoline b-B2* variant groups were consistent across all *Puroindoline D1* haplotypes for thousand-kernel weight, grain number per spike and grain weight per spike (Table 3). However, in no case did the varieties with *Pinb-B2v2* have significantly higher values than those varieties with *Pinb-B2v3* (i.e. a reverse of the generalized trend).

Distributions of the grain traits and flag leaf size parameters that were significant for the *Pinb-B2* contrast are presented in

Fig. 1. All distributions tended to approximate normality, and illustrated further the differences between the *Pinb-B2v2* and *Pinb-B2v3* groupings. Some very modest positive and negative skewness were evident for most traits. A significant number of *Pinb-B2v3* varieties exceeded 46 g whereas very few *Pinb-B2v2* exceeded this weight. Grain diameter showed a distinct positive skewness for *Pinb-B2v2* varieties; the majority of the *Pinb-B2v3* varieties had mean diameters greater than 2.5 mm. The *Pinb-B2v3* varieties appeared to have a potential yield advantage in their greater grain number per spike (mean = 50.3 kernels) which was also reflected in greater grain weight per spike (mean = 2.18 g).



**Fig. 1.** Distribution of thousand-kernel weight (A), grain diameter (B), grain weight per spike (C), grain number per spike (D), flag leaf area (E), flag leaf width (F) of varieties with either *Pinb-B2v2* (*Pinb-A2v4/Pinb-B2v2/Pinb-D2v1*) and *Pinb-B2v3* (*Pinb-A2v4/Pinb-B2v3/Pinb-B2v1*).

The width, length and area of the flag leaf were investigated due to the direct relationship of photosynthetic capacity to crop yield (Dere and Yildirim, 2006; Mohiuddin and Croy, 1980; Richards, 1983). In this survey, the *Pinb-B2v3* varieties had significantly wider flag leaves with a considerable proportion greater than 30 cm<sup>2</sup> (mean = 28.8 cm<sup>2</sup>). Among the varieties with *Pinb-B2v2*, a few had a very narrow flag leaf (<1.25 cm), whereas the majority of *Pinb-B2v3* varieties had flag leaves greater than 1.75 cm, and a few with greater than 2.25 cm. Flag leaf length between the two *Pinb-B2* haplotypes did not differ significantly.

#### 4. Discussion

Prior to this study, we analyzed the association of *Puroindoline b-2* variants with milling quality and Farinograph parameters of flour doughs. No significant differences were observed between the two types of varieties grouped according to the *Pinb-B2v2* and *Pinb-B2v3* haplotypes (data not shown). However, the results presented here indicate significant differences in grain-yield-related traits between them.

The grain yield of wheat can be dissected into its components, including thousand-kernel weight, grain number per spike and grain weight per spike, etc. Of them, thousand-kernel weight and grain number per spike showed the most stable association with grain yield in bread wheat (Briggs and Aytenfisu, 1980), especially in terms of heritable genetic factors. In this study, varieties with *Pinb-B2v3* possessed significantly heavier kernels (about 10% heavier), and more kernels per spike (again, about 10% more). Whether these traits have contributed to the greater preponderance of the *Pinb-B2v3* type in varieties in this survey of varieties from the largest wheat production of China, the Yellow and Huai Valleys, cannot be resolved here. Conversely, there was no difference in spikelet number per spike or grain weight per plant between the two *Pinb-B2* haplotypes (Table 3).

According to report of Chen et al. (2010a), *Pinb-2v1*, *Pinb-2v2*, *Pinb-2v3* and *Pinb-2v4* are located on chromosomes 7DL, 7BL, 7B and 7AL in bread wheat, respectively. Coincidentally, the strongest QTL effects controlling grain yield, especially for grain weight, were found on chromosomes 7AL and 7BL in the report of Quarrie et al. (2005), and a QTL associated with grain yield has also been identified on chromosome 7D in the study of Kuchel et al. (2007). Very recently, several QTLs including 7A and 7B associated with grain yield and its components have been evaluated in a recombinant inbred line population (McIntyre et al., 2010). However, more research will be required to evaluate further the apparent association of the *Pinb-2* loci with grain hardness and morphology, and grain yield.

The flag leaf is one of the important factors affecting wheat grain yield. According to Dere and Yildirim (2006), flag leaf width was significant while flag leaf length traits were insignificant among the genetic components of variation dependent upon dominance effects or both additive and dominance effects, suggesting that flag leaf width possessed higher inheritance than flag leaf length. Interestingly, we observed a significant association between *Puroindoline b-B2* variants and flag leaf width, but not flag leaf length in this study.

#### 5. Conclusions

In the wheat producing areas of the Yellow and Huai Valleys, *Pinb-B2v3* (vs. *Pinb-B2v2*) is the predominant genotype. *Pinb-B2* variants are associated with a significant difference in grain hardness in soft wheat varieties, but much less so in hard. *Pinb-B2v3* varieties possessed significantly superior wheat yield traits compared to *Pinb-B2v2* varieties, including thousand-kernel

weight, grain diameter, and grain number and weight per spike. *Pinb-B2v3* varieties also had wider and larger flag leaves. Lastly, the data in this report are consistent with the *Puroindoline b-2* loci consisting of a homoeologous series with *Pinb-B2v2* and *Pinb-B2v3* being allelic. As with any trait-genotype association study, the results may be due to linkage, direct or indirect effect of the genes themselves or simply an unknown structure within the surveyed germplasm. In the future, additional studies should be able to address, support or refute the results reported here.

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