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Chromosome 5H of *Hordeum* species involved in reduction in grain hardness in wheat genetic background

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Abstract Grain hardness is an important factor affecting end-use quality in wheat. Mutations of the puroindoline genes, which are located on chromosome 5DS, control a majority of grain texture variations. Hordoindoline genes, which are the puroindoline gene homologs in barley, are located on chromosome 5HS and are also responsible for grain texture variation. In this study, we used three types of wheat–barley species (*Hordeum vulgare*, *H. vulgare* ssp. *spontaneum*, and *H. chilense*) chromosome addition lines and studied the effect of chromosome 5H of these species on wheat grain characteristics. The 5H chromosome addition lines showed significantly lower grain hardness and higher grain weight than the corresponding wheat parents. The effect of enhancing grain softness was largest in the wheat–*H. chilense* line regardless of having an increase in grain weight similar to those in the wheat–*H. vulgare* and wheat–*H. spontaneum* lines. Our results indicated that chromosome 5H of the *Hordeum* species plays a role in enhancing grain softness and increasing grain weight in the wheat genetic background, and the extent of effect on grain hardness depends on the type of *Hordeum* species. Protein analysis of hordoindolines indicated that profiles of 2D-electrophoresis of hordoindolines were different among *Hordeum* species and hordoindolines in the addition lines appeared to be most abundant in wheat–*H. chilens*e line. The differences in enhancing grain softness among the *Hordeum* species might be attributed to the quantity of hordoindolines expressed in the 5H chromosome addition lines. These results suggested that the barley hordoindolines located on chromosome 5HS play a role in reducing grain hardness in the wheat genetic background.

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Introduction

Grain hardness is a major factor influencing end-use quality in wheat. It is inherited and controlled primarily by the *Hardness* locus (*Ha*) located on the short arm of chromosome 5D (Mattern et al. 1973; Law et al. 1978). The *Ha* locus encodes the grain softness protein-1 (*Gsp-1*), puroindoline-a (*Pina*), and puroindoline-b (*Pinb*) genes (Morris 2002, for review). All hard wheats have been reported to carry a mutation in either *Pina* or *Pinb* (Giroux and Morris 1997; Lillemo and Morris 2000; Tranquilli et al. 2002; Ikeda et al. 2005). These findings indicate that puroindolines are the primary genetic elements controlling grain hardness in wheat.

Puroindoline homologs have been found in diploid ancestor wheats and related species, except for the tetraploid *Triticum* species (Gautier et al. 2000). In barley, hordoindolines, which are the puroindoline homologs of barley, have been identified and mapped to the short arm of chromosome 5H (Beecher et al. 2001). Beecher et al. (2002) reported that the quantitative trait loci (QTLs) associated with grain hardness mapped to this region. A recent study reported that allelic variation exists in barley in both *hordoindoline a* (*Hina*) and *hordoindoline b* (*Hinb*) sequences (Caldwell et al. 2006; Turuspekov et al. 2008). Takahashi et al. (2010) found that a null mutation of one of *Hinb* genes increased grain hardness. These results suggested that hordoindolines are the genetic elements controlling grain hardness in barley.

Wheat–alien chromosome addition lines have been produced from various interspecific and intergeneric crosses to introduce valuable genes from wild or cultivated relatives into wheat. Barley is one of the potential gene sources for wheat improvement, and sets of wheat–barley chromosome addition lines have been produced to transfer agronomically important characteristics of barley, such as disease resistance and early maturity to wheat (Islam et al. 1981; Miller et al. 1982; Taketa and Takeda 2001). However, their effect on flour quality has not been studied except for the effect of *H. chilense* on dough strength (Alvarez et al. 1994).

In this study, we used three types of wheat–barley species (*Hordeum vulgare*, *H. vulgare* ssp. *spontaneum*, and *H. chilense*) chromosome addition lines and studied the effect of chromosome 5H on grain characteristics in the wheat genetic background.

Materials and methods

Plant materials

We used three types of wheat–barley chromosome addition lines: *Triticum aestivum* cv. Chinese Spring–*H. vulgare* cv. Betzes chromosome (CS–Hv; Islam et al. 1981), *T. aestivum* cv. Shinchunaga–*H. vulgare* ssp. *spontaneum* strain H602 chromosome (Scn–Hs; Taketa and Takeda 2001), and *T. aestivum* cv. Chinese Spring–*H. chilense* accession H1 (4010001) chromosome (CS–Hc; Miller et al. 1982). These lines and the corresponding wheat parents were kindly gifted by Dr. S. Taketa, Okayama University, Japan. Both wheat parents are soft wheats and the puroindoline genotypes are *Pina-D1a/Pinb-D1a*. The above chromosome addition lines and the corresponding original parents were planted in three replicates in November 2006 and harvested in June 2007. Harvested seeds appeared to be sound without sprouting damage.

Analysis of grain characteristics

Grain characteristics (hardness, weight, and diameter) were measured using a single–kernel characterization system (SKCS4100; Perten) using 100 grains. Grain protein content was determined by near infrared spectroscopy (Infratec 1275; Foss).

Milling of samples and analysis of flour characteristics

Samples of the three replicates were collected for milling. These samples were tempered to a moisture content of 14.5% and milled with a Brabender Quadrumat Jr Laboratory mill. Flour was obtained at approximately 60% extraction rate, and its moisture and protein content were measured by near infrared spectroscopy (InfraAlyzer 500; Bran+Luebbe). Flour particle size distribution was analyzed with a laser diffraction particle size analyzer (HELOS and RODOS; Sympatec).

Analysis of hordoindolines expressed in wheat

A Triton X-114-soluble fraction was extracted from crushed grains of the wheat–barley chromosome addition lines according to the method reported by Giroux and Morris (1998). Two-dimensional (2D) gel electrophoresis (IEF/SDS–PAGE) was performed as reported previously by Ikeda et al. (2005). Hordoindolines were identified by N–terminal amino acid sequencing using the PPSQ-21 amino acid sequencer (Shimadzu). Multiple sequence alignments of HINA and HINB amino acid sequences were performed using ClustalW (Thompson et al. 1994).

Statistical analysis

Statistical analysis was performed using StatView 5.0 (SAS Institute). Student's *t* test was performed between the chromosome addition lines and the corresponding wheat parents.

Results

Table 1 shows grain, milling and flour characteristics of the wheat–barley chromosome addition lines and the corresponding wheat parents. All the chromosome addition lines, except 5H, had grain hardness higher than or equivalent to that in the corresponding wheat parents (data not shown). Chromosome addition lines with 5H showed significantly lower grain hardness than the corresponding wheat parents in all the three pairs. The difference in grain hardness between the chromosome addition lines and the corresponding wheat parents was largest for the CS–Hc pair followed by the Scn–Hs pair. Chinese Spring showed higher grain hardness than Shinchunaga. Grain weight of the chromosome addition lines was significantly higher than that of the corresponding wheat parents in all the three pairs. The difference in grain weight between the chromosome addition lines and the corresponding wheat parents was largest for the Scn–Hs pair.

Grain diameter of the chromosome addition lines was larger than that of the corresponding wheat parents in all three pairs, but significantly so in the Scn–Hs and CS–Hc pairs. Grain protein content of the 5H chromosome addition lines was significantly higher than that of the corresponding wheat parents in the CS–Hv and Scn–Hs pairs.

Table 1 Grain, milling and flour characteristics of the wheat–barley 5H chromosome addition lines and the wheat parents

	Grain hardness (HI)	Grain weight (mg)	Grain diameter (mm)	Grain protein content (%)	Flour yield (%)	Flour particle size (um)
Chinese Spring–*H. vulgare* (CS–Hv)						
5H	33.3**	37.5**	2.61	13.9**	59.1	53.3
Chinese Spring	39.7	31.4	2.50	12.0	62.6	58.1
Shinchunaga–*H. spontaneum*(Scn–Hs)						
5H	24.1*	39.2**	2.71**	13.6**	50.2	35.6
Shinchunaga	34.3	32.2	2.53	11.1	58.1	45.1
Chinese Spring–*H. chilense* (CS–Hc)						
$5H^{ch}$	18.3***	35.4**	2.53*	11.7	60.6	33.4
Chinese Spring	41.0	29.7	2.41	11.1	65.0	56.2

Grain data express means of three replicates. Wheats of the three replicates were collected and milled. Flour particle size means median of flour particle size distribution

*, **, *** Significant difference at $P<0.05$, 0.01 and 0.001, respectively, by *t* test between the chromosome addition line and the wheat parent

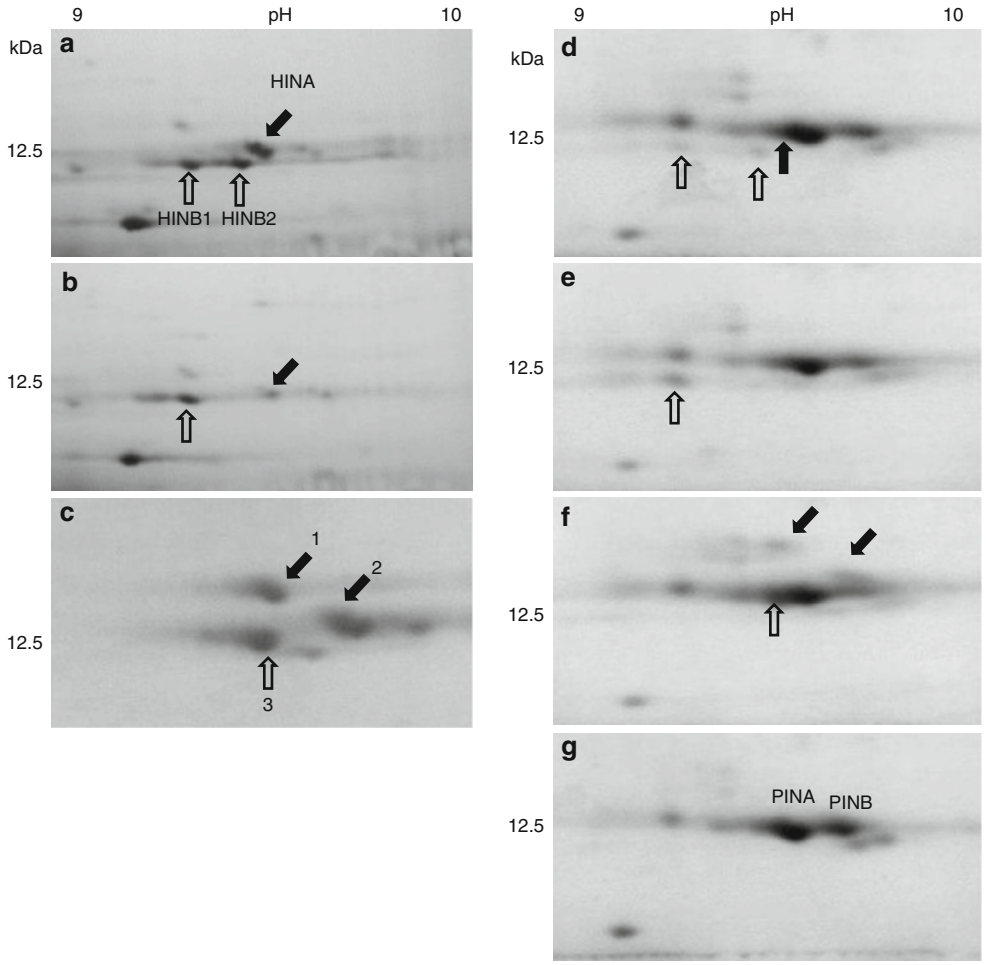

Fig. 1 Profiles of 2D-gel electrophoresis of Triton X-114 soluble fractions isolated from donor parents, wheat–barley 5H chromosome addion lines and wheat parent. **a–c** Donor parents, **a** *H. vulgare* cv. Betzes; **b** *H. vulgare* ssp. *spontaneum* strain H602; **c** *H. chilense* accession H1 (4010001); **d–f** addition lines, **d** CS–Hv; **e** Scn–Hs; **f** CS–Hc; **g** wheat parent, *T. aestivum* cv. Chinese Spring. Puroindolines (PINs) and hordoindolines (HINs) were identified by previous reports (Ikeda et al. 2005; Takahashi et al. 2010). *Solid* and *open arrows* indicate HINA and HINB, respectively. The N-terminal amino acid sequences of hordoindolines of *H. chilense* were identified as follows: *1* YGEVVGSYEGGAGGGXAQQX (HINA), *2* EGGAGGG GAQQXPLEKKLNNXRNYL (HINA), and *3* NEVGGGGGSQ (HINB) + EVGGGGGSQQXP (HINB)

These results indicate that 5H chromosomes play a role in enhancing grain softness as well as increasing grain weight, grain diameter, and grain protein content. Chromosome 5H had a significant influence on grain hardness and grain weight in all three pairs, on grain diameter in the Scn–Hs and CS–Hc pairs, and on grain protein content in the CS–Hv and Scn–Hs pairs.

Flour yield of the addition lines was lower than that of the corresponding wheat parents. Decrease in flour yield was largest in the Scn–Hs pair. The median flour particle size of the addition lines was lower than that of the corresponding wheat parents in all three pairs. Similar to grain hardness, the effect of *H. chilense* 5H chromosome on the median flour particle size was largest.

Protein analysis of Triton X-114-soluble proteins in the wheat–barley chromosome addition lines revealed spots corresponding to hordoindolines as well as puroindolines (Fig. 1). Profiles of 2D-gel electrophoresis of hordoindolines were different among donor parents. Hordoindolines of *H. chilense* had iso-electric points and molecular sizes different from those of *H. vulgare* and *H. spontaneum*. *H. spontaneum* showed less amount and smaller molecular weight of HINA spots and only one HINB spot when compared with *H. vulgare*. Although hordoindolines are expressed with puroindolines in the wheat genetic background, the amount of hordoindolines is quite less than that of puroindolines. The relative amount of hordoindolines in the addition lines appeared to be most abundant in the CS–Hc. Comparison of the deduced amino acid sequences of hordoindolines indicated that hordoindolines except HINB1 of *H. spontaneum* had several amino acid in/dels and substitutions when compared with those of *H. vulgare* (Fig. 2). The positions of ten cysteine residues and the tryptophan-rich domain, which were considered to be important for wheat puroindoline function, were conserved among these materials (Fig. 2).

Discussion

We expected the hordoindolines located on chromosome 5HS to have a function similar to that of puroindolines in wheat. All chromosome addition lines, except 5H, had grain hardness higher or equivalent to that in the corresponding wheat parents (data not shown). We also confirmed that these hordoindolines were expressed in the wheat–barley chromosome addition lines used in this study. Addition of chromosome 5H to wheat resulted in reduced grain hardness and increased grain weight. QTLs for grain weight, grain diameter, and grain protein content have been identified on chromosome 5H using barley populations (Tinker et al. 1996; Mather et al. 1997; Pillen et al. 2003). Some of these QTLs have been mapped to the distal end of chromosome 5HS where the hordoindoline genes are located (Mather et al. 1997). Although we could not determine whether these QTLs play a role in the wheat genetic background, the addition lines generally have higher grain weight, larger grain diameter and higher grain

HINA and PINA

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Hv-a    MKAFFLVGLLALVASAAFAQYGEVVGSYEGGAGGGGAQQCPLGTKLDSCRNYLLDRCTTMKDFPVTWRWWTWWKG 75
Hs-a    MKAFFLIGLLALVARAAFAQYGEVVGSYEG-----GAQQCPLGTKLDSCRNYLLDRCTTMKDFPVTWRWWTWWKG 70
Hc-a    MKAFFLIGLLALVASAAFAQYGEVVGSYEGGAGGGGAQQCPLEKKLNSCRNYLLDRCTTMKDFPVTWRWWTWWKG 75
Ta-a    MKALFLIGLLALVASTAFAQYSEVVGSYDV-AGGGGAQQCPVETKLNSCRNYLLDRCSTMKDFPVTWRWWKWWKG 74
        *** ** *******  ***** ******       ******   ** ********** ********** ****

Hv-a    GCEELLHDCCSQLSQMPPQCRCNIIQGSIQRDLGGFFGFQRDRTVKVIQAAKNLPPRCNQGPACNIPS-TTGYYW 149
Hs-a    GCEELLHDCCSQLGQIPPQCRCNIIQGSIQRDLGGVFGFQRDRTVKVIQAAKNLPPRCNQGPACNIPSTTTGYYW 145
Hc-a    GCEELLRECCSQLGQLPPQCRCNIIQGSIQRDLGGVFGFQRDRTFKVIQAAKNLPPRCNQGPACNIPS-TSGYY- 148
Ta-a    GCQELLGECCSRLGQMPPQCRCNIIQGSIQGDLGGIFGFQRDRASKVIQEAKNLPPRCNQGPPCNIPG-TIGYYW 148
        ** ***  *** * * ************** **** ******* ***** ********** ****  * ***
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HINB and PINB

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Hv-b1   MKTLFLLAILALVASTTFAQYS-VGGGYNDVGGGGGSQQCPQERPNLGSCKDYVMERCFTMKDFPLTWPTKWWKG 74
Hs-b1   MKTLFLLALLALVASTTFAQYS-VGGGYNDVGGGGGSQQCPQERPNLGSCKDYVMERCFTMKDFPLTWPTKWWKG 74
Hv-b2   MKTLFLLALLALVASTTSAQYS-VGGGYNDVGGGGGSQQCPQERPNLGSCKDYVMERCFTMKDFPVTWPTKWWKG 74
Hs-b2   MKTLFLLALLALVASTTSAQYS-VGGGYNDVGGGGGSQQCPQERPNLGSCKDYVMERCFTMKDFPVTWPTKWWKG 74
Hc-b    MKTLFLLALLTLVAGTTFAQYL-VGGGYNEVGGGGGSQQCPQERPNLGSCKDYVMERCFTMKDFPITWPTKWWKG 74
Ta-b    MKTLFLLALLALVASTTFAQYSEVGGWYNEVGGGGGSQQCPQERPKLSSCKDYVMERCFTMKDFPVTWPTKWWKG 75
        ******** * *** ** ***  *** ** *************** * ***************** ********

Hv-b1   GCEQEVREKCCQQLSQIAPQCRCDAIRGVIQGKLGGIFGIGGGDVFKQIQRAQILPSKCNMGADCKFPSGYYW 147
Hs-b1   GCEQEVREKCCQQLSQIAPQCRCDAIRGVIQGKLGGIFGIGGGDVFKQIQRAQILPSKCNMGADCKFPSGYYW 147
Hv-b2   GCEHEVREKCCQQLSQIAPHCRCDAIRGVIQGKLGGIFGIGGGDVFKQIQRAQILPSKCNMGVDCRFPSGYYW 147
Hs-b2   GCEHEVREKCCEQLSQIAPHCRCDAIRGVIQGKLGGIFGIGGGAVFKQIQRAQILPSKCNMGADCKFPSGYYW 147
Hc-b    GCEHEVREKCCQQLSQIAPQCRCDSIRRVIQSKLGGIFGIWRGEVYKQIQRAQSLPSKCNMGADCKFPSGYYW 147
Ta-b    GCEHEVREKCCKQLSQIAPQCRCDSIRRVIQGRLGGFLGIWRGEVFKQLQRAQSLPSKCNMGADCKFPSGYYW 148
        *** ******* ******* **** ** ***  ***  **  * * ** **** ******** ** *******
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Fig. 2 Deduced amino acid sequences of hordoindolines (HINA and HINB) of *H. vulgare*, Betzes (Hv), *H. vulgare spontaneum* strain H602 (Hs), *H. chilense* accession H1 (4010001) (Hc), and puroindolines (PINA and PINB) of *T. aestivum* (Ta). *Asterisks* indicate identical amino acids. Dashes indicate deleted amino acids. The conserved tryptophan-rich hydrophobic domain is indicated by a *rectangle*. Amino acid sequences determined by amino acid sequencing are *underlined*. These sequences were submitted to DDBJ (AB611024, AB611026, AB611027, AB611029, AB611030, AB611032, BAG55290, BAG55291)

protein content. Grain protein content of the addition lines was higher probably because of their low yield (data not shown). Grain hardness is positively correlated with grain protein content in wheat (Slaughter et al. 1992; Giroux et al. 2000; Gazza et al. 2008). When compared with the corresponding wheat parents, the 5H addition lines showed significantly lower grain hardness, while the protein content of these lines was significantly higher in the CS–Hv and Scn–Hs pairs, and similar in the CS–Hc pair (Table 1). These results suggest that reduced grain hardness is independent of grain protein content. Grain hardness is also negatively correlated with grain weight in cultivars and recombinant inbred lines of wheat (Martin et al. 2001; Gazza et al. 2008). However, the increase in grain weight did not necessarily reduce grain hardness. The two appeared to be coincidental. The increase in grain weight is largely explained by the presence of QTLs on chromosome 5H. Using 5H ditelosomic addition lines, 5H dissection lines carrying chromosome 5H segments (Ashida et al. 2007) or transgenic wheat with hordoindoline genes, we will be able to evaluate the effect of hordoindolines on grain characteristics in wheat more accurately, minimizing the effect of other chromosomal regions and genes.

Our results indicate that the effect of chromosome 5H on grain softness is dependent on the source of the *Hordeum* species. Reduction in grain hardness in the addition lines was largest in the CS–Hc pair. Chinese Spring showed higher grain hardness than Shinchunaga (Table 1) and it agrees with the previous reports that Chinese Spring has relatively harder grain texture among soft wheats (Giroux and Morris 1997). Although the genetic background of wheat is different, chromosome 5H of *H. chilense*, a wild relative of barley, had a larger effect on grain softness than wild and cultivated barley. The profiles of 2D gel electrophoresis of hordoindolines were different among donor parents and the amount of hordoindolines in the CS–Hc pair appeared to be more abundant than those in the CS–Hv and in Scn–Hs pairs (Fig. 1). The amino acid sequence variation in hordoindolines of *H. chilense* and *H. spontaneum* should change the 2D profiles of the hordoindolines. The HINA amino acid sequence including a deletion (GAGGG) and the HINB-2 amino acid sequence in *H. spontaneum* were also reported by Caldwell et al. (2006) and Li et al. (2010). The amino acid changes might also bring changes in the amount of hordoindolines as reported in PINB of *Pinb-D1c* allele in wheat (Ikeda et al. 2005). Since only HINB-1 protein was detected by 2D analysis in this strain, the amino acid sequence variation in HINB-2 might also affect their expression. Larger effect of chromosome 5H of *H. chilense* on grain softness might be attributed to the quantity or characteristics of hordoindolines expressed in the addition lines. Further analysis of hordoindolines in the addition lines will be necessary to estimate the effect of hordoindolines on grain hardness.

The flour particle size of the addition lines was smaller than that of the corresponding wheat parents. Since flour particle size is positively correlated with grain hardness (Devaux et al. 1998), the decrease in flour particle size can be attributed to the reduction in grain hardness. In our study, the addition lines produced a lower flour yield than the corresponding wheat parents. The effect of chromosome 5H on flour yield and other milling characteristics must be further studied in large-scale milling.

Use of chromosome 5H to enhance grain softness could lead to large variations in wheat grain hardness. In a soft wheat background, "supersoft" wheat can be developed to produce flour with smaller particle size and lower damaged starch content, which in turn can be used to develop new products in the food industry.

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