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Research Note

# A new puroindoline b mutation present in Chinese winter wheat cultivar Jingdong 11

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**Abstract**

Kernel hardness is one of the most important characteristics in determining utilization and marketing of bread wheat. Genes coding for puroindoline a and b (PINA and PINB) were located at the *Ha* locus and designated as *Pina-D1* and *Pinb-D1*, respectively. The coding sequence of the *Pinb* gene in a Chinese winter wheat cultivar Jingdong 11 (*Triticum aestivum* L.) was amplified with polymerase chain reaction (PCR), and the obtained 447-bp fragment sequenced from two strands, and compared with the eight known *Pinb* alleles. The results showed that Jingdong 11 possessed a new *Pinb* allele not reported previously, and was designated as *Pinb-D1q*. It is characterized by a single base T to G substitution, which results in a tryptophan to leucine substitution (TGG to TTG) at position 44 and is most likely the cause of hard grain texture in Jingdong 11. The characterization of *Pinb-D1* alleles would be helpful in manipulating grain hardness of bread wheat in breeding programs.



*Keywords:* Bread wheat; Kernel hardness; Puroindolines; *Pinb* mutation

Kernel texture is one of the most important quality parameters in bread wheat, and has a profound effect on milling, end use quality, and marketing classification (Mattern et al., 1973; Morris, 2002). The present understanding of the molecular genetic basis for this trait came with the discovery of friabilin which is composed primarily of puroindoline a and b (PINA and PINB). A thorough review of friabilin, puroindolines and grain hardness from a molecular genetic viewpoint has been provided by Morris (2002).

Significant genetic variation at the *Pinb-D1* locus is present in bread wheat. Up to now, eight single nucleotide mutations in *Pinb* and one *Pina* null mutation (designated as *Pina-D1b*) have been reported. These mutations result in a change in kernel hardness from soft to hard (Giroux and Morris, 1998; Lillemo and Morris, 2000; Morris et al., 2001; Xia et al., 2005). The eight mutations in *Pinb* were designated *Pinb-D1b*, *Pinb-D1c*, *Pinb-D1d*, *Pinb-D1e*, *Pinb-D1f*, *Pinb-D1g*, *Pinb-D1l* and *Pinb-D1p*, respectively. However, the *Pinb-D1l* mutation reported by Pan et al. (2004), was not confirmed in our previous study (Xia et al., 2005). Recently, five different *Pina* alleles and six unique *Pinb* alleles were detected in *Aegilops tauschii*, all of which are associated with soft endosperm (Gedye et al., 2004; Massa et al., 2004). It is important for wheat breeding programs to identify alleles of these genes present in elite germplasm since different hard genotypes may confer differences in functional quality (Cane et al., 2004; Martin et al., 2001; Nagamine et al., 2003).

*Abbreviations* PCR, polymerase chain reaction; PINA, puroindoline a protein; *Pina*, gene coding for puroindoline a protein; PINB, puroindoline b protein; *Pinb*, gene coding for puroindoline b protein; SKCS, single kernel characterization system.

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Table 1
Nucleotide and deduced amino acid sequence changes in the currently reported puroindoline b alleles in bread wheat

| Allele | Position | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 |
| *Pinb-D1a* | TGG | CCC | ACA | AAA | TGG | TGG | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CTG | AGC | CAG |
| (Soft) | W | P | T | K | W | W | K | G | G | C | E | H | E | V | R | E | K | C | C | K | Q | L | S | Q |
| *Pinb-D1b* | TGG | CCC | ACA | AAA | TGG | TGG | AAG | AGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CTG | AGC | CAG |
| (Hard) | W | P | T | K | W | W | K | S | G | C | E | H | E | V | R | E | K | C | C | K | Q | L | S | Q |
| *Pinb-D1c* | TGG | CCC | ACA | AAA | TGG | TGG | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CCG | AGC | CAG |
| (Hard) | W | P | T | K | W | W | K | G | G | C | E | H | E | V | R | E | K | C | C | K | Q | P | S | Q |
| *Pinb-D1d* | TGG | CCC | ACA | AAA | TGG | AGG | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CTG | AGC | CAG |
| (Hard) | W | P | T | K | W | R | K | G | G | C | E | H | E | V | R | E | K | C | C | K | Q | L | S | Q |
| *Pinb-D1e* | TGA | CCC | ACA | AAA | TGG | TGG | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CTG | AGC | CAG |
| (Hard) | * | P | T | K | W | W | K | G | G | C | E | H | E | V | R | E | K | C | C | K | Q | L | S | Q |
| *Pinb-D1f* | TGG | CCC | ACA | AAA | TGG | TGA | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CTG | AGC | CAG |
| (Hard) | W | P | T | K | W | * | K | G | G | C | E | H | E | V | R | E | K | C | C | K | Q | L | S | Q |
| *Pinb-D1g* | TGG | CCC | ACA | AAA | TGG | TGG | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGA | TGC | AAG | GAG | CTG | AGC | CAG |
| (Hard) | W | P | T | K | W | W | K | G | G | C | E | H | E | V | R | E | K | * | C | K | Q | L | S | Q |
| *Pinb-D1p* | TGG | CCC | AC-A | AAT | GGT | GGA | AGG | GCG | GCT | GTG | AGC | ATG | AGG | TTC | GGG | AGA | AGT | GCT | GCA | AGG | AGC | TGA | GCC | AG |
| (Hard) | W | P | T | N | G | G | R | A | A | V | S | M | R | F | G | R | S | A | A | S | S | * | A | |
| *Pinb-D1q* | TGG | CCC | ACA | AAA | TGG | TTG | AAG | GGC | GGC | TGT | GAG | CAT | GAG | GTT | CGG | GAG | AAG | TGC | TGC | AAG | GAG | CTG | AGC | CAG |
| (Hard) | W | P | T | K | W | L | K | G | G | C | E | H | E | V | R | E | K | C | C | K | Q | L | S | Q |

Chinese wheat germplasm is characterized by a broad diversity and adaptation to different environments, and until recently, there has been little germplasm exchange between China and other countries. Both hard, medium or mixed, and soft types are found in various parts of China, since there has been little effort to select for quality parameters. Grain texture is often not matched with protein content and quality (He et al., 2004). Therefore, improvement of wheat quality, especially hardness, has become a major target for Chinese wheat breeding programs. Previously, we reported the distribution of puroindoline alleles in Chinese winter wheats; *Pinb-D1b* was found to be the most dominant type for hard texture, and *Pinb-D1p* was detected in 10 hard genotypes (Xia et al., 2005). Here we report a new type of mutation at the *Pinb* locus in the winter wheat cultivar Jingdong 11.

The bread wheat cultivar Jingdong 11, was developed from Changfeng 3/041//Jingdong 6 in the wheat breeding program at the Beijing Academy of Agricultural and Forestry Sciences. The cultivar shows outstanding dry white noodle quality and was released as noodle wheat in Beijing in 2002. Seeds of Jingdong 11 were harvested at the Anyang Experimental Station, Chinese Academy of Agricultural Sciences in 2004.

Grain hardness of 300 kernels was measured by the Perten Single Kernel Characterization System (SKCS) 4100, following the manufacturer's operational procedure (Perten Instruments North America, Inc., Springfield, IL). Average, standard deviation, and distribution of SKCS hardness data were automatically obtained from the measurements. The SKCS produces a four-class frequency distribution of hardness data for each sample, with class limits of $<33$, 34–46, 47–59, and $>60$.

Soft and hard kernels were chosen from Jingdong 11 to examine their puroindoline alleles according to their degree of vitreousness. Genomic DNA of 10 hard and 3 soft kernels from Jingdong 11 was extracted separately from pulverized kernels, following a modified method of Lagudah et al. (1991), to verify the purity of the sample and to detect the alleles. Two pairs of allele-specific primers were used for detection of *Pinb-D1b* (Gly46 to Ser46), i.e. 5′-ATGAAGGCCCTCTTCCTCA-3′ (upstream primer) and 5′-CTCATGCTCACAGCCGCT-3′ (downstream primer) for detection of the substitution of G to A at position 223 in nucleotide sequence of *Pinb* gene, and 5′-ATGAAGGCCCTCTTCCTCA-3′ (upstream primer) and 5′-CTCATGCTCACAGCCGCC-3′ (downstream primer) for the detection of the absence of nucleotide change at position 223 of *Pinb* sequence (Giroux and Morris, 1997, 1998). Amplification of the *Pinb* full-length sequence was performed using the sense-strand terminal primer 5′-ATGAAGACCTTATTCCTCCTA-3′ and the anti-sense terminal primer 5′-TCACCAGTAATAGCCACTAGG-GAA-3′ (Gautier et al., 1994). The sense-strand terminal primer 5′-ATGAAGGCCCTCTTCCTCA-3′ and the anti-sense-strand terminal primer 5′-TCACCAGTAATAGC-CAATAGTG-3′ were used to amplify the *Pina* gene (Gautier et al., 1994).

For PCR amplifications a PTC-200 Peltier Thermocycler (Gene Company) was used. PCR reaction was performed in 25 μl volumes containing 10 pmol of each primer, 250 μM of each of dNTP, 1×PCR buffer, 1.5 mM of $MgCl_2$, 0.5 unit of *Taq* DNA polymerase (Promega), and 100 ng of genomic DNA. The samples, denaturated at 94 °C, were submitted to 35 cycles of 45 s denaturation at 94 °C, 1 min annealing at 58 °C, and 1 min elongation at 72 °C, with a final extension of 5 min at 72 °C at the end. PCR products were analyzed on 1.5% (w/v) agarose gels, stained with ethidium bromide, and detected using UV light.

A 447-bp specific fragment amplified from the template DNA of 10 hard and 3 soft kernels of Jingdong 11 with full-length *Pina* and *Pinb* primers was sequenced from both strands by Augct Biotechnology Company (http://www.augct.com) and Bioasia Biotechnology Company (http://www.bioasia.cn) to check the sequences of *Pina* and *Pinb* genes, respectively. Sequence alignments were performed using DNAMAN software.

The SKCS hardness index (mean ± SD) and the frequency distribution of Jingdong 11 were 51 ± 12 and 07-22-45-26, respectively. The cultivar was classified as class 3, a medium type. The frequency distribution of the hardness data suggested that the cultivar is a mixture of soft and hard types.

PCR amplification with two pairs of *Pinb-D1b* specific primers did not show a *Pinb-D1b* mutation in the hard kernels of Jingdong 11. However, subsequent sequencing indicated a point mutation in hard kernels of Jingdong 11 involving a base substitution of G to T at the 218th nucleotide in the coding sequence of the *Pinb* gene, resulting in a tryptophan to leucine change (TGG to TTG) at position 44 in the deduced amino acid sequence of puroindoline b. Sequencing of three soft kernels showed that they all had the wild type of *Pinb* (*Pinb-D1a*), whereas the wild-type allele of *Pina* (*Pina-D1a*) was found in both the soft and the hard kernels of Jingdong 11. Comparison with all reported *Pinb* mutations (Table 1), shows that the *Pinb* gene in hard kernels from Jingdong 11 is different from any of the known puroindoline b alleles. According to the 2004 Supplement of the Wheat Gene Catalogue (McIntosh et al., 2004), the single nucleotide mutation with G to T substitution at the 218th nucleotide of *Pinb* gene should be designated as *Pinb-D1q* (Table 1).

Seeds obtained from the breeder, Dr Sun Jiazhu, at Beijing Academy of Agricultural and Forestry Sciences have further confirmed these results. It is therefore concluded, that wheat cultivar Jingdong 11 carries two types of puroindoline alleles. This is possible since no selection for grain hardness was made before it was released in 2002 (Dr. Sun Jiazhu, personal communication).

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