Comparison of five puroindoline alleles on grain hardness and flour properties using near isogenic wheat lines

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Grain texture is one of the most important characteristics that affect the end-use quality of wheat (Triticum aestivum L.). Mutations in the puroindoline-a and puroindoline-b genes are associated with hard grain texture. The expression patterns of the PINA and PINB proteins differ among Pin alleles. We studied the effect of Pin alleles on grain hardness, and milling flour properties using near isogenic lines grown at two different locations. The genotype was found to significantly affect quality parameters related to grain hardness. Grain hardness, flour particle size, damaged starch content were significantly low in Pin-D1b, Pin-D1p, Pina-D1b, and Pin-D1k. Grain hardness of Pina-D1k, lacking both PINA and PINB, were the highest, followed by Pina-D1b lacking PINA. Pina-D1k and Pina-D1b showed high damaged starch contents and CO₂ production. Damaged starch is associated with water absorption of flour and CO₂ production, an important characteristic in bread-making. The alleles might be useful for improving bread-making quality. These results indicate that the grain texture of hard wheat is affected by the amount of PINs each allele.

Key Words: Triticum aestivum L., puroindoline, grain hardness, quality, near isogenic line.

Introduction

Grain texture is one of the most important characteristics that affect the end-use quality of wheat (Triticum aestivum L.). The Ha (Hardness) locus, located on the short arm of chromosome 5D, is known to control grain hardness (Mattern et al. 1973). This locus encodes a 15 kDa protein called friabilin, which consists of puroindoline-a (Pina), puroindoline-b (Pinb), and grain softness protein 1 (Gsp-1) (Greenwell and Schofield 1986, Giroux and Morris 1998). The expression of both wild-type PINA and PINB proteins is required for soft kernel texture. Mutations in the Pina and Pinb genes have been associated with hard kernel texture (Giroux and Morris 1997, Lillemo and Morris 2000, Tranquilli et al. 2002). Major Pina and Pinb alleles differ among regions (Lillemo and Morris 2000, Ram et al. 2002, Cane et al. 2004, Ikeda et al. 2005, Xia et al. 2005). The deletion of Pina (Pina-D1b) results in a harder grain texture than the point mutation in Pinb-D1b (Giroux et al. 2000). The effects of these alleles on quality properties have primarily been studied with Pina-D1b and Pinb-D1b. Pinb-D1b is considered to be more suitable for milling and bread making than Pina-D1b (Martin et al. 2001, Eagles et al. 2006, Chen et al. 2007).

Tranquilli et al. (2002) reported that simultaneous deletion of Pina and Pinb resulted in harder grain texture than the single deletion of Pina-D1. Ikeda et al. (2005) showed that the expression patterns of PINA and PINB differed among Pin alleles. However, a large difference in grain texture is often observed with the same allele (Morris et al. 2001, Chen et al. 2007, Lillemo et al. 2006). Therefore, it is necessary to compare effect of these alleles to grain texture in the same genetic background.

Thus, we developed near isogenic lines (NILs), each of which carries one of five different Pina or Pinb alleles, including both Pina and Pinb deletions (Pina-D1k). Using these lines, we studied the effects of the puroindoline alleles on grain hardness, milling, and flour properties. This study provides useful information for the breeding and processing of bread wheat.

Materials and Methods

The recurrent parent Fukuhonoka (Pina-D1a, Pinb-D1a) with soft grain texture was crossed with donor parents of puroindoline alleles: Chugoku 140 (Pina-D1a, Pinb-D1b), Kitamiharu 63 (Pina-D1a, Pinb-D1c), Shijiazhuang 34 (Pina-D1a, Pinb-D1p), Wildcat (Pina-D1b, Pinb-D1a), and

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Table 1. Characteristics of Pina and Pinb alleles used this study

<table>
<thead>
<tr>
<th>Source</th>
<th>Genotypes (G)</th>
<th>Locations (L)</th>
<th>G × L</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain hardness</td>
<td>118.3 **</td>
<td>258.1 **</td>
<td>14.38</td>
<td>6.033</td>
</tr>
<tr>
<td>Grain weight</td>
<td>1.132</td>
<td>273.0 **</td>
<td>1.857</td>
<td>1.450</td>
</tr>
<tr>
<td>Grain diameter</td>
<td>0.001</td>
<td>0.374**</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Grain protein</td>
<td>0.193</td>
<td>5.208**</td>
<td>0.092</td>
<td>0.142</td>
</tr>
<tr>
<td>Flour yield</td>
<td>4.422*</td>
<td>3.730</td>
<td>0.293</td>
<td>1.372</td>
</tr>
<tr>
<td>Flour particle size</td>
<td>255.6**</td>
<td>513.3**</td>
<td>3.102</td>
<td>11.35</td>
</tr>
<tr>
<td>Damage Starch</td>
<td>2.780**</td>
<td>0.708**</td>
<td>0.173*</td>
<td>0.054</td>
</tr>
<tr>
<td>CO₂ production</td>
<td>446.1 **</td>
<td>61.5</td>
<td>24.1</td>
<td>17.47</td>
</tr>
<tr>
<td>Peak viscosity</td>
<td>169.0</td>
<td>1101.1 **</td>
<td>233.1</td>
<td>87.80</td>
</tr>
<tr>
<td>Break down</td>
<td>283.0 *</td>
<td>4825.8 **</td>
<td>76.5</td>
<td>82.07</td>
</tr>
<tr>
<td>Setback</td>
<td>118.2</td>
<td>429.2 *</td>
<td>158.6</td>
<td>67.73</td>
</tr>
</tbody>
</table>

* and ** indicate significant differences at 5% and 1% levels, respectively.
only found for damaged starch content. Six quality parameters that showed significant differences by genotype were analyzed by a multiple range test among alleles (Table 3). Data of recurrent parent Fukuhonoka are also shown for reference in Table 3. Grain hardness of NIL Pinb-D1b was significantly the lowest among alleles examined. Effects of Pinb-D1c and Pinb-D1p on grain hardness were not significantly different from those of Pinb-D1b. Grain hardness of NIL Pina-D1k, which was expected to be the hardest allele, was in fact the highest among them, but it was not statistically significantly different from NIL Pina-D1b. For flour yield, NIL Pinb-D1c was significantly higher than that of NIL Pina-D1b, which was the lowest. For flour particle size, NIL Pinb-D1b was significantly the lowest. NILs Pinb-D1c, Pinb-D1p, and Pina-D1b were significantly higher than that of NIL Pinb-D1b, as found in grain hardness. NIL Pina-D1k showed the highest among the alleles. Grain hardness is known to correlate with flour particle size (Pomeranz et al. 1985); there was also significant correlation in this study (Table 4).

For damaged starch content, NIL Pinb-D1b was significantly lower than the other alleles. Although there was no significant difference between NIL Pinb-D1c and NIL Pinb-D1p, NIL Pina-D1b showed significantly higher damaged starch content than Pinb-D1c or Pinb-D1p. NIL Pina-D1k showed significantly the highest damaged starch content among these alleles. Damaged starch showed a significant correlation coefficient with grain hardness ($r = 0.982**$), flour particle size ($r = 0.980**$) and CO$_2$ production ($r = 0.979**$) (Table 4). CO$_2$ production of NIL Pinb-D1b was the lowest and that of NIL Pina-D1k was the highest. There was no significant difference in CO$_2$ production between NIL Pinb-D1c and NIL Pinb-D1p. The CO$_2$ production of NIL Pina-D1b was higher than that of NIL Pinb-D1p. It was not significantly different from NIL Pinb-D1c or NIL Pina-D1k. The order of CO$_2$ production was consistent with that of damaged starch content. Change in CO$_2$ production from bread dough without sugar in NILs is shown in Figure 1. Although CO$_2$ production during fermentation was not different within 60 min among the alleles, the CO$_2$ production of NIL Pinb-D1b was significantly lower after 120 min. The CO$_2$ production changed between 120 and 180 min among the alleles. Although the CO$_2$ production of NIL Pinb-D1b was less than 7.2 mL for the time tested, NIL Pina-D1k produced 24.1 mL. The CO$_2$ production per 60 min reduced after 180 min for all alleles.

The breakdown of NIL Pina-D1k was significantly lower than that of NIL Pinb-D1b. There was no significant difference among the others.

### Discussion

Because grain weight, grain diameter, and grain protein content, which vary considerably among donor parents of puroindolines, were not significantly different among the NIL genotypes, these characteristics may not be controlled by the Ha locus. Grain hardness and related characteristics, such as flour particle size, damaged starch content, and CO$_2$ production, showed significant differences by genotype. There was no significant difference in interaction between genotype and location, except damaged starch content. This suggests that the differences found in quality parameters among NILs were primarily due to differences in genotype.

Ikeda et al. (2005) reported that the amount of PIN differed among Pina and Pinb alleles; Pina-D1a/Pinb-D1b showed normal amounts of PINA and reduced amounts of PINB. Pinb-D1c and Pinb-D1p lacked PINB. Pina-D1b and Pina-D1k lacked PINA and both PINA and PINB, respectively. On the other hand, Pinb-D1b showed the least effects on the quality parameters in this study. Pinb-D1c and Pinb-D1p, both lacking PINB, were not significantly different in grain hardness or other parameters. Pinb-D1c has

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**Table 3. Results of quality parameters of near isogenic lines with puroindoline alleles**

<table>
<thead>
<tr>
<th>Puroindoline</th>
<th>Grain hardness</th>
<th>Flour yield</th>
<th>Flour particle size</th>
<th>Damaged starch</th>
<th>CO$_2$ production</th>
<th>Breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pina-D1a</td>
<td>(HI) ±SE</td>
<td>(%) ±SE</td>
<td>(µm) ±SE</td>
<td>(%) ±SE</td>
<td>(ml) ±SE</td>
<td>(RVU) ±SE</td>
</tr>
<tr>
<td>Pinb-D1b</td>
<td>6</td>
<td>61.5 ±1.43 a</td>
<td>67.8 ±0.43 ab</td>
<td>76.2 ±2.16 a</td>
<td>5.5 ±0.06 a</td>
<td>84.0 ±2.34 a</td>
</tr>
<tr>
<td>Pinb-D1c</td>
<td>6</td>
<td>67.0 ±1.27 b</td>
<td>68.4 ±0.20 a</td>
<td>83.8 ±2.37 b</td>
<td>6.2 ±0.12 b</td>
<td>96.4 ±1.66 b</td>
</tr>
<tr>
<td>Pinb-D1p</td>
<td>6</td>
<td>67.0 ±0.93 b</td>
<td>67.8 ±0.26 ab</td>
<td>84.1 ±2.20 b</td>
<td>6.1 ±0.08 b</td>
<td>92.6 ±1.79 b</td>
</tr>
<tr>
<td>Pinb-D1a</td>
<td>6</td>
<td>70.5 ±1.46 bc</td>
<td>66.2 ±0.79 b</td>
<td>85.4 ±2.60 b</td>
<td>6.6 ±0.11 c</td>
<td>101.2 ±1.96 ed</td>
</tr>
<tr>
<td>Pina-D1k</td>
<td>6</td>
<td>73.3 ±2.81 c</td>
<td>66.9 ±0.39 ab</td>
<td>94.5 ±1.81 c</td>
<td>7.3 ±0.21 d</td>
<td>106.7 ±1.27 d</td>
</tr>
</tbody>
</table>

Values showed mean ± standard error. Values followed by the same letters in the same column are not significantly different (P < 0.05) by Ryan-Einot-Gabriel-Welsch multiple range test. Bottom row shows the data of recurrent parent Fukuhonoka for reference.

**Table 4. Correlation coefficients among quality parameter in relation to grain texture based on alleles (n = 5)**

<table>
<thead>
<tr>
<th>Quality parameter</th>
<th>Flour particle size</th>
<th>Damage starch</th>
<th>CO$_2$ production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain hardness</td>
<td>0.957**</td>
<td>0.982**</td>
<td>0.988**</td>
</tr>
<tr>
<td>Flour particle size</td>
<td>0.980**</td>
<td>0.944**</td>
<td>0.979**</td>
</tr>
</tbody>
</table>

** shows significantly different at 1% level.
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been reported to have a harder grain texture than Pinb-D1b (Limmelo and Morris 2000) among European cultivars. Ma et al. (2009) also observed that Pinb-D1c had harder grain texture than Pinb-D1b, using NILs. We found that NILs having Pinb-D1c or Pinb-D1p had higher grain hardness than those having Pinb-D1b, and, moreover, the former had larger flour particle size, higher damaged starch content, and more CO2 production than the latter. It was suggested that both Pinb-D1c and Pinb-D1p were associated with harder kernel texture than Pinb-D1b. Although Pinb-D1c and Pinb-D1p have different mutations, both alleles result in lack of PINB. This suggests that these alleles had the same effect on grain texture.

Pina-D1b had a harder texture than Pinb-D1b as described in previous reports (Groux et al. 2000, Martin et al. 2001). Ma et al. (2009) also reported that grain hardness of a NIL having Pina-D1b was significantly harder than one having Pinb-D1c. However, in this study, the grain hardness of NIL Pina-D1b was not significantly different from that of NIL Pinb-D1c. The difference might not be due to materials or cultivation, but the different multiple range test used. The damaged starch content of NIL Pina-D1b was higher than that of NILs Pinb-D1c and Pinb-D1p. Grain hardness was affected by strength of adhesion between starch granules and the gluten matrix in endosperms (Anjum and Walker 1991). Damaged starch was caused by physical damage during roller milling, due to strong adhesion. The hard texture of the NIL Pina-D1b might result in higher damaged starch content. Because the quantity of PINA in wheat endosperm was more than that of PINB (Ikeda et al. 2005), the amount of PINs in both Pinb-D1c and Pinb-D1p, lacking PINB were larger than that of Pina-D1b, lacking PINA. The difference in the total amount of PINs might explain the difference in the quality properties between the alleles.

Martin et al. (2001) also reported that flour yield of cultivars having Pina-D1b was lower than those of Pinb-D1b. However, no significant difference on flour yield was seen among the NILs, except for Pinb-D1c, in this study. It is necessary to confirm on the milling property using a Bühler test mill. Because the Bühler test mill can analyze more details than the Brabender Jr. test mill on milling and flour properties.

Tranquilli et al. (2002) reported that the grains of lines with double-null deletions of Pin-a and Pin-b were harder than those with a single Pin-a deletion. Although there was no significant difference in grain hardness between lines having Pina-D1k and Pina-D1b, the former was the highest grain hardness among the NILs tested here. NIL Pina-D1k had significantly larger flour particles and higher damaged starch content than NIL Pina-D1b. It was considered that Pina-D1k was ranked harder than Pina-D1b, based on hardness related characteristics. The results of our study suggest that the grain texture in hard wheat was affected by the amount of PINs in the endosperm, although the alleles lose binding property to the starch surface against the wild-type. The five alleles can be assigned in order of hardness as follows: Pina-D1k, Pina-D1b, Pinb-D1c = Pinb-D1p, Pinb-D1b.

It corresponded to a final proof though the CO2 production from bread dough showed large differences among NILs from 120 to 180 min. The final proof is an important stage for loaf volume in the baking process. Damaged starch content influences CO2 production: a significant correlation was observed among damaged starch content, CO2 production, and alleles in this study. Moreover, damaged starch absorbs several times more water than intact starch. Water absorption of dough is an important characteristic in baking (Greer 1959). Cane et al. (2004) reported that water absorption of Pina-D1b was higher than that of Pinb-D1b. The difference in damaged starch content was 1.1% between NIL Pina-D1b and NIL Pinb-D1b, and 1.8% between NIL Pina-D1k and NIL Pinb-D1b. Pina-D1k and Pinb-D1b have higher water absorption capacity than Pinb-D1b. The effects of Pina-D1k and Pinb-D1b on flour properties are useful in bread-making unless they increase the stickiness of dough due to their high water absorption. The quality of bread crumbs is also affected by puroindoline (Dubreil et al. 2002). The puroindoline alleles affect the water absorption capacity of dough, fermentation, and the quality of bread crumbs. Further study is necessary to clarify the relationship between the alleles and bread- or noodle-making.

Literature Cited


