Comparison of the effects of puroindoline genotypes on grain and flour properties using near isogenic lines

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ABSTRACT

We studied the effect of puroindoline alleles on grain hardness, milling and flour properties using near isogenic lines (NILs) of five kinds of *Pina* or *Pinb* genes; *Pina-D1b*, *Pinb-D1b*, *Pinb-D1c*, *Pinb-D1p* and *Pina-D1k/Pinb-D1q* (double null). The different effects were observed on the grain hardness, flour particle size and damaged starch content among them. The effect of puroindoline genes to those quality traits was estimated as follows; *Pina-D1k/Pinb-D1q >= Pina-D1b>Pinb-D1c= Pinb-D1p>Pinb-D1b*.

INTRODUCTION

Grain texture is one of the most important characteristics for end-use quality of wheat. The *Ha* (Hardness) locus located on the short arm of chromosome 5D is known to control grain hardness. This locus encodes 15 kDa protein called friabilin which consists of puroindoline a (*Pina*), puroindoline b (*Pinb*) and grain softness protein 1 (*Gsp-1*) genes. Expression of both *Pina* and *Pinb* gene is required for soft grain texture. The mutations of *Pina* or *Pinb* genes were associated with hard texture. Some mutations of *Pina* and *Pinb* have been reported varying grain hardness among hard wheat cultivars[1, 2, 3, 4]. Although *Pinb-D1b* is considered to be suitable for milling and bread making compared with *Pina-D1b* [5], there is no report of the effects of *Pin* genotypes on end-use quality in the same genetic background. We have developed near isogenic lines (NILs), each of which carries one of five different *Pina* or *Pinb* genes. Using these lines, we studied the effect of puroindoline alleles on grain hardness, milling and flour properties.

MATERIALS AND METHODS

Fukuhonoka, a soft wheat cultivar with good white salted noodle properties, was crossed with five donor parents having different kinds of *Pin* genes as follows; Wildcat (*Pina-D1b*), Chugoku 140 (*Pinb-D1b)*, Kitamiharu 63 (*Pinb-D1c)*, Shijiazhuang 34 (*Pinb-D1p*) and Bindokku (*Pina-D1k/Pinb-D1q* (double null)) (Table 1). The recurrent parent was backcrossed to the BC$_n$F$_1$ plants having the *Pina-D1k/Pinb-D1q*, *Pina-D1b*, *Pinb-D1b*, *Pinb-D1c* or *Pinb-D1p* genes. The presence of *Pinb-D1b*, *Pinb-D1c*, or *Pinb-D1p* in the BC$_n$F$_1$ seeds was checked by *Pinb* mutant-specific PCR-markers and DNA sequencing. As for *Pina* null mutants of *Pina-D1k/Pinb-D1q* and *Pina-D1b*, the grain hardness of BCnF$_2$ bulk seeds was measured by an SKCS 4100 (Perten Co. Ltd., USA.) to confirm the BC$_{n1}$F$_1$ plant having heterozygote, and the homozygote of *Pina-D1k/Pinb-D1q* or *Pina-D1b* in the BCnF$_2$ seeds were also checked by a *Pina*-specific PCR-marker. The PCR-markers were described by Ikeda *et al*.[3]. Six successive backcrosses were made with the recurrent parents and then five kinds of NILs were developed. The recurrent parent Fukuhonoka and the NILs were sown in triplicate at the National Agricultural Research Center for Western Region, Fukuyama, in November 2006 and harvested in June 2007. The donor parents were grown without replicate. The grain characteristics (weight, diameter and hardness) were measured with an SKCS 4100. The grain protein content and grain moisture were determined using an Infratec 1275 analyzer (Foss Tecator, Denmark). Grain samples to which water was added so as to make their content 16% moisture for hard grain and 14.5% moisture for soft grain were milled on a Brabender Jr. test mill (Brabender Inc., Germany). Flour samples were used to measure flour particle size, SDS-sedimentation volume and damaged starch content. Flour particle size expressed as median of flour particle size distribution was measured by a laser-diffraction Heros and Rodos particle-size analyser (Sympatec GmbH, Germany). A SDS-sedimentation test was performed as described by Takata *et al*.[6]. Damaged starch was measured by a starch damage assay kit (Megazyme Ltd., Ireland). The analyses of particle size, SDS-sedimentation volume and damaged starch content were repeated twice for each sample.

Table1. The puroindline genotypes and the grain propertiesof the donor parents

Varieties	Puroindoline genotype		Grain weight (mg)	Grain diameter (mm)	Grain hardness (HI)	Grain protein content (%)
	Pina	*Pinb*				
Bindokku	*Pina-D1k*	*Pinb-D1q*	33.7	2.60	83	10.9
Wildcat	*Pina-D1b*	*Pinb-D1a*	42.1	2.77	79	12.8
Chugoku 140	*Pina-D1a*	*Pinb-D1b*	40.6	2.73	54	11.1
Kitamiharu 63	*Pina-D1a*	*Pinb-D1c*	39.0	2.69	80	13.8
Shijiazhuang 34	*Pina-D1a*	*Pinb-D1p*	49.9	3.01	58	11.8

RESULTS

Grain characteristics and protein content were different values among the donor parents (Table 1).The grain hardness of Bindokku (*Pina-D1k/Pinb-D1q*) was the highest among them and that of Chugoku 140 (*Pinb-D1b*) was the lowest. Wildcat (*Pina-D1b*) and Bindokku (*Pina-D1k/Pinb-D1q*) were the highest for the flour particle size, and the content of damaged starch of Wildcat (*Pina-D1b*) was the highest. Those of Chugoku 140 were the lowest (Table 2).

Table2. The flour characteristics of the donor parents

Varieties	Flour yield (%)	Flour particle size (µm)	Damaged starch content (%)	SDS-sedimentation volume 0hr (ml)	SDS-sedimentation volume 24hr (ml)
Bindokku	65.9	98	6.8	48.3	47.7
Wildcat	65.6	98	7.2	43.5	49.9
Chugoku 140	63.9	66	4.4	38.0	23.8
Kitamiharu 63	64.5	86	6.8	70.0	79.4
Shijiazhuang 34	64.1	81	6.3	44.4	36.2

The grain characteristics of the recurrent parent Fukuhonoka and the NILs are presented in Table 3. There were no significant differences in the grain weight and the grain protein content between the NILs and Fukuhonoka (*Pina-D1a*, *Pin-D1a*). The grain diameter of NIL with *Pina-D1k/Pinb-D1q* was significantly higher than that of Fukuhonoka. There was no significant difference in it among the others. The grain hardness of Fukuhonoka was definitely lowest value (29 HI). That of *Pinb-D1b* was significantly the lowest among the hard grain genotypes. The flour characteristics of Fukuhonoka and the NILs are presented in Table 4. The flour yield of Fukuhonoka was the lowest. There was no significant difference in the flour yield among the NILs. For the median of flour particle size, Fukhonoka was 43µm that was the lowest among them. The NIL with *Pina-D1k/Pinb-D1q* was significantly higher than the other NILs. There was no significant difference among *Pina-D1b*, *Pinb-D1c* and *Pinb-D1p*. The particle size of NIL with *Pinb-D1b* was significantly the lowest among the NILs. The correlation coefficient between the grain hardness and the flour particle size among the NILs was r=0.71** (Fig. 1.). For the damaged starch content of Fukuhonoka was the lowest. There were significant differences among the NILs. The correlation coefficient between the damaged starch and the grain hardness was significantly high (r=0.95**, n=18). The correlation coefficient between the damaged starch and the flour particle size was also significantly high (r=0.96**, n=18) (Fig. 2.). Except for soft grain genotype Fukuhonoka, those correlation coefficients were also significant r=0.77** (n=15) and r=0.86** (n=15), respectively. For the SDS-sedimentation volume, there was no significant difference at standard time. However, the volume at 24 hours of Fukuhonoka was significantly lower than the that of NILs with *Pina-D1k/Pinb-D1q, Pina-D1b* or *Pinb-D1c*. Although the volumes of the NILs with *Pinb-D1b* or *Pinb-D1p* were not significantly different from Fukuhonoka, the volume of former were 7ml or much higher than a latter value.

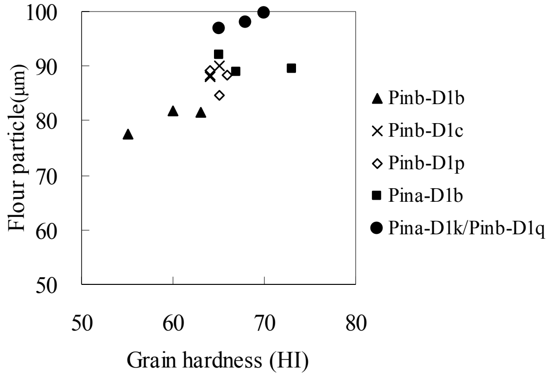

Fig. 1. Relationship between the grain hardness and the flour particle size in the NILs.

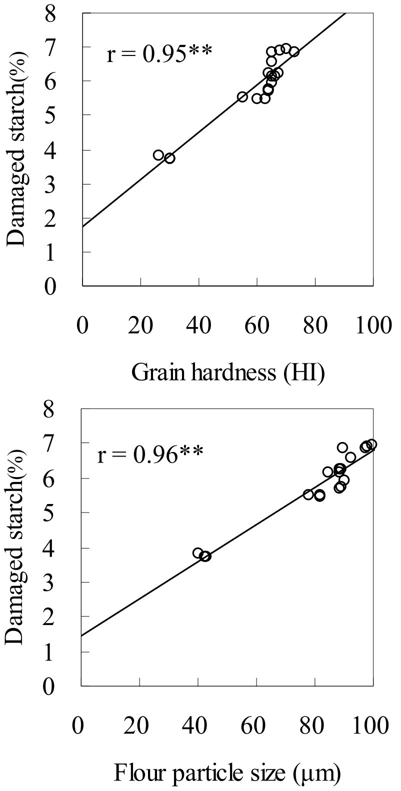

Fig. 2. Relationships between the damaged starch content and the grain hardness or the flour particle size.

**** Significant at 0.1% level.**

DISCUSSION

The flour yield of Fukuhonoka was significantly lower than the NILs except for *Pina-D1b*. Flour particle size considerably affects to flour yield of the test mill because a Brabender Jr. test mill separates flour with only one roll screen.

Table 3. Grain characteristics of Fukuhonoka and the near isogenic lines in relation to puroindline genes

NILs and recurrent parent	Grain weight (mg)		Grain diameter (mm)		Grain hardness (HI)		Grain protein content (%)	
Pina-D1k/Pinb-D1q	43.2	a	2.85	a	68	a	10.1	a
Pina-D1b	42.2	a	2.80	ab	68	a	10.1	a
Pinb-D1b	41.8	a	2.80	ab	59	b	10.5	a
Pinb-D1c	42.5	a	2.82	ab	64	a	10.2	a
Pinb-D1p	41.2	a	2.81	ab	65	a	10.2	a
Fukuhonoka	41.2	a	2.72	b	29	c	10.4	a

Values followed by the same letters in the same column are not significantly different ($P<0.05$) by Tukey-Kramer test.

Table 4.Flour characteristics of Fukuhonoka and the near isogenic lines in relation to puroindline genes

NILs and recurrent parent	Flour yield (%)		Flour particle size (μm)		Damaged starch content (%)		SDS-sedimentation volume			
							0hr (ml)		24hr (ml)	
Pina-D1k/Pinb-D1q	67.4	a	98	a	6.9	a	31.9	a	29.4	a
Pina-D1b	66.8	ab	90	b	6.6	ab	33.3	a	29.0	a
Pinb-D1b	67.9	a	80	c	5.5	d	37.1	a	30.5	ab
Pinb-D1c	68.6	a	89	b	6.0	cd	35.2	a	30.6	a
Pinb-D1p	68.1	a	87	b	6.1	bc	34.2	a	28.5	ab
Fukuhonoka	65.0	b	43	d	3.8	e	31.0	a	21.4	b

Values followed by the same letters in the same column are not significantly different ($P<0.05$) by Tukey-Kramer test.

The grain hardness of NIL with *Pinb-D1b* was significantly lower than that of the other hard genotypes. On the other hand, NILs without PIN-a protein, *Pina-D1k/Pinb-D1q* and *Pin-a-D1b*, showed the highest grain hardness among NILs. Ikeda *et al.* (2005) reported that *Pinb-D1b* expressed PIN-b protein while *Pinb-D1c* and *Pinb-D1p* did not express PIN-b protein, and *Pina-D1b* did not express PIN-a protein. Furthermore, *Pina-D1k/Pinb-D1q* did not express both PIN-a and PIN-b proteins. It suggested that the expression of PIN-a and PIN-b proteins is associated with the grain hardness.

The flour particle size of *Pinb-D1b* was significantly lower than that of the other NILs as well as the grain hardness. The flour particle size was significantly different between *Pina-D1k/Pinb-D1q*, and *Pina-D1b*, *Pinb-D1c*, *Pinb-D1p*, although the grain hardness was not significantly different among them. *Pina-D1k/Pinb-D1* is absent both PIN-a and PIN-b proteins while *Pinb-D1c*, *Pinb-D1p* and *Pina-D1b* are absent either of PIN. It assumed that PIN-a and PIN-b proteins was a close relation to the flour particle size compared with the grain hardness.

The damaged starch content was also significantly different among the NILs. The damaged starch content was highly correlated with both the grain hardness and the flour particle size. It is associated with water absorption and fermentation of dough on bread-making. We should consider which genotypes are suitable for bread and noodle-making as reported by Martin *et al.*[5].

The SDS-sedimentation volume at 24 hours of the NILs was higher than that of Fukuhonoka. Since they had the same glutenin subunit composition and protein content, gluten protein did not affect to it. It seemed that the flour particle size, namely hard or soft, affected to the volume, although the volume at standard time was not significantly different between hard and soft genotype.

There are significant differences among the hard grain genotypes on grain hardness, flour particle size and damaged starch in this study. We are preparing the further study using a Bühler test mill.

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