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Effect of the puroindoline locus and environment on Chinese fresh noodle texture

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Abstract. Grain produced from doubled-haploid (DH) wheat lines, developed from a hard- and a soft-grained wheat cultivar, were bulked according to *Pinb* (puroindoline b) genotypes for an assessment of Chinese fresh noodle texture by a trained taste panel. Each DH line was designated as 'soft' or 'hard' grained, based on a PCR amplification of the wildtype, soft allele, or the mutant, hard allele. Theoretically, the soft and hard grain bulks represented respective *Pinb* alleles and an independent assortment of unlinked alleles from the parents, Sunco and Chuanyu 12. Grains from the parents and DH lines were grown at 2 locations in Queensland, Australia, and one in Sichuan, China. The grains were milled and processed for a taste panel evaluation in Chengdu, Sichuan. Results suggest the *Pinb* alleles had a significant effect on noodle softness and explained 30% of the variation; the 'soft' *Pinb* allele conferred a softer noodle texture. Location had a significant effect on noodle smoothness; wheat grain grown at Biloela, Queensland, produced a smoother noodle texture than grain grown in Sichuan. The effect of location confirms the importance of environment as a variable for this quality character. This investigation exemplifies the utility of *Pinb* markers for specifically altering Chinese Fresh Noodle texture.

Additional keywords: Triticum aestivum, grain hardness, bulked segregant analysis, taste panel, *Pinb*, noodle softness.

Introduction

Wheat grown in Sichuan province, south-west China, typically yields the second largest crop, after rice. Sichuan-grown wheat grain is primarily used for the production of Chinese fresh noodles and steamed breads. The quality of these products varies and is frequently poor due to the use of wheat cultivars with poor end product quality attributes. Crop improvement programs have historically emphasised higher yields until recent consumer demands and international competition have partly shifted the emphasis in China. Wheat breeders are now placing more emphasis on selection criteria for higher quality noodles and steamed breads (He 2001; Y. Zou, unpublished data).

Cultivars developed in Australia, Canada, and the US are commonly considered to have superior quality characteristics and are used to improve Chinese wheat (He 1999; Liu *et al*. 2003). Australian cultivars have been classified according to specific bread and noodle requirements. For example, Australian Standard White (ASW) from Western Australia is preferred for Udon Japanese white salted noodles (Morris and Rose 1996). The quality parameters for Udon noodles have been relatively well documented in the literature

Abbreviations used: BSA, bulked segregant analysis; DH, doubled haploid; PCR, polymerase chain reaction; *Pinb*, Puroindoline b.

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(Oda *et al*. 1980; Toyokawa *et al*. 1989*a*, 1989*b*; Yamamori *et al.* 1992; Konik *et al*. 1993; Yun *et al*. 1997). These parameters are based on starch and protein composition (including storage proteins, granule bound starch synthase, puroindolines, polyphenol oxidases, and total protein) of the grain and are measured using biochemical or instrumental analyses (Williams *et al*. 1970; Oda *et al*. 1980; Crosbie 1991; Zhao and Sharp 1994; Bettge *et al*. 1995; Turnbull and Rahman 2002).

Genetic control of important starch and protein compositions has been determined with classical and molecular methods (Symes 1965; Chao *et al*.1989). Recent investigations have relied on the tools of molecular biology for identification of genes controlling quality traits (Sourdille *et al*. 1996; Zhao *et al*. 1998; Campbell *et al*. 1999; Batey *et al*. 2001). With this information, breeders may specifically and expeditiously select genes and alter the genetics of wheat (Briney *et al*. 1998; McLauchlan *et al*. 2001; Nagamine *et al*. 2003).

Grain hardness is an important quality variable. Its effects on bread and noodle flour characteristics have been elucidated by several investigations (Pomeranz and Williams 1990; Konik *et al*. 1993; Morris and Rose 1996; Liu *et al*. 2003; Nagamine *et al*. 2003). Grain hardness is determined by a starch surface protein, friabilin, encoded by a gene on chromosome 5D (Greenwell and Schofield 1986). Friabilin is composed of 2 proteins, puroindolines a and b (reviewed by Morris 2002). Wildtype puroindolines confer the development of soft grains, while mutations in either puroindoline a or b have been associated with hard grains (Giroux and Morris 1998). Nagamine *et al*. (2003) suggest that *Pinb* (the Puroindoline b gene) has a significant effect on flour colour and starch viscosity—important indicators of Japanese noodle quality.

The type of wheat grown in Chinese regions reflects agronomic factors (He 1997) and end product quality preferences (Liu *et al*. 2003). The southern growing region of Sichuan province in China grows mostly soft and medium-hard spring wheat (He 2001). The first objective of this study was to determine if the *Pinb* gene (and grain hardness) has an effect on noodle texture, as assessed by a taste panel. Due to the limitations on the number of samples panelists can accurately evaluate, genotypic bulks based on *Pinb* alleles were used to address this objective. Commonly, this method of bulked segregate analysis (BSA) has been used successfully in the reverse direction, to identify genetic markers associated with qualitative trait loci, based on phenotypic bulks (Michelmore *et al*. 1991).

A second objective was to determine whether the growing environment has an effect on noodle texture. Several investigations have studied effects of the environment on quality characteristics: dough rheology, baking quality, protein composition, and content (Lukow and McVetty 1991; Peterson *et al*. 1992; Graybosch *et al*. 1996; Grausgruber *et al*. 2000; Mikhaylenko *et al*. 2000; Panozzo and Eagles 2000). In this study, wheat was grown at 2 locations in Queensland, Australia, and 1 location in Sichuan, China, and tested for noodle texture characteristics by a taste panel in Sichuan.

Materials and methods

Sunco/Chuanyu 12 DH lines

One hundred and twenty doubled-haploid lines were derived from Sunco (*Wx-B1* = wildtype; glutenin profile = a, b, a, b, b, b), an Australian hard white spring wheat with very good yellow alkaline noodle characteristics (Mares and Campbell 2001), and Chuanyu 12 (*Wx-B1* = wildtype; glutenin profile = a, b, d, d,?(ambiguous), a), a Chinese soft white spring wheat with good Asian noodle characteristics.

Field design

Sunco, Chuanyu 12, and 120 DH lines were planted in an alpha lattice design, replicated twice at each of 2 sites in Queensland (Roma and Biloela), and 1 site in Sichuan (Wenjiang).

Particle size index

The softness of grains produced by each line was quantified using the particle size index (PSI) according to approved method 55-30 (AACC 2000).

Molecular analysis

DNA was extracted using a commercial genomic DNA extraction kit (Qiagen). Polymerase chain reaction (PCR) primer sequences *PinB-D1*-forward, ATGAAGACCTTATTCCTCCTA (Gautier *et al*.1994), and *PinB-D1*-reverse, CTCATGCTCACAGCCGCC (described as the reverse complement in Giroux and Morris 1997), were used to amplify a *Pinb-D1a* wildtype (soft) or a *Pinb-D1b* mutant (hard) allele. The puroindoline-b sequences differ at amino acid 101, where the wildtype encodes a glycine and the mutant a serine. The PCR solution contained 1.5 units of Taq (Bioline Inc.), 1× NH_2 reaction buffer, 4 mM $MgCl_2$, 0.2 mM dNTP, 0.4 μM primer, and 20 ng genomic DNA. The *PinB-D1* forward and reverse primers were subjected to a touchdown thermocycling program: 1 cycle of 94°C, 3 min; 10 cycles of 94°C, 30 s; 65°C (−1°C for subsequent cycles), 1 min; 72°C, 1 min; 25 cycles of 94°C, 30 s; 55°C, 30 s; 72°C, 30 s; 1 cycle of 72°C, 7 min.

Bulking of grain for taste panel evaluation

Reliance on a taste panel is constrained by the number of samples panelists can accurately evaluate. In this study it was possible to evaluate only 8 samples per day, including a control. Hence, lines were consolidated into informative bulks based on puroindoline alleles. Twenty g of each DH line from each replication of each site was bulked according to *Pinb* marker genotypes; lines showing the wildtype, Chuanyu 12 genotype were designated ‘soft’ and lines showing the mutant, Sunco genotype were designated ‘hard’. The bulk method was based on a principle described by Michelmore *et al*. (1991); homozygous lines were combined into a bulk according to a common characteristic. In this investigation, the characteristic was a common genotype rather than a common phenotype. The Queensland-grown bulked grains were shipped to Sichuan for conditioning and milling.

Falling number and grain protein analysis

Hard and soft grain samples were conditioned to 15.0 and 13.5%, respectively, using AACC method 26-95 (AACC 2000). After the conditioning treatment, samples (20 g of each line within each bulk comprised a total of 980 g of the soft, wildtype genotypes and 880 g of the hard, mutant genotypes) were milled in a Quadrumat Junior mill (Duisburg, Germany; AACC method 26-21A) and stored at RT for

1 week prior to use. The quality of the flour was assessed for sprouting damage and/or α-amylase activity using the Falling Number apparatus (Perten Instruments AB, Huddinge, Sweden) according to ICC method 107/1 (ICC 1997). Grain protein content was measured with a near infrared (NIR) analyser (1241 Grain Analyzer, FOSS Inc., Denmark; AACC method 39-10A). Samples for the falling number and protein were replicated 2 times.

Noodle preparation

The noodle sheet was prepared according to the method described by Konik *et al*. (1993), with some modification. Flour was mixed with water (32% by weight of flour) and NaCl (2% by weight) with a Kenwood (UK) tabletop mixer using a flat mixing paddle. Flour moisture was balanced for 30 s using a slow mixing speed (speed 1) before water was added and mixed into the flour for 30 s at the same slow speed; subsequently the flour and water blend (dough crumb) was mixed at the high speed (speed 3) for 2 min, followed by the slow speed for a final 2 min. The dough crumb was hand-kneaded (aggregated) for 1 min and pressed 4 times into a 4-mm noodle sheet using a YM12.5 Noodle machine (Rongji, China). The noodle sheet was placed in a sealed plastic bag and set aside for 30 min at room temperature before it was further pressed to a series of thinner sheets: 3, 2, and 1 mm. The final 1-mm-thick sheet was cut into noodle strips 2 mm wide and 20 cm long, placed in a plastic bag, sealed, and set aside for 30 min. Each noodle sample was weighted to 120 g, placed in Rui Yuan (China) wire baskets, boiled in distilled water for 6.5 min, immediately immersed in distilled ice water for 2 min., distributed to labelled bowls, and covered with distilled water (RT) for the panelist evaluation 15 min after the cooking procedure.

Taste panel training

Fifteen individuals who worked at the Crop Research Institute in Chengdu were trained for descriptive testing of noodle textural characteristics during a 5-day period. Trainees learned to discern noodle smoothness, softness, and elasticity, evaluating cooked noodles comprising flours varying for added components—gluten, malt flour, and tapioca starch—to adjust elasticity, hardness, and smoothness textural characteristics. Trainees initially discerned larger textural contrasts followed by gradually reduced contrasts. Trainees who could discern the more subtle differences were chosen for sample evaluation.

Taste panel design and evaluation

Six panelists evaluated noodle samples during a 3-day period; each day was used to evaluate a trial site and was separated into 2 sessions, 1 replication per session. Each panelist had a designated 'control' for each evaluation. (The control noodles were made from the same batch of flour, a blend of Sichuan-grown Sunco and Tasman grain. The control was used for comparing unknown, test samples on a relative scale.) The 4 samples, Sunco, Chuanyu 12, 'hard' bulk, and 'soft' bulk, were randomised amongst the 6 panelists; each panelist tested 3 samples, not including the control. Scores were indicated on a linear scale of 15 cm. The control was allotted a residence at the 12th cm along the scale, and the unknown samples were placed relative to the control and other unknowns. Samples allotted higher positions than the control were deemed to have more softness, smoothness, or elasticity (higher scores imply increased 'preference', although this is a subjective description), depending on the measured trait. All scores were divided by the control score to indicate the relationship between sample and control.

Statistical analysis

Panelist scores were standardised (sample scores were divided by control scores) for each genotype and textural characteristic; standardised scores may be easily interpreted for relative differences between samples. Scores were statistically compared using GLM-ANOVA (SPSS 12.1, SPSS Inc., Chicago, IL) multiple comparison function; dependent variables were textural categories, 'softness', 'smoothness', and 'elasticity'; independent variables were 'genotype', 'environment', and 'panelist'. An estimate of the variation explained by *Pinb* was derived from the proportion of sum-squared variation attributable to the bulked lines, relative to the variation attributable to the parents, for a textural characteristic.

Results

PSI and Pinb analysis

Pinb primers were used to amplify DNA from Sunco, Chuanyu 12, and the DH lines. Forty-four DH samples amplified a hard-grain genotype and 49 amplified a soft-grain genotype. Twenty-seven DH samples produced ambiguous amplifications and were not included in the analysis. The hard-grain DH genotypes had significantly lower PSI scores than the soft-grain DH genotypes (Fig. 1). These results confirm the effect of puroindoline gene on grain softness, although overlapping distributions suggest other factors affect softness in this population. Morris *et al*. (2001) conclude the *Hardness* locus on chromosome 5D confers most of the variation between hardness classes. For this investigation, DH lines were bulked according to *Pinb* genotypes and consequent hardness classes for the noodle analysis.

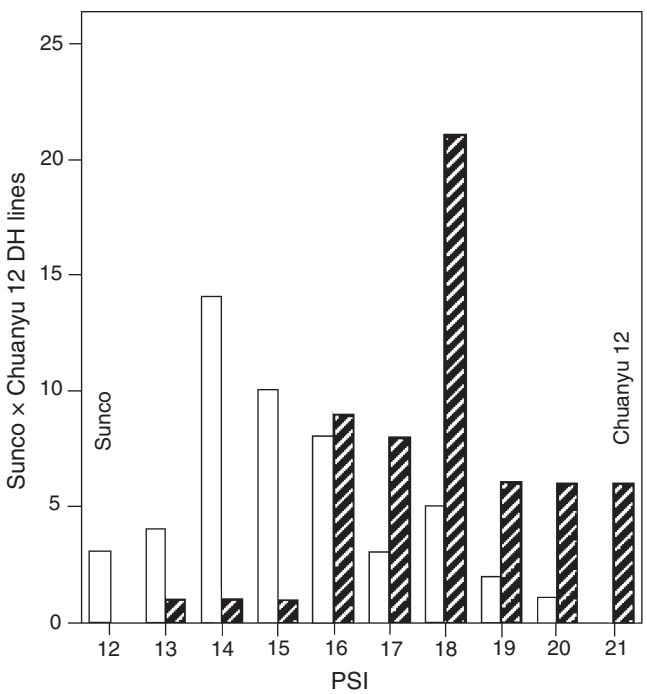

Fig. 1. Distribution of Particle Size Index (PSI) scores measured from grain of the Sunco × Chuanyu 12 DH lines designated 'hard' or 'soft', according to the *Pinb* genotypes. A statistical comparison indicates the soft lines produced a significantly higher particle size than the hard lines ($P < 0.001$). Open bars represent 'hard' genotypes, and diagonal-striped bars represent 'soft' genotypes.

Taste panel textural analysis

Fresh Asian noodles made from soft-grained genotypes (bulked and parental) had significantly higher scores for noodle 'softness' than noodles made from hard-grained genotypes (Table 1). Noodles made from the soft-grained bulk had a mean score 10% higher than noodles made from the hard-grained bulk. These results suggest the *Pinb* locus affects noodle softness and explains 30% of the variation (SS bulked samples/SS parents = 0.098/0.322) between Sunco and Chuanyu 12 for noodle softness. These results corroborate Konik *et al*. (1993), who found the grain hardness variable improved their model for predicting quality parameters, including noodle softness scores.

For noodle 'smoothness' and 'elasticity', noodles made from soft-grained or hard-grained, bulked genotypes had the same scores, statistically (Table 1). Smoothness scores between the parents, Chuanyu 12 and Sunco, were significantly different, implying genetic differences unrelated to *Pinb*; differences were not detected for noodle elasticity. These results suggest the *Pinb* locus had no effect on noodle smoothness or elasticity.

Sunco × Chuanyu 12 DH lines were grown at 3 locations, 2 in Queensland and 1 in Sichuan. Parental and bulked grain samples from each location were milled and processed for flour and noodles in Sichuan. Taste panel scores for samples derived from each location were analysed for environmental effects. Compared to Biloela samples, Wenjiang samples had significantly lower scores (by about 4%) for noodle smoothness (Table 2). These 2 sites did not significantly differ in their scores for either elasticity or softness. Scores for samples from the Roma site did not differ significantly from those from the other 2 sites for any textural characteristic. Genotype × location interaction was not detected for any noodle texture character between the three sites.

Discussion

An explanation for differential effects of grain hardness on noodle softness may be related to differences in flour protein content, despite there being no differences between bulks for grain protein content (Table 3). Nagamine *et al*. (2003) indicated that flour yield and protein content were significantly lower in flour derived from soft *v.* hard grains. Hogg *et al*. (2005) transformed a hard-grained cultivar with puroindoline constructs and compared milling and end-use characteristics. Their results suggest puroindoline content was correlated with decreased flour yield and decreased protein and ash content. These investigations suggest that textural differences detected in this study may partly result from protein differences caused by the relative inefficiency of flour extraction from hard and, in particular, soft grains using small-scale experimental mills. The significance of this effect in practice is likely to be minimal since there is substantial anecdotal evidence that commercial mills achieve similar flour yields and protein recovery for both hard and soft grains.

The effect of the environment on noodle texture may be due to alterations of grain chemical composition. Grain protein contents differed between some of the sites, but these differences did not correspond with site-related textural differences (Table 4). Igrejas *et al*. (2001) suggest wheat grown at 4 locations in France produced different total flour protein contents but the same puroindoline a and b contents between locations. Panozzo and Eagles (2000) suggest flour protein content and, more specifically, the proportion of glutenin and gliadin proteins varied significantly between 15 environments in Victoria, Australia.

Table 1. Mean scores (± s.d.) and statistical comparisons of hard and soft genotypes for noodle texture

Textural scores of hard and soft wheat genotypes grown at 3 locations were determined by taste panelists and statistically analysed using GLM-ANOVA. Scores were standardised by dividing estimated scores of the hard and soft samples by score of the standard sample. Within columns, means followed by the same letter are not significantly different at $P = 0.05$ using the Bonferroni multiple comparison tests

Genotype	*n*	Elasticity	Smoothness	Softness
Bulk (hard)	21	1.00a ± 0.10	1.05a ± 0.05	0.92a ± 0.10
Bulk (soft)	21	0.95a ± 0.12	1.03a ± 0.06	1.02b ± 0.09
Sunco (hard)	23	1.00a ± 0.08	0.97b ± 0.07	0.90a ± 0.09
Chuanyu12 (soft)	23	0.94a ± 0.12	1.06a ± 0.05	1.05b ± 0.07

Table 2. Mean scores (± s.d.) and statistical comparisons of all genotypes for noodle texture at each location

Textural scores of hard and soft wheat genotypes grown at 3 locations were determined by taste panelists and statistically analysed using GLM-ANOVA. Scores were standardised by dividing estimated scores of the hard and soft samples by score of the standard sample. Within columns, means followed by the same letter are not significantly different at $P = 0.05$ using the Bonferroni multiple comparison tests

Location	*n*	Elasticity	Smoothness	Softness
Biloela, Qld	30	0.98a ± 0.10	1.05a ± 0.06	1.00a ± 0.11
Roma, Qld	29	0.95a ± 0.14	1.02ab ± 0.07	0.97a ± 0.11
Wenjiang, Sichuan	29	0.98a ± 0.08	1.01b ± 0.06	0.94a ± 0.10

Table 3. Grain protein percentage for genotypes Sunco, Chuanyu12, hard and soft bulks, averaged between sites

Grain was harvested at ~12% moisture. Means followed by the same letter are not significantly different at $P = 0.05$ using the Bonferroni multiple comparison tests

Genotype	*n*	Grain protein content (%)	s.d.
Bulk (hard)	6	16.0a	1.1
Bulk (soft)	6	15.8a	1.1
Sunco (hard)	4[A]	16.0a	0.9
Chuanyu 12 (soft)	4[A]	14.5b	1.0

[A]Samples for one site, Roma, were not analysed for protein content.

Table 4. Grain protein percent for sites Biloela, Roma, and Wenjiang, averaged between genotypes

Grain was harvested at ~12% moisture. Means followed by the same letter are not significantly different at $P = 0.05$ using the Bonferroni multiple comparison tests

Location	*n*	Grain protein content (%)	s.d.
Biloela, Qld	8	15.1a	0.23
Roma, Qld	4[A]	17.3b	0.26
Wenjiang, Sich	8	15.5a	1.1

[A]Samples for Sunco and Chuanyu 12 were not analysed for protein content at one site, Roma.

Sichuan-grown wheat is susceptible to effects of pre-harvest sprouting (PHS) and α-amylase activity due to typical springtime (harvest) rainfalls. Falling number scores of the soft and hard bulks were 293 and 339 s, respectively. Scores for the soft bulks fall on the border of acceptability for the ASW wheat grain classification, according to minimum standards (300 s) established by the Australian Wheat Board. Scores for Sunco and Chuanyu 12 grain grown at Roma and Biloela during a typical dry season, similar to 2003, ranged between 350 and 400 s for Sunco and 300 and 340 s for Chuanyu 12. The scores of bulks grown in Sichuan suggest α-amylase was not an important factor contributing to the differences between samples grown in Sichuan *v.* Biloela.

Panelist scores indicate the growing environment had an effect on noodle smoothness, based on 1 year of data collection. Subsequent investigations may determine whether the environmental effect on texture is consistent over trial years and if any genotype × environment effect becomes detectable.

Genotypic bulked segregant analysis, based on *Pinb* genotypes, facilitates the detection of an associated phenotype, noodle softness. Panelist scores indicate noodles made from bulked 'soft' grains have a softer texture than those made from bulked 'hard' grains. Scores reflect objective differences and similarities between samples. Higher scores for softness imply an increased preference, although a determination of preference was not an objective; the objective was to determine whether panelists could detect differences in bulked samples based on the *Pinb* genotypes. Theoretically, the only distinction between bulks are puroindoline a and b and linked genes; it is possible that unknown linked genes conferred an effect on noodle texture. It is more likely the puroindoline genes, which affect other quality traits as cited in the introduction, are involved with the variation for noodle softness under the experimental conditions imposed by this investigation.

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