

## Relationships between Quality Characteristics in the New Two-rowed, Hull-less Barley Cultivar “Yumesakiboshi”

Takashi NAGAMINE<sup>1,3\*</sup>, Takashi YANAGISAWA<sup>1,4</sup>, Toyotaka MINODA<sup>2</sup>, Osamu SHIGEMATSU<sup>2</sup>, Kazuyamu TOKURA<sup>2</sup> and Tohru KATOU<sup>2</sup>

<sup>1</sup> Barley Research Team, Western Region Agricultural Research Center, National Agriculture and Food Research Organization (NARO) (Zentsuji, Kagawa 765–8508, Japan)

<sup>2</sup> Paddy Field Agriculture Research Institute, Saitama Prefectural United Agricultural Research Center (Kumagaya, Saitama 360–0831, Japan)

### Abstract

The cultivation variations and interrelations among quality characteristics in two-rowed, hull-less pearling barley were studied to obtain basal information for high quality production of this new class of barley in Japan. Samples of the two-rowed, hull-less cultivar “Yumesakiboshi” grown under various cultivation conditions were pearled and analyzed for the relationships between different quality characteristics. The key quality problem for two-rowed hull-less barley appeared to be the high rate of grain breakage by pearling, the rate of which positively correlated with the thousand-grain weight and negatively correlated with grain hardness. Samples with a thousand-grain weight of >40 g showed an extremely high broken-grain rate. The pearl whiteness had a negative correlation with the steely grain rate. Both the steely grain rate and the pearl whiteness showed significant correlations with grain hardness. However, the effect of grain protein content on the steely grain rate showed yearly fluctuation. The hard grain characteristics reduced the broken-grain rate, but adversely affected the steely grain rate and the pearl whiteness. The results of this study indicate that for the high quality production of two-rowed, hull-less barley, the cultivation techniques of limiting the thousand-grain weight to less than 40 g and reducing the steely grain rate are the most promising approaches.

**Discipline:** Crop production

**Additional key words:** broken-grain rate, grain hardness, pearling, SKCS, steely-grain rate

### Introduction

On a global scale, most barley is used for malting and feed. In Japan meanwhile, pearled barley is also employed as a rice extender and in traditional food processing. For example, the fermentation of the seasoning “Mugimiso” and of the Japanese spirit “Shochu” are important uses for barley in Japanese food culture<sup>1</sup>. Recent research into the health functionality of barley, including its positive effects on serum lipid levels<sup>7, 9, 17, 19</sup> and on the control of blood sugar levels<sup>29</sup>, has boosted barley consumption in Japan.

The amount of hull-less barley production (around 20,000 tons/year) represents just a fraction of Japanese barley production. However, hull-less barley has several advantages over hulled cultivars, including higher crop prices for the farmers and more stable demand from barley food manufacturers. Until the release of the first two-rowed, hull-less cultivar “Yumesakiboshi” in 2009, all hull-less barley grown in Japan was six-rowed. The release of the first two-rowed cultivar was expected to meet manufacturers’ needs for a larger grain size and an improved low plump-grain ratio, which has often been a severe problem, especially for barley grown in moisture-damaged fields.

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Present address:

<sup>3</sup> Crop Development Division, Hokuriku Research Center, NARO Agricultural Research Center, NARO (Jyouetsu, Niigata 943–0193, Japan)

<sup>4</sup> Wheat and Barley Research Division, NARO Institute of Crop Science, NARO (Tsukuba, Ibaraki 305–8518, Japan)

\*Corresponding author: e-mail [naga@affrc.go.jp](mailto:naga@affrc.go.jp)

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For steady growth in demand for two-rowed, hull-less barley as a new barley class, it is important to produce stable, high-quality cultivars. Similar to the six-rowed, hull-less barley, the two-rowed barley is processed after pearling. Quality characteristics such as pearling time, the broken-grain ratio, and pearl whiteness are important for manufacturers. For farmers, attaining the quality standard values, which are expressed in terms of four quality characteristics (steely grain rate, volume weight, pearl whiteness, and plumped-grain rate) is important to obtain the quality bonus subsidies. The influences of environmental conditions and yearly fluctuations on these quality characteristics have been reported for six-rowed barley<sup>16</sup>. Moreover, the relationships between the quality characteristics have been studied using both six-rowed cultivars<sup>8,15</sup> and Canadian ones<sup>3</sup>.

In this study, the relationships between the quality characteristics of the two-rowed, hull-less cultivar “Yumesakiboshi” were studied using various samples from different growth conditions.

## Materials and methods

### 1. Barley samples

In 2007 and 2008, “Yumesakiboshi” was grown in the field at the Saitama Prefectural Agriculture Research Center in Kumagaya, Saitama prefecture. Plants were grown after drill seeding in 15 rows (row intervals, 30 cm; length, 4.5 m). Each experimental block was set for two replications. Various combinations of the different cultivation methods were applied to cover the cultivation variation in practical production in a farmer’s field. For basal fertilization, the fertilizer level was modified from 6 to 16 kg/10 a (nitrogen level), and for dressing fertilization, from 0 to 4 kg/10 a. The amount of sowing seed ranged from 4 to 10 kg/10 a, and the sowing dates were set in late October, early and mid-November, and early and late December. Further, the number of times of barley plant treading was changed from 0 to 10 times. Using these conditions in various combinations, 43 and 44 samples were prepared in the 2008 and 2009 harvest seasons, respectively, for further quality analysis. All samples were sized and cleaned over a 2.2 mm screen prior to quality analysis and pearling.

For a comparison of quality characteristics with a six-rowed, hull-less cultivar “Ichibanboshi” under the same and standard conditions, both cultivars was sown at the beginning of November with 8 kg/10a of sowing seed. Basal fertilization was applied for 6 kg/10 a (nitrogen level), and for dressing fertilization for 2 kg/10 a at the stage of seventh leaf emergence.

### 2. Analysis of the grain characteristics

The volume weight of the samples was measured using a Brouwer weight-to-volume balance (Fuji Kinzoku Co., Ltd, Tokyo, Japan). To determine the steely grain ratio, 100 whole grains were cross-sectioned using a Heinsdorf grain cutter (Fujiwara Scientific Company Co., Ltd, Tokyo, Japan) and scored as follows: 1.0 for steely, 0.5 for intermediate, and 0.0 for mealy grains. The results were expressed as a percentage of the sum of all scores from 100 grains, while grain hardness was measured for 300 grains using a Single Kernel Classification System (SKCS) 4100 instrument (Perten Instruments Inc., Springfield, USA). The protein and water contents of the samples were measured by near-infrared reflectance spectroscopy using a Infratec 1241 Grain Analyzer (FOSS Tecator AB, Hillerød, Denmark); the application NB180262 was used for hull-less barley.

### 3. Pearling and analysis of pearled grains

The grain was pearled in a Satake TM-05 test mill (Satake Co. Ltd., Higashi Hiroshima, Japan). For each sample, 200 grams was pearled to 60% extraction using a #36 stone running at 1150 rpm. The pearling and subsequent analysis were performed in duplicate, with the average time required for pearling recorded as the pearling time. A 20-g sample of the pearled grain was examined for broken grains, the criterion for which was any piece less than two-thirds of a whole pearled grain. The broken-grain rate was expressed as a percentage by weight. The pearl whiteness of the grains was measured using the Kett whiteness tester model C-300-3 (Kett Electric Laboratory, Tokyo, Japan).

## Results and discussion

### 1. Cultural variations in grain and quality characteristics

The climate conditions of the 2008 harvest crop were within the normal range of conditions. In contrast, the 2009 harvest crop experienced warm temperatures and significant rainfall over winter (Fig. 1). A comparison of the averages of grain and quality characteristics between the 2 years reflected these climate differences. In 2009, smaller averages were observed in grain protein content, thousand-grain weight, grain hardness, pearling time, and volume weight (Table 1). This difference in quality between the 2 years was similar to the results of Nakamura et al. (2006)<sup>16</sup>. For that study, increased rainfall had a negative effect on the values for thousand-grain weight, volume weight, and grain hardness for six-rowed hulled cultivars. In 2009, six of eight characteristics showed larger coefficients of variation (CVs), which rep-

resent the degree of variation among different cultural treatments. The larger CVs in 2009 likely reflect the adverse climate conditions resulting in significant growth differences among the treatments.

Among the different characteristics, the CV of the broken-grain rate (66.5% in 2008 and 82.3% in 2009) was the highest in both years, hence the broken-grain rate appears to be a characteristic largely influenced by cultiva-

tion conditions. The broken-grain rate is one of the key quality characteristics for barley pearling manufacturers; it directly influences the production efficiency. In addition to the large CV values, the average broken-grain rate (7.8% in 2008 and 8.8% in 2009) is a major quality problem for “Yumesakiboshi.” In most manufacturers’ evaluations of a new pearling barley cultivar, the broken-grain rate must be less than 3% for promising breeding lines of both hulled and hull-less six-rowed barley. This higher broken-grain rate in two-rowed cultivars relative to six-rowed ones has also been previously reported<sup>3, 14, 28</sup>.

Among the quality bonus characters, the pearl whiteness did not attain the standard quality value of 43.0 in 60.4% of samples in 2008 and 29.5% in 2009, while the steely grain rate had a high CV characteristic (67.2% in 2008 and 78.5% in 2009). Nonetheless, even with significant variation, 100% of samples in 2008 and 75% in 2009 attained the standard value of 50.0. Conversely, for the volume weight, none of the samples in either year attained the quality standard value (840 g/L) that was set originally for the six-rowed, hull-less cultivars. The volume weight of two-rowed barley is naturally considered to be much smaller than that of the six-rowed one, because the larger grains of two-rowed barley are difficult to pack densely in a volume instrument, hence a new cri-

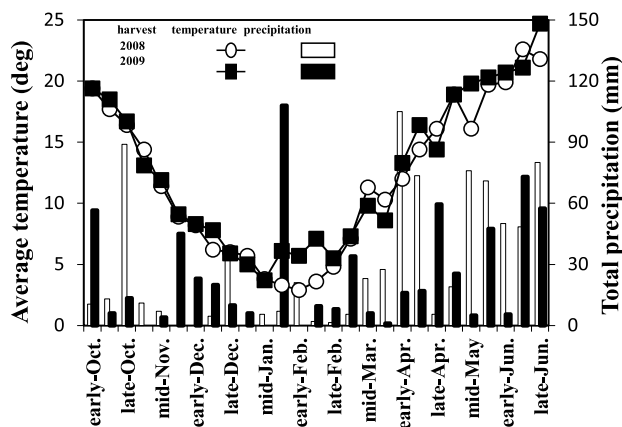


Fig. 1. Average temperature and precipitation during barley growth for 2008 and 2009 harvests

Table 1. Statistical values of quality characteristics for 2008 and 2009 harvests

Characteristics	Harvest year	Grain <sup>1)</sup> protein content (%)	Thousand-grain weight (g)	Grain hardness (SKCS Unit)	Pearling <sup>2)</sup> time (sec)	Broken-grain rate(%)	Steely grain rate(%)	Pearl whiteness	Volume weight (g/l)
Quality standard value <sup>3)</sup>		-	-	-	-	-	50.0	43.0	840
Quality allowable value <sup>4)</sup>		-	-	-	-	-	60.0	40.0	-
Average	2008	10.8	41.3	67.0	775	7.8	18.5	42.5	822
Maximum		13.9	45.8	75.0	910	19.1	45.5	45.7	839
Minimum		8.3	34.9	60.1	660	0.6	1.0	39.2	803
Range		5.6	10.9	14.9	250	18.6	44.5	6.5	36
Coefficient of variation (%)	2008	14.9	6.2	6.5	7.0	66.5	67.2	3.4	1.2
Average	2009	8.9	40.5	57.5	755	8.8	27.3	44.1	806
Maximum		11.0	43.8	81.8	960	31.5	69.0	51.1	837
Minimum		7.1	36.6	35.0	430	1.4	0.0	38.5	757
Range		3.9	7.2	46.8	530	30.2	69.0	12.6	80
Coefficient of variation (%)	2009	11.4	4.6	18.9	17.3	82.3	78.5	6.6	2.6

Number of samples: 43 (2008), 44 (2009)

1): The value of grain protein content was adjusted by the water content constant to be 13.5%.

2): Time to grind 180 g of grain to 60% pearl yield

3): Values for barley quality subsidies

4): Values for barley allowable for the shipment of barley

terion of volume weight should be prepared for the two-rowed, hull-less cultivar.

## 2. Factors influencing the quality characteristics

### (1) Quality characteristics of two-rowed

“Yumesakiboshi” in comparison with six-rowed “Ichibanboshi”

To understand the quality characteristics of two-rowed, hull-less cultivar “Yumesakiboshi”, the values of quality characteristics were compared with six-rowed, hull-less cultivar “Ichibanboshi” which was grown under the same and standard condition for two years.

“Yumesakiboshi” had characteristics such as larger thousand-grain weight, shorter pearling time, lower steely grain rate, brighter pearl whiteness and smaller volume weight (Table 2). These characteristics closely matched those of Yanagisawa et al. (2010)<sup>28</sup>.

### (2) Pearling related characters—broken-grain ratio and pearling time

The broken-grain rate had a significant positive correlation with thousand-grain weight ( $r=0.78^{**}$  in 2008,  $r=0.65^{**}$  in 2009) (Table 3, Fig. 2). In both 2008 and 2009, the samples with a thousand-grain weight exceeding 40 g showed a particular increase in the broken-grain rate. Further, there was a significant negative correlation between the broken-grain rate and the grain hardness ( $r=-0.62^{**}$  in 2008,  $r=-0.76^{**}$  in 2009) (Fig. 3). The positive effect of small and/or hard grains on the reduction of the broken-grain rate has also been reported for six-rowed hulled cultivars<sup>15</sup> and Canadian cultivars<sup>3</sup>.

Inversely, with regard to pearling time, both the smallness and hardness of the grain had the undesirable effect of extending the pearling time. The thousand-grain weight had a significant negative correlation with

pearling time ( $r=-0.62^{**}$  in 2008,  $r=-0.53^{**}$  in 2009) (Fig. 4). Moreover, the grain hardness was positively correlated with pearling time ( $r=0.74^{**}$  in 2008,  $r=0.87^{**}$  in 2009) (Fig. 5). These effects of grain hardness and/or thousand-grain weight on pearling time matched those reported by many previous studies<sup>3, 8, 15, 24</sup>.

### (3) Grain and pearl appearance-related characters—pearl whiteness and steely grain rate

Pearl whiteness had a significant negative correlation with steely grain rate ( $r=-0.52^{**}$  in 2008 and  $r=-0.73^{**}$  in 2009, Fig. 6), whereas grain steel (vitreousness) reflects the degree of internal compactness of the endosperm. Even after removal of the outer grain fractions by the pearling procedure, the steely endosperm appeared translucent, while the mealy endosperm reflected the light diffusely, as measured by the degree of whiteness.

The other endosperm compactness-related characteristics such as grain hardness and pearling time also showed correlations with the pearl whiteness. Significant negative correlations were apparent with grain hardness ( $r=-0.58^{**}$  in 2008 and  $r=-0.79^{**}$  in 2009, Fig. 7) and pearling time ( $r=-0.52^{**}$  in 2008 and  $r=-0.78^{**}$  in 2009, Table 3).

In previous studies, the effects of grain protein content on the steely grain rate varied. Some studies indicated clear, positive relationships between them<sup>8, 10, 12, 24</sup>, while no significant relation was observed by Edney et al. (2002)<sup>3</sup>. In our experiments, the value of the correlation coefficients differed significantly between harvest years ( $r=0.63^{**}$  in 2008,  $r=0.27^{ns}$  in 2009, Table 3). In addition to the grain protein content, some factors for the steely grain rate will need to be resolved in further research because high protein samples with lower steely grain rates were observed in both years (Fig. 8).

**Table 2. Quality comparison between two-rowed “Yumesakiboshi” and six-rowed “Ichibanboshi” under the same growth condition**

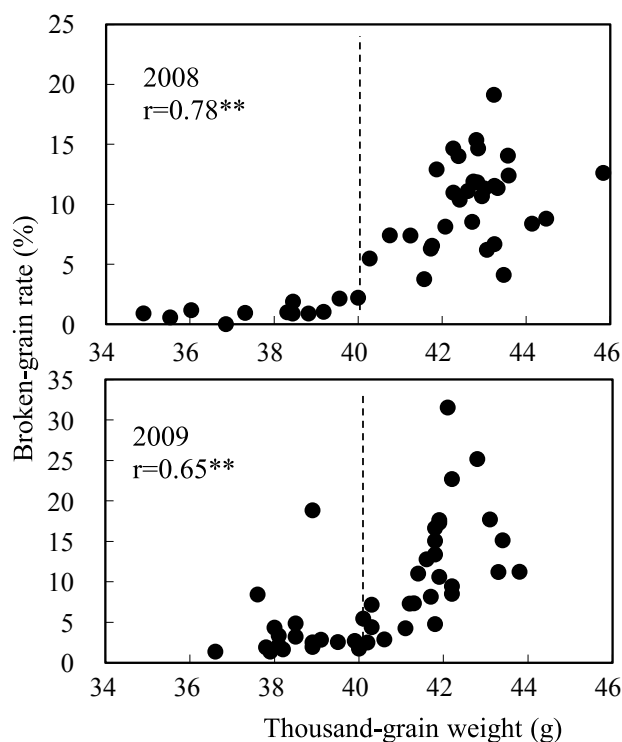
Cultivar	Harvest year	Grain protein content (%)	Thousand-grain weight (g)	Grain hardness (SKCS Unit)	Pearling time (sec)	Broken-grain rate (%)	Steely grain rate (%)	Pearl whiteness	Volume weight (g/l)
Yumesakiboshi (Two-rowed)	2008	9.6	39.2	68.4	790	1.0	4.0	43.5	833
	2009	7.3	38.9	58.5	820	2.5	3.0	44.6	832
	Ave.	8.5	39.1	63.5	805	1.8	3.5	44.1	833
Ichibanboshi (Six-rowed)	2008	9.4	30.2	65.0	860	0.8	28.0	42.4	835
	2009	7.8	31.0	59.1	1080	5.7	64.0	41.4	844
	Ave.	8.6	30.6	62.1	970	3.3	46.0	41.9	840

Samples for this comparison were grown under a standard growth condition. Sowing was at the beginning of November with a seed density of 8kg/10 a. Chemical fertilizer was applied for 6 kg/10 a (nitrogen level) with dressing fertilization 2 kg/10 a at the stage of stem elongation.

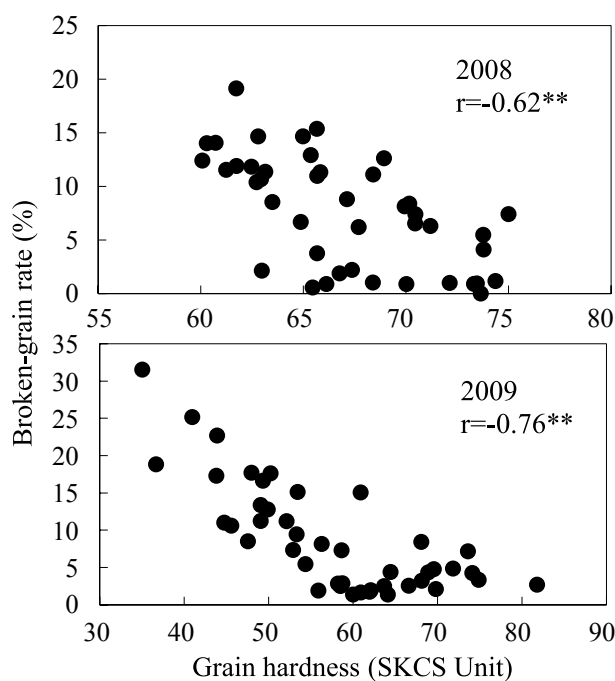
**Table 3. Correlation coefficients among the quality characteristics in 2008 and 2009**

Characteristics	Harvest year	Grain protein content	Thousand-grain weight	Grain hardness	Pearling time	Broken grain rate	Steely grain rate	Pearl whiteness	Volume weight
Grain protein content	2008	1.00							
	2009	1.00							
Thousand-grain weight	2008	0.67	1.00						
	2009	0.63	1.00						
Grain hardness	2008	-0.17	-0.49	1.00					
	2009	0.07	-0.56	1.00					
Pearling time	2008	-0.23	-0.62	0.74	1.00				
	2009	0.11	-0.53	0.87	1.00				
Broken-grain rate	2008	0.51	0.78	-0.62	-0.67	1.00			
	2009	0.20	0.65	-0.76	-0.83	1.00			
Steely grain rate	2008	0.63	0.60	0.15	-0.08	0.29	1.00		
	2009	0.27	-0.25	0.74	0.59	-0.46	1.00		
Pearl whiteness	2008	-0.50	-0.15	-0.58	-0.52	0.12	-0.52	1.00	
	2009	-0.08	0.47	-0.79	-0.78	0.67	-0.73	1.00	
Volume weight	2008	-0.41	-0.32	0.20	0.06	-0.56	-0.11	0.21	1.00
	2009	-0.65	-0.44	0.14	0.00	-0.45	-0.04	-0.14	1.00

Number of samples : 43 (2008), 44 (2009)



**Fig. 2. Relationship between thousand-grain weight and broken-grain rate**



**Fig. 3. Relationship between grain hardness and broken-grain rate**

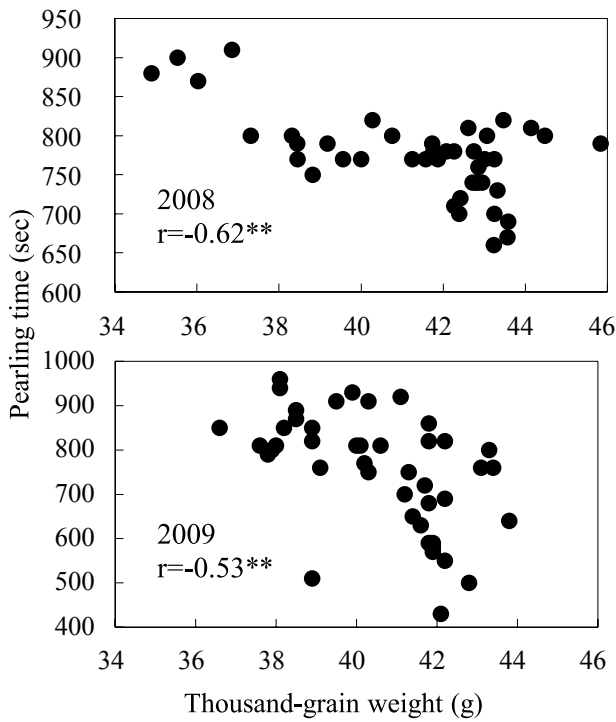


Fig. 4. Relationship between thousand-grain weight and pearling time

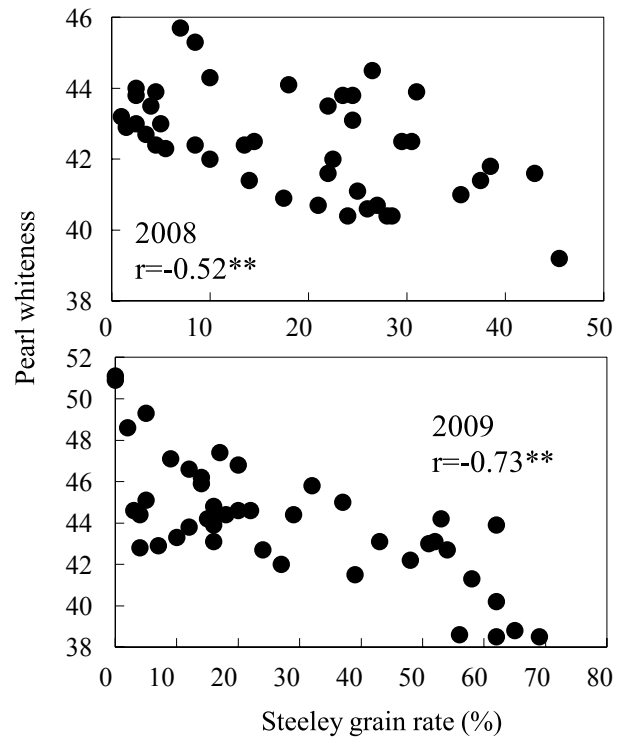


Fig. 6. Relationship between steely grain rate and pearl whiteness

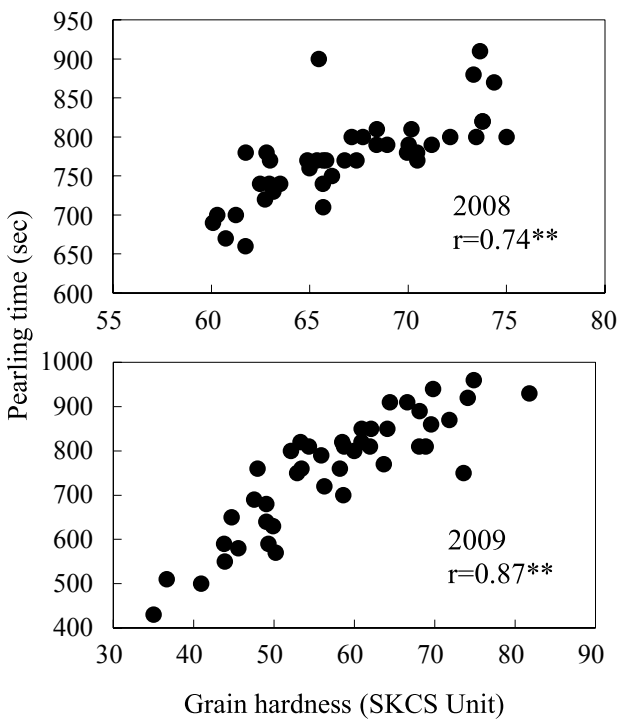


Fig. 5. Relationship between grain hardness and pearling time

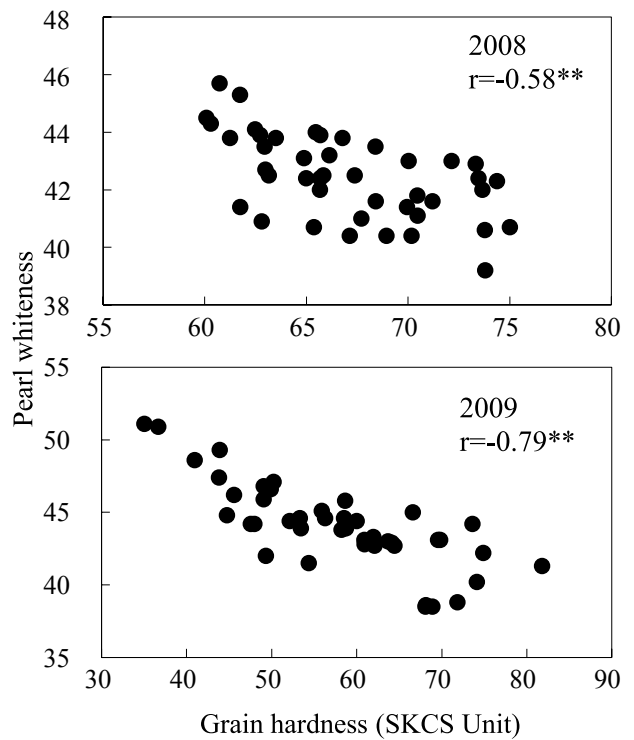
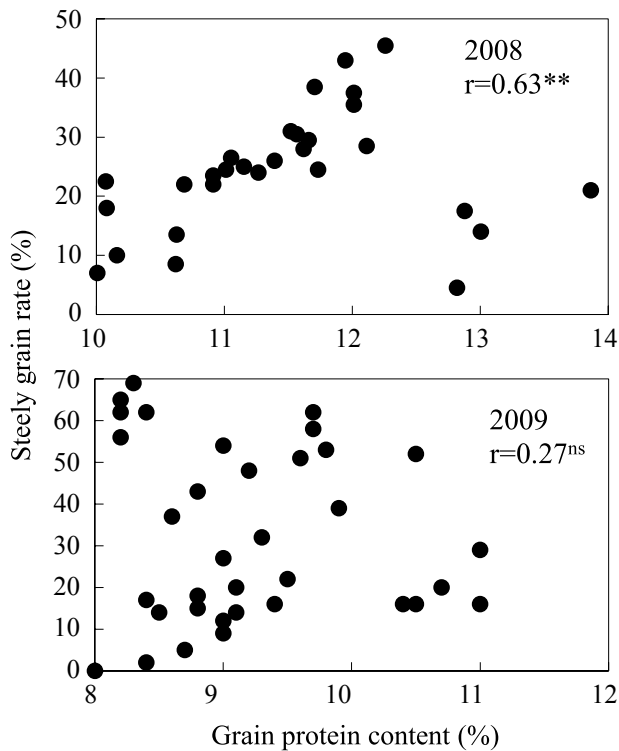


Fig. 7. Relationship between grain hardness and pearl whiteness



**Fig. 8. Relationship between grain protein content and volume weight**

### Problems and resolution for the high quality production of two-rowed hull-less barley

In order to clarify the quality problems for new type barley as the two-rowed, hull-less one, the variations of quality characteristics and their relationships were analyzed using samples of the first two-rowed, hull-less cultivar “Yumesakiboshi”, which were grown under various cultivation conditions. Among the quality problems, the reduction of the broken-grain rate was considered especially important in two-rowed, hull-less barley. In our experiment, the maximum broken-grain rate was as high as 31.5%, while for the six-rowed, hull-less ones, less than 3% tends to be the manufacturer’s request. Actually in this experiment, the limit was 5.7% for “Ichibanboshi”. The higher frequencies of grain broken by the pearling procedure were also shown for hulled, two-rowed barley<sup>3,14</sup>.

To reduce the broken-grain rate, a reduction in the thousand-grain weight and/or an increase in the grain hardness were indicated as effective through analyses examining the relationship between the quality characteristics. For the thousand-grain weight, it was clearly shown that 40 g was the upper threshold for keeping the broken-grain rate low (Fig. 2). The thousand-grain weight is one

of the yield-composing factors, and various cultivation techniques for controlling the yield-composing factors, including fertilizer application levels, additional fertilization, sowing density, and sowing date, have already been used for the production of two-rowed malting barley<sup>21, 26, 27, 21</sup>. Further for “Yumesakiboshi,” Minoda et al. (2011)<sup>11</sup> reported a significant positive correlation between thousand-grain weight and the total amount of fertilizer nitrogen ranging from 6 to 16 kg/10a. The increase in total fertilizer application not only kept the thousand-grain weight within the optimum range but also had a positive effect by increasing the yield. They also showed the superior effect to increase the thousand-grain weight by the application of fertilizer at the stage of spike emergence than the earlier stages.

In contrast to the cultivation control of the thousand-grain weight, the option of increasing grain hardness to reduce the broken-grain rate is less attractive due to the lack of information on cultivation methods for increasing grain hardness. In addition, as shown in this study, increase in grain hardness had unfavorable pleiotropic outcomes such as an increase in steely grain rates and decrease in pearl whiteness.

However, the evidence that grain hardness reduces grain breakage during pearling is meaningful for barley breeders, since a major genetic factor controlling grain hardness has recently been revealed as the genotype of hordoinoline (*Hinb2*)<sup>2, 22</sup> which was the homoeologous of “friabilin” in wheat<sup>5, 6, 13, 18</sup>. An effective breeding strategy to reduce the broken-grain rate as a major quality problem for two-rowed pearling barley is the use of *Hinb2b* allele, which hardens the endosperm texture.

Color is the other important quality for pearling barley. Regarding color-related characteristics, the relation between steely grain hardness and pearl whiteness indicates the importance of maintaining a low steely grain rate through cultivation control. An increase in the steely grain rate has been reportedly stimulated by the application of nitrogen fertilizer at the late stage<sup>23</sup>, an excess of fertilizer application<sup>20</sup>, and cultivation in Andosol upland fields<sup>25</sup>. To overcome this problem from a breeding perspective, Tohnooka et al. (2010a)<sup>24</sup> revealed a drastic decrease in the steely grain rate for the lines possessing *fra* allele (fractured starch granules).

Volume weight is a key quality characteristic, especially for farmers, as one of the quality bonus characteristics. However, none of the samples in this study attained the standard value (840 g/L), which was originally set for six-rowed, hull-less barley.

Larger grains of two-rowed, hull-less barleys than six-rowed ones have disadvantages when it comes to attaining the same volume weight, since it is more difficult

to compactly fill in a volume with larger grains. A new optimum standard volume weight for two-rowed, hull-less ones should be determined. As for hulled barleys, different standard volume weight values have actually been used respectively for six- and two-rowed ones. The establishment of a new standard value for two-rowed, hull-less barley is considered necessary; not only for farmers but also barley manufacturers, because excessive volume weight would likely bring unfavorable characteristics such as high steely-grain rate and low pearl whiteness, as shown in the correlation analysis among the quality characteristics in this study.

### Acknowledgments

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