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

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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; Mejhede 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). 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 amplifying *Pinb* genomic sequence (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 Jia 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' - cagaaagcaacctgtaaatc-3' |
| PaR | 5' - aatgtcttcacggccaancc-3' |
| PbF | 5' - ccacgnaactagtgcaggaatgtaaaaggtg-3' |
| PbR | 5' - aagttgcggagtgcaggaataggt-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

<table>
<thead>
<tr>
<th>Outcome</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment Ia</td>
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<td>Cleavage</td>
<td>No cleavage</td>
</tr>
<tr>
<td>Experiment Ib</td>
<td>No cleavage</td>
<td>Cleavage</td>
<td>No cleavage</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>Pinb-D1b</td>
<td>Pinb-D1a</td>
<td>Other known</td>
<td>Pinb-null or new alleles</td>
</tr>
</tbody>
</table>

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REFERENCES