Addition of chromosome 5H of *Hordeum* species to wheat enhances grain softness

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ABSTRACT

Grain hardness is an important factor affecting end-use quality in wheat. Variation of the genes for puroindolines which is located on chromosome 5DS controls the majority of grain texture variation. The puroindoline homologs in barley, hordoindolines are located on chromosome 5HS and appeared to be involved in grain texture. In this study, we used three sets of wheat-*Hordeum* species (*H. vulgare*, *H. vulgare ssp. spontaneum*, and *H. chilense*) chromosome addition lines and studied the effect of chromosome 5H of *Hordeum* species on wheat grain hardness. The addition lines of 5H chromosome resulted in significantly lower grain hardness than the corresponding wheat parents. The addition lines also showed significantly higher kernel weight than the corresponding wheat parent. Significant negative correlations between grain hardness and kernel weight were found in wheat-barley addition lines containing 5H and other chromosomes. However, the effect of enhancing grain softness was largest in wheat-*H. chilense* addition line regardless of similar increase of kernel weight compared with wheat–*H. vulgare* and wheat–*H. spontaneum* addition lines. Our results indicated that chromosome 5H of *Hordeum* species increases grain softness and kernel weight in the genetic background of wheat and the effect on grain hardness seemed to depend on *Hordeum* species. The barley hordoindolines on chromosome 5H may play a role in reducing grain hardness.

INTRODUCTION

Grain hardness is an important factor affecting end-use quality in wheat. Puroindoline a (PINA) and puroindoline b (PINB) proteins are the major determinants of grain hardness and the genes for PINA and PINB are located on chromosome 5DS[1]. It is reported that hard wheats have mutations in either *PinA* or *PinB*[1,2,3,4]. The puroindoline homologs of barley, the hordoindolines have been identified and locate on the short arm of chromosome 7 (5H) [5,6]. This chromosomal region appears to be involved in grain texture-dependent traits such as milling energy and the level of fine grind extract, as well as malts-extract viscosity[6,7,8,9]. In this study, we used three sets of wheat-barley (*Hordeum* species: *H. vulgare*, *H. vulgare* ssp. *spontaneum*, and *H. chilense*) chromosome addition lines and studied the effect of chromosome 5H of *Hordeum* species on wheat grain hardness.

MATERIALS AND METHODS

We used three sets of wheat-barley chromosome addition lines as follows: *Triticum aestivum* cv. Chinese Spring-*H. vulgare* cv. Betzes chromosome addition lines (CS-*H. vulgare*)[10], *T. aestivum* cv. Shinchunaga-*H. vulgare* ssp. *spontanium* chromosome addition lines (Scn-*H. spontaneum*)[11], and *T. aestivum* cv. Chinese Spring-*H. chilense* chromosome addition lines (CS-*H. chilense*) lacking chromosome 3H. A ditelosomic addition line for the 1H short arm (1HS) was used for each wheat-barley addition line except for CS-*H. chilense* chromosome addition line. Disomic addition lines for the other chromosomes were used. An addition line of chromosome 2H of CS-*H. vulgare* and the ditelosomic addition line of 1HS of CS-*H. chilense* were not used because we couldn't prepare sufficient amount of seeds. These materials are kindly gifted by Dr. Taketa in Okayama university in Japan. These chromosome addition lines and their parental wheats were planted in three replicates in November 2006 and harvested in June 2007. Harvested seeds appeared to be sound without sprouting damage.

Grain characteristics were measured by single kernel characterization system (SKCS4100, Perten Co. Ltd., USA) using one hundred kernels of each line. Protein content (%) was determined by near-infra-red spectroscopy (Infratec 1275, Foss Tecator, Denmark). The *t*-test was performed between the addition line and the corresponding wheat parent for protein content, hardness index and kernel weight using StatView program.

RESULTS AND DISCUSSION

Grain characteristics of the three pairs of the wheat-barley addition line of 5H chromosome and the corresponding wheat parent was shown in Table 1. Grain hardness of the addition line was significantly lower than that of the corresponding wheat parent in all pairs of wheat-barley addition line. In CS–*H. vulgare*, grain hardness (hardness index, HI) of the addition line was 33.3 HI, significantly lower than that of the parent (39.7 HI). In Scn–*H. spontaneum*, the addition line showed significantly lower grain hardness (24.1 HI) than the parent (34.3 HI). In CS–*H. chilense*, grain hardness of the addition line (18.3 HI) was significantly lower than that of the parent (41.0 HI). Difference of grain hardness between the addition line and the parent was largest in CS–*H. chilense*. A positive correlation between grain hardness and grain protein concentration has often been reported[13,14,15,16]. However, in CS-*H. chilense*, the

addition line showed significantly lower grain hardness than the parent despite similar protein content (11.7%) compared with the wheat parent (11.1%). In CS-*H. vulgare* and Scn-*H. spontaneum*, the addition lines showed significantly lower grain hardness although protein contents of the addition lines (13.9 and 13.6%, respectively) were significantly higher than those of the parents (12.0 and 11.1%, respectively). No significant correlation between grain hardness and protein content was found in all sets of wheat-barley addition lines containing 5H and other chromosomes, while positive correlations were found without 5H chromosome in CS-*H. vulgare* and Scn-*H. spontaneum* (r=0.62* and 0.49*, respectively, Fig. 1). Fig. 1 demonstrates that the addition of barley 5H chromosome to wheat led to a reduction in grain hardness regardless of protein content. It suggests that hordoindolines, puroindoline homologs in barley located on chromosome 5HS may play a role in reducing grain hardness in wheat.

Table 1. Grain characteristics of three pairs of wheat-barley 5H chromosome addition line and the corresponding wheat parent.

	Hardness index (HI)		Kernel weight (mg)		Kernel diameter (mm)		Protein content (%)	
CS-*H. vulgare*								
5H	33.3	*	37.5	*	2.61		13.9	*
Chinese Spring	39.7		31.4		2.50		12.0	
Scn-*H. spontaneum*								
5H	24.1	*	39.2	*	2.71	*	13.6	*
Schinchunaga	34.3		32.2		2.53		11.1	
CS-H. chilense								
5H	18.3	*	35.4	*	2.53	*	11.7	
Chinese Spring	41.0		29.7		2.41		11.1	

Asterisks express significant difference at P<0.05 according to *t*-test.

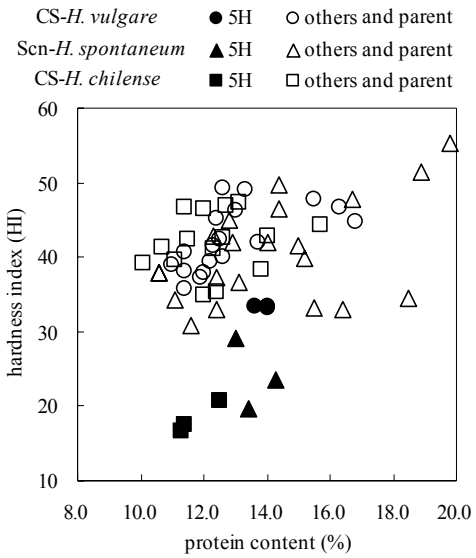

Fig. 1. Relationship between grain hardness index and protein content among wheat-barley chromosome addition lines containing 5H and other chromosomes and the wheat parents.

Kernel weight increased significantly by the addition of 5H chromosome in all pairs of wheat-barley addition lines. In CS–*H. vulgare*, kernel weight of the addition line (37.5mg) was significantly higher than that of the parent (31.4mg). In Scn–*H. spontaneum*, kernel weight of the addition line (39.2 mg) was significantly heavier than that of the parent (32.2 mg). In CS–*H. chilense*, the addition line showed significantly heavier kernel weight (35.4 mg) than the parent (29.7 mg). Positive correlation has been reported between kernel weight and grain protein concentration[15,18]. Although protein content of the addition line was not significantly different from the parent in CS-*H. chilense*, the line showed significantly heavier kernel weight. No significant correlation between grain hardness and protein content was found in each set of addition lines containing all chromosome addition lines, while positive correlation was found in Scn-*H. spontaneum* without 5H chromosome (r=0.76**, Fig. 2). As shown in Fig. 2, addition line of 5H showed heavier kernel weight regardless of protein content. Kernel diameter of the addition line of 5H (2.71 and 2.53mm) was significantly larger than that of the parent (2.53 and 2.41mm) in Scn-*H. spontaneum* and CS-*H. chilense*, respectively. Significant correlations was found between kernel diameter and kernel weight (r=0.92***, 0.91***, and 0.81** in CS–*H. vulgare*, Scn–*H. spontaneum* and CS-*H. chilense*, respectively, data not shown). Larger kernel diameter (kernel size) might be involved in larger kernel weight. This result suggested that the regions which control kernel weight exist on 5H chromosome.

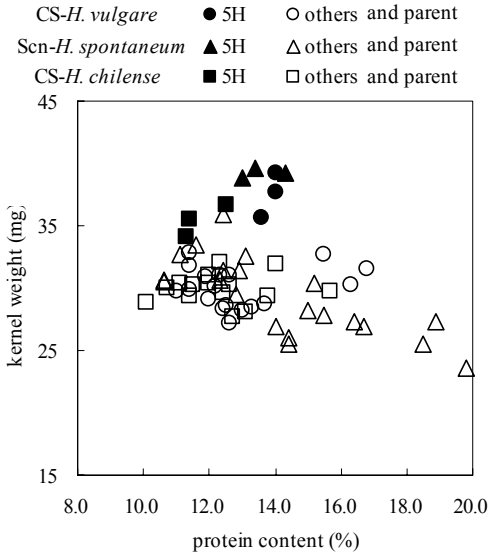

Fig. 2. Relationship between kernel weight and protein content among wheat-barley chromosome addition lines containing 5H and other chromosomes and the wheat parents.

Our results showed that barley 5H chromosome enhances grain softness and increases kernel weight in the wheat genetic background. It has been reported that SKCS hardness index was negatively correlated with kernel weight[16,17]. Significant negative correlations

between grain hardness and kernel weight were found in all sets of wheat-barley addition lines containing 5H and other chromosomes (Fig. 3, r=0.67**, 0.80*** and 0.85*** in CS–*H. vulgare*, Scn–*H. spontaneum* and CS-*H. chilense*, respectively), while this correlation was not significant without 5H chromosome in CS–*H. vulgare* and CS-*H. chilense* (r=0.62** in Scn–*H. spontaneum*). First, we expected barley hordoindoline located on 5HS plays a role in reducing wheat grain hardness. However, the effect of barley hordoindolines on wheat grain hardness was not clear because of significant correlations between grain hardness and kernel weight. As for the barley 5H chromosome, 5DL and 5DS chromosome addition lines and dissection lines were developed[19,20]. Using these materials, we will be able to determine which regions of 5H chromosome have these functions and whether barley hordoindolines play a role in enhancing grain softness in wheat.

Our results also suggested that the effect of 5H chromosome on grain softness is dependent on the source of *Hordeum* species (Fig. 3). Reduction of hardness index was largest in CS-*H. chilense* regardless of similar increase of kernel weight compared with CS–*H. vulgare* and Scn–*H. spontaneum*. Although genetic background of wheat was different, barley 5H chromosome enhanced grain softness more in wild relatives, especially in *H. chilense* than in barley cultivars (*H. vulgare*). Analysis of hordoindolines of *H. chilense* might explain these differences.

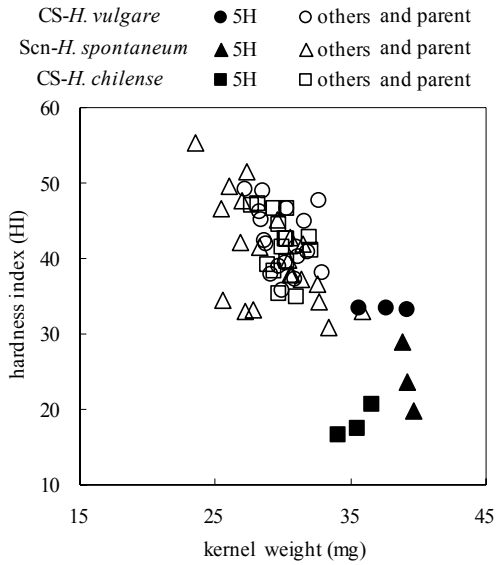

Fig. 3. Relationship between grain hardness index and kernel weight among wheat-barley chromosome addition lines containing 5H and other chromosomes and the wheat parents.

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