Analysis of *Pina* and *Pinb* alleles in the micro-core collections of Chinese wheat germplasm by Ecotilling and identification of a novel *Pinb* allele

Jing Wang[1, 2], Jiazhu Sun[1], Dongcheng Liu[1], Wenlong Yang[1], Daowen Wang[1], Yiping Tong[1, *], Aimin Zhang[1, *]

[1]The State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

[2]Graduate School of Chinese Academy of Sciences, Yuquan Road, Beijing 100039, China

ABSTRACT

Kernel hardness is mainly conditioned by allelic variations of *Pina-D1* and *Pinb-D1* genes. The Ecotilling approach was optimized to investigate *Pina* and *Pinb* alleles in the micro-core collections (MCC) of Chinese wheat germplasm. As a result, three *Pina* and eight *Pinb* alleles were found. A novel variant (designated as *Pinb-D1x*) was discovered in one of the accessions from Xinjiang winter-spring wheat region. Generally, more *Pinb* alleles were detected in the accessions coming from the regions that grow winter or a mixture of spring and winter wheats.

Keywords:Wheat, Pina and Pinb alleles, Ecotilling

INTRODUCTION

Common wheat varieties can be classified as hard or soft types based on their kernel hardness. Soft wheat varieties possess wild type (WT) forms of both Pin proteins (Bhave and Morris, 2008a). In contrast, hard wheat varieties display mutations in one or both *Pin* genes, which lead to changes in Pin protein structure and/or expression pattern (Bhave and Morris, 2008b). To date, 17 *Pina* (named 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 found in common wheat and related species (Morris and Bhave, 2007). To better use this resource for improving wheat kernel texture through molecular breeding, the natural variations of *Pina* and *Pinb* genes among the MCC are studied. According to successful applications of Ecotilling in the studies published so far (Comai et al., 2004; Gilchrist et al., 2006; Mejlhede et al., 2006), the Ecotilling approach was optimized and employed to reveal the distribution of *Pina* and *Pinb* alleles in the micro-core collections (MCC) that consisted of 262 accessions.

MATERIAL AND METHOD

The distribution of MCC in the ten wheat cultivation regions of China is illustrated in Fig. 1. In addition, there were 17 accessions originally imported from foreign countries, which had been frequently used in Chinese wheat breeding programs (Zhuang 2003).

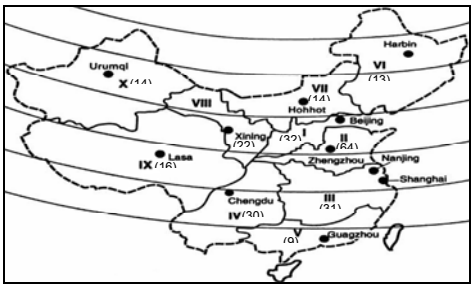

Fig.1. MCC distribution in ten wheat cultivation regions.

I,	Northern winter wheat region
II,	Yellow and Huai River Valley winter wheat region
III,	Middle and Low Yangtze Valley winter wheat region
IV,	Southwestern winter wheat region
V,	Southern winter wheat region
VI,	Northeastern spring wheat region
VII,	Northern spring wheat region
VIII,	Northwestern spring wheat region
IX,	Qing-Tibetan Plateau spring-winter wheat region
X,	Xinjiang winter-spring wheat region

Kernel hardness of 262 MCC accessions was determined by the Perten Single Kernel Characterization System (SKCS) 4100. Genomic DNA samples of 225 MCC were isolated by the DNeasy 96 Plant DNA Purification Kit (Gold Chain BioTech Centre, Beijing, China). Ecotilling was conducted following Comai et al., (2004) with little modification. Two pairs of oligonucleotide PCR primers were synthesized and fluorescently labeled commercially (LI-COR Biosciences, Lincoln, USA), one (PaF and PaR) for amplifying *Pina* genomic sequence (1239 bp) and the other (PbF and PbR) for that of *Pinb* (1421 bp) (Table 1).

Different strategies were applied in identifying *Pina* and *Pinb* alleles in the MCC accessions. For *Pina*, *Pina-D1b* (*Pina* null) was excluded by three independent amplifications. Then, DNA pools of three MCC accessions with Chinese Spring (containing WT *Pina* allele *Pina-D1a*, Giroux and Morris, 1997) were used as the templates for screening the *Pina* alleles. For *Pinb*,

two references *Pinb-D1a* (WT allele carried by Chinese Spring, Giroux and Morris, 1997) and *Pinb-D1b* (in Jinan 17, Xia et al., 2005) were used and four cleavage results were observed (table 2). Once a polymorphism had been identified, the corresponding allele from several individual seeds was directly sequenced commercially. Multiple sequence alignment was conducted using the ClustalW software. Conceptual translation of DNA sequence and prediction of protein molecular mass were performed at the ExPASy website (http://www.expasy.ch/tools/).

Table 1. PCR primers for Ecotilling

PaF	5′- caggaagcgacatgtatctcaat -3′
PaR:	5′- aatggtatcctcacggcaaactca -3′
PbF:	5′-ccaacgaaactaatgagaaataaaaaggtg-3′
PbR:	5′-aagttgttggatggacgaataaggtt- 3′

RESULTS AND DISCUSSION

The frequencies of soft, hard and mixed genotypes were 30.2%, 55.7%, and 14.1%, respectively, among the MCC accessions based on the SKCS data. Because of the heterogeneities present in the mixed genotypes, more efforts were given to the 225 accessions.

Following the optimized strategies listed above, *Pina* and *Pinb* alleles in the 225 MCC accessions were reliably determined. The distribution of *Pina* and *Pinb* alleles in the examined MCC accessions sorted by origins and cultivation regions. Several conclusions could be drawn from the data. First, three *Pina* alleles, namely *Pina-D1a*, *Pina-D1b* and *Pina-D1l*, were detected in the 225 MCC accessions, with the highest frequency found for *Pina-D1a* (83.6%). Second, eight *Pinb* alleles were detected in the 225 MCC accessions, with high frequencies found for *Pinb-D1a* (50.2%), *Pinb-D1b* (24.4%) and *Pinb-D1p* (22.7%). Third, in general, the diversity of *Pinb* alleles was higher in the accessions collected from the winter wheat regions or the ones cultivating both winter and spring wheats, compared to that found for the accessions from the spring wheat regions. The allelic diversity of *Pinb* was particularly high in the Southwestern winter wheat region. One of the accessions from this region was found to contain *Pinb-D1e*, which had previously been detected in only two spring wheat genotypes from North America (Morris et al., 2001), and two endemic wheat lines from Yunnan province, China (Chen et al., 2007a). In addition, a recently described *Pinb* allele, *Pinb-D1u* (Chen et al., 2007a), was also detected in the two accessions from this region. Interestingly, *Pinb-D1u* was also found present in one of the accessions collected from the Qing-Tibetan Plateau spring-winter wheat region, which connects the Southwestern winter wheat and the Xinjiang winter-spring wheat regions. Fourth, the allelic diversity of *Pinb* was also relatively high in the accessions from Xinjiang winter-spring wheat region, and those from the Yellow and Huai River Valley winter wheat region. Finally, the allelic diversities of *Pina* and *Pinb* appeared to be quite low in the 14 introduced foreign accessions.

One new *Pinb* allele was identified from the accession Kashibaipi that came from Xinjiang winter-spring wheat region. After nucleotide sequence comparisons with the known *Pinb* alleles (Morris and Bhave, 2007), this new variant was designated as *Pinb-D1x* (EMBL accession number AM909618). Compared to *Pinb-D1a* coding sequence, two nucleotide changes occurred in that of *Pinb-D1x*, one being a G to A substitution at the nucleotide (nt) position 257 and the other being a C to T substitution at the nt position 382. The two mutations in *Pinb-D1x* were confirmed by seven individual sequences. While the first mutation resulted in the replacement of the WT cysteine (C) residue at position 57 by tyrosine (Y), the second mutation truncated the deduced Pinb-D1x protein by 21 residues (relative to Pinb-D1a). Consequently, the predicted molecular mass of Pinb-D1x was substantially lower than that of Pinb-D1a. Further comparisons revealed that the first nucleotide substitution observed in *Pinb-D1x* was not found in any of the previously described *Pinb* alleles. However, the second nucleotide substitution occurred in *Pinb-D1x* was identical to the one found in *Pinb-D1ab*. *Pinb-D1ab* was originally detected in a Japanese wheat line KU3062 (EMBO accession AB302894). This allele was also found in the MCC accession Tuokexun 1 by this work. Kashibaipi and Tuokexun 1 were both from Xinjiang winter-spring wheat region. The SKCS hardness index values (means ± SD) of Kashibaipi (70± 15) and Tuokexun 1 (78±12) were both significantly higher than that of Chinese Spring (25±17).

Table 2. Analysis of *Pinb* allele based on the four outcomes of two separate Ecotilling experiments

	Outcome			
	1	2	3	4
Experiment I a	Cleavage	No cleavage	Cleavage	No cleavage
Experiment I I	No cleavage	Cleavage	Cleavage	No cleavage
Allele	Pinb-D1b	Pinb-D1a	Other known or new alleles	Pinb-null

ACKNOWLEDGEMENTS

We thank Professors Xueyong Zhang and Jizeng Jia (Institute of Crop Science, Chinese Academy of Agricultural Sciences) for providing the wheat micro-core collections. The authors are grateful to Drs Craig Morris (Washington State University, USA), Zhonghu He (Institute of Crop Science, Chinese Academy of Agricultural Sciences) and Feng Chen (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences) for constructive suggestions to this work and manuscript preparation. Financial support for this work is provided by the Ministry of Science and Technology of China (2006AA02Z142,

2002CB11130102) and the National Natural Science Foundation of China (30521001).

REFERENCES

Bhave M, Morris C F, 2008a. *Plant Mol Biol*, 66, 205-19.

Bhave M, Morris C F, 2008b. *Plant Mol Biol*,66, 221-31.

Chen F, He Z H, Xia X C, *et al*. 2006. *Theor Appl Genet*, 112, 400-409.

Chen F, Yu Y X, Xia X C, 2007a. *Euphytica* 156, 39-46.

Comai L, Henikoff S. 2001. *Plant Physio1*. 126: 480-484.

Gilchrist E J, Haughn G W, Ying C C, *et al*. 2006. *Mol Ecol* 15: 1367-1378.

Giroux M J, Morris C F, 1997. *Theor Appl Genet*, 95, 857-864.

Mejlhede N, Kyjovska Z, Backes G, *et al*. 2006. *Plant Breeding* 125: 461-467.

Morris C F, Lillemo M, Simeone M C, *et al*. *Crop Science* 41, 218-228.

Morris C F, Bhave M. 2007. J Cereal Sci doi:10.1007/s11103-007-9264-6.

Xia L Q, Chen F, He Z H, *et al*. 2005. Cereal Chemistry 82, 38-43.

Zhuang Q S. 200. *Chinese Agriculture Press*, Beijing, China, pp 1-681 (in Chinese)