

Relationship Between Quality Parameters and SKCS Hardness Index in Malting Barley

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ABSTRACT

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To make an efficient and laborsaving quality selection in a malting barley breeding program, the relationship between malting quality parameters and the hardness index (HI) measured by Single Kernel Characterization System (SKCS) 4100 was studied with barley genetic resources and a group of descendant lines from “Yon Rkei 1363” that possess the high lysine allele *lysI* and a very high diastatic power (DP). There was a highly significant correlation between the grain HI and the malt extract both in the genetic resources ($r = -0.48^{**}$) and in the *lysI* parent-derived lines ($r = -0.50^{**}$). Malt HI showed a highly significant correlation with malt extract ($r = -0.70^{**}$ in genetic resources, $r = -0.57^{**}$ in the *lysI* parent-derived lines) and malt total protein ($r = 0.73^{**}$, 0.84^{**} , respectively). For the selection of very high DP lines from the *lysI* parent-derived group, high grain HI selection was effective as all the very high DP lines had much harder grain characteristics. Therefore SKCS was regarded as a useful tool for quality selection in malting barley breeding.

Key words: Diastatic power, grain hardness, *Hordeum vulgare*, malt quality, SKCS.

INTRODUCTION

High quality is the most important objective of Japanese malting barley breeding. Many parameters are used to determine malt quality, namely, malt extract (EX), diastatic power (DP), total protein content (TP), soluble nitrogen content (SN), Kolbach index (KI; the percentage of SN in total malt nitrogen), wort beta-glucan content (BG), and wort viscosity (VI). Laborious procedures such as malting, mashing and analysis are required to evaluate these quality parameters. To make high quality breeding efficient, a quick quality estimation method that involves the use of grain or malt itself is required. To meet this requirement, a friabilimeter that uses malt for the prediction of EX and VI, and a pearlograph using malt for hot water extract have been developed and used in breeding^{1,5,12}. A Single Kernel Characterization System 4100 (SKCS) has

also been used in recent times for cereal quality estimation using grains of barley, wheat, rice, oats and sorghum^{2,7,8,10,11}. SKCS is advantageous with regard to the small quantity of sample that is required for analysis, and it can be used to study several parameters such as hardness index, weight, thickness and the water content of single grains. However, limited information is available regarding the usability of SKCS for determining quality in malting barley breeding. One of the objectives of this paper was to clarify the relationship between malt quality and SKCS parameters, particularly the SKCS hardness index.

Another research objective was the investigation of the relationship between the quality parameters and SKCS hardness indices for the lines derived from high lysine parents possessing the *lysI* allele. The pleiotropic effect due to the high lysine allele *lysI* has been reported to increase the beta-amylase activity in the grain⁴. The beta-amylase stored in the grain is the largest contributor to malt diastatic power, which is a major and important parameter in malt quality evaluation. The main use of the *lysI* allele in breeding has been to improve the nutrient value of the feed barley. No malting cultivar with a very high diastatic power has been developed by employing the pleiotropic effect of *lysI*.

Considering these requirements, we studied the relationship between malt quality parameters and SKCS hardness indices using both barley genetic resources and the lines derived from the *lysI* parent.

MATERIALS AND METHODS

Plant materials

Two groups of barley material were used: group 1 comprised barley genetic resources, and group 2 included lines derived from the *lysI* parent. Group 1 included 55 cultivars/lines including the major malting cultivars, high quality breeding lines, and other genetic resources with superior agronomic traits. These were often used as the cross parent in our practical breeding program. Group 2 comprised 36 lines that were either secondary lines or those that were directly derived from the “Yon Rkei 1363”, a breeding line that possesses the high lysine allele *lysI* from “Hipoly” barley. All of these lines were F6 or later generations and not all lines were believed to possess the *lysI* allele.

All the materials were grown in the field of the Tochigi branch of the Tochigi prefectural agricultural experiment

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station in 2002–2003. Plants were spaced 10 cm apart within rows, and rows were spaced 65 cm apart. Chemical fertilizer was applied before the sowing at 1.2 kg N/10a, 12 kg P/10a and 11.7kg K/10a.

Micromalting

Malting and quality analysis were carried out by the small-scale evaluation method (50 g sample method) used in the Japanese public breeding program. Micromalting was performed with the Phoenix Micromalting Apparatus (Phoenix Biosystems, Sydney, Australia). Fifty gram aliquots of cleaned seeds, retained on a 2.2 mm sieve, were steeped for 36, 40, or 44 h at a temperature of 15 °C with the cycles of 2-h flood and 2-h air rest to obtain a moisture content of 43.5%. To optimize the moisture content, the optimum steeping time was estimated by the preliminary steeping test using 10 g of the sample. At the end of steeping of 50 g of the sample, the wet grain weight was adjusted by wiping or addition of the grain surface water. Germination was carried out for 96 h at 15°C. Kilning was conducted sequentially at 40°C for 12 h, at 60°C for 3 h, and 80°C for 5 h.

Malt analysis

Each 10 g sample of malt was fine ground using a Cyclotec 1093 sample mill equipped with a 1.0 mm mesh screen (Foss Tecator, Hoganas, Sweden). Malt total protein content (TP) was measured for malt flour by the NIR method using an Infracac 1241 Grain Analyzer (Foss Tecator). The mashing temperature program was the Congress mash EBC 4.5 method³; however, the mashing volume was reduced to 1/20 to use 2.5 g malt in 20 mL deionized water, the same method as used in the breeding programs. Eighty samples were mashed simultaneously in 50 mL capped flasks on reciprocal shaking equipment in the mashing bath (Sigma Tech, Tokyo, Japan). Malt extract (EX) was measured with a DMA 5000 density meter (Anton Paar GmbH, Graz, Austria) by employing EBC method 4.5.1³. Soluble nitrogen of malt (SN) was determined by the spectrophotometric method (EBC 4.9.2)³ by using a U-2001 spectrophotometer equipped with an AS-3000 autosampler (Hitachi, Tokyo, Japan). Wort beta-glucan (BG) was determined by the improved Congo red method⁶. Wort viscosity (VI) was measured by EBC method 4.8³ using a Y-VM91 viscosity meter (Yayoi Co Ltd., Tokyo, Japan). Diastatic power (DP) was measured by the method of Kawaguchi et al.⁹ using the 15th EBC malt as the standard.

SKCS analysis of barley grain and malt

The single-kernel characterization system 4100 (Perten Instruments, Huddinge, Sweden) was operated in accordance with the manufacturer's manual. Approximately 10 g of grain or malt of each line was used for the analysis.

RESULTS AND DISCUSSION

Relationship between the malt quality and SKCS hardness indices in barley genetic resources

Correlation coefficients between SKCS hardness indices and malt quality parameters are shown in Table I. EX

showed significant correlation with the grain hardness index (Grain HI) ($r = -0.48^{**}$) at the 1% level. The EX was lower in harder grain cultivars, particularly most cultivars that had a grain HI larger than 70 SKCS units had an EX less than 82% (Fig. 1). It was considered that the grain HI selection was effective in eliminating the low malt EX lines.

Malt SKCS hardness index (malt HI) showed a significant negative correlation with EX and KI, and a significant positive correlation with TP, BG and VI (Table I, Fig. 2). The EX, KI, BG and VI are the parameters that indicate the degree of modification during malting. Therefore, except for TP, all these correlations indicated that the malt HI was influenced by the degree of modification. However, the other modification parameter, malt soluble nitrogen (SN), which is also known to be influenced by TP, did not show a significant correlation with malt HI ($r = 0.04$).

It may be possible to eliminate inferior lines having low EX and KI and high BG and VI by the elimination of the high malt HI lines. However, the selection for only the smaller malt HI would involve the risk of selecting lines with a very high KI (Fig. 2). Currently, Japanese maltsters have experienced serious problems with some domestic barleys that showed a very high KI value, and they have requested new cultivars having the optimum KI value.

Table I. Correlation coefficients between SKCS hardness index and malt quality parameters in 55 barley genetic resources.

Quality parameter	Grain HI	Malt HI
Grain SKCS hardness index (Grain HI)	1.00	-
Malt SKCS hardness index (Malt HI)	0.55 ^a	1.00
Single grain weight (GW)	-0.46 ^a	-0.37 ^a
Malt extract (EX)	-0.48 ^a	-0.70 ^a
Malt total protein (TP)	0.17	0.73 ^a
Malt soluble nitrogen (SN)	-0.15	0.04
Kolbach index (KI)	-0.23	-0.63 ^a
Diastatic power (DP)	0.35 ^a	-0.01
Wort beta-glucan content (BG)	0.19	0.45 ^a
Wort viscosity (VI)	0.27	0.53 ^a

^a Value is significant at a 1% level.

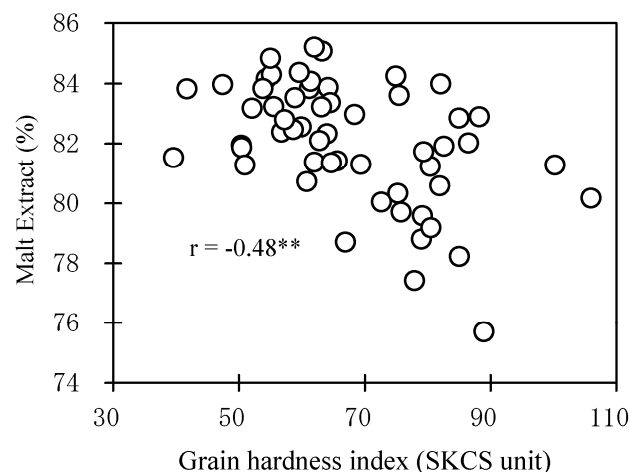


Fig. 1. Relationship between grain hardness index and malt extract in 55 barley genetic resources.

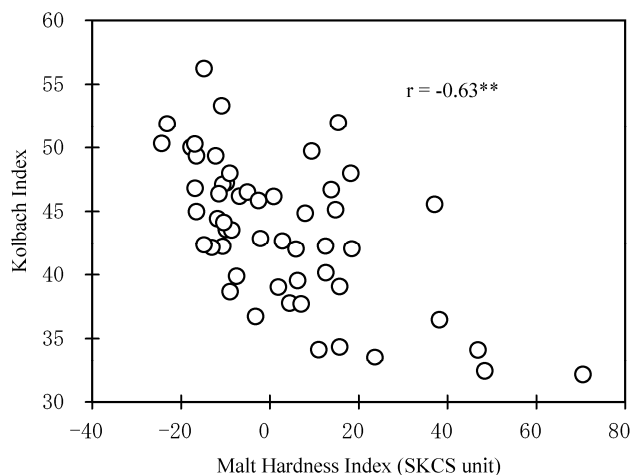


Fig. 2. Relationship between malt hardness index and Kolbach index in 55 barley genetic resources.

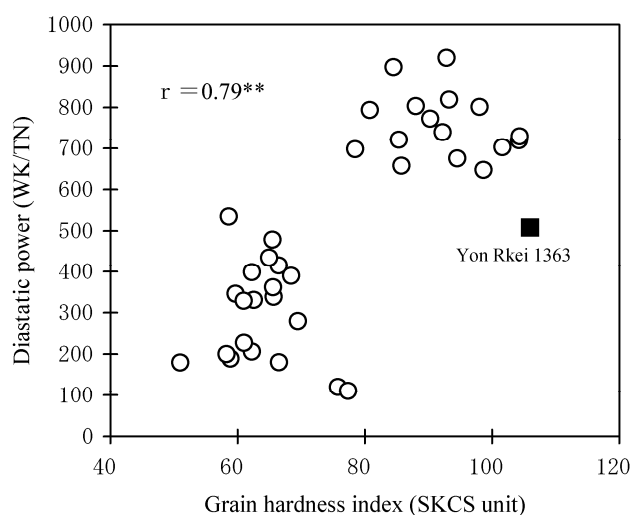


Fig. 3. Relationship between grain hardness index and diastatic power of 36 lines derived from *lys1* line “Yon Rkei 1363”.

Relationship between malt quality and SKCS hardness indices in *lys1* parent-derived lines

By using a combination of grain HI and malt diastatic power, 36 lines derived from *lys1* parent “Yon Rkei 1363” were divided into 2 groups: 1) high grain HI and high DP group and 2) low grain HI and low DP group (Fig. 3). High grain HI lines (HI: larger than 70 SKCS units) had a DP value that was larger than 600 WK/TN. The DP values of the current Japanese cultivars ranged between 200 and 300 WK/TN. The high DP value (larger than 600WK/TN) lines could be expected to possess the *lys1* allele, since the allele is known to have the pleiotropic effect of very high beta-amylase activity⁴.

Therefore, for the breeding of very high DP cultivars, high grain HI selection from the *lys1* parent-derived lines was considered effective. However, the indirect selection of DP based on grain HI was less effective in the case of normal (non *lys1*) lines. As shown in Table I, the correlation coefficient was much smaller in normal cultivars ($r = 0.35^{**}$).

Table II. Correlation coefficients of SKCS hardness indices and malt quality traits in 36 lines derived from *lys1* line “Yon Rkei 1363”.

Quality parameter	Grain HI	Malt HI
Grain SKCS hardness index (Grain HI)	1.00	-
Malt SKCS hardness index (Malt HI)	0.54 ^a	1.00
Single grain weight (GW)	-0.79 ^a	-0.37 ^a
Malt extract (EX)	-0.50 ^a	-0.57 ^a
Malt total protein content (TP)	0.33	0.84 ^a
Malt soluble nitrogen content (SN)	0.05	0.23
Kolbach index (KI)	-0.34	-0.62 ^a
Diastatic power (DP)	0.79 ^a	0.31
Wort beta-glucan content (BG)	-0.01	0.27
Wort viscosity (VI)	-0.45 ^a	-0.04

^a Value is significant at a 1% level.

In the case of the other malt quality parameters, the grain HI of these *lys1* parent-derived 36 lines showed a significant correlation with EX ($r = -0.50^{**}$) and VI ($r = -0.45^{**}$) (Table II); this was similar to the observations in normal cultivars. Additionally, for malt HI, the same parameters, namely, EX ($r = -0.57^{**}$), TP ($r = 0.84^{**}$) and KI ($r = -0.62^{**}$) showed a significant correlation. The correlation coefficient between malt HI and DP was not significant ($r = 0.31$). Therefore, the selection of very high DP lines based on grain HI was considered easier than selection based on malt HI.

Quality parameters of very high diastatic power *lys1* lines

Since the development of high lysine cultivars possessing the *lys1* allele for feed barley, the malting quality of the present *lys1* cultivars such as “Hipoly” and “Yon Rkei 1363” has been inferior with regard to most of the quality parameters, except for DP. Particularly, the low EX, high TP, and high BG of these cultivars are major quality problems for malting. To clarify the possibility of using *lys1* in malting barley breeding and the problems associated with its use, quality parameters were compared between the high and low DP groups for which a DP value of 600 WK/TN served as the differentiation point. The differences between the group averages were significant at the 1% level with regard to EX, VI and single grain weight (GW) (Table III). The high DP group had a lower EX and smaller GW, a major problem. The average of the malt extract (EX), the most important quality parameter in our breeding program, was 1.0% lower in the high DP group compared with the low DP group. Among the lines of the high DP group, no line had a malt extract greater than 84%; lines with this value were regarded as high EX lines in our breeding program (Fig. 4). However, several lines in the high DP group showed a malt extract value that was larger than 83%; this is similar to the value of “Mikamo Golden,” which is currently the leading cultivar, and the acceptable level in our breeding criteria. The average GW was 4.4 mg (11%) less than in the high DP group. A large grain size is preferred due to an increase in the plump-grain percentage, which is an important parameter that impacts the income of farmers. Additionally, maltsters know that smaller grain tends to have a lower EX in the same cultivars (genotypes). A negative correlation was observed between GW and EX ($r = -0.37^{*}$) among the 55 genetic resources used in this research.

Table III. Comparison of averages of quality traits for high and low diastatic power lines derived from *lysI* line “Yon Rkei 1363”.

Quality parameter	Average		Variance analysis ^a
	High DP group (>600 WK/TN)	Low DP group (<600 WK/TN)	
Number of lines	16	20	—
Grain SKCS hardness index (Grain HI)	92.1	64.1	**
Malt SKCS hardness index (Malt HI)	11.7	-3.9	*
Single grain weight (GW)	35.8	40.2	**
Malt extract (EX)	81.7	82.7	*
Malt total protein content (TP)	10.0	9.6	NS ^b
Malt soluble nitrogen content (SN)	0.71	0.69	NS
Kolbach index (KI)	44.3	45.2	NS
Diastatic power (DP)	756	303	**
Wort beta-glucan content (BG)	54	56	NS
Wort viscosity (VI)	1.55	1.61	*

^a Differences are significant between the groups at the 1% (**) and 5% (*) levels.

^b Not specified.

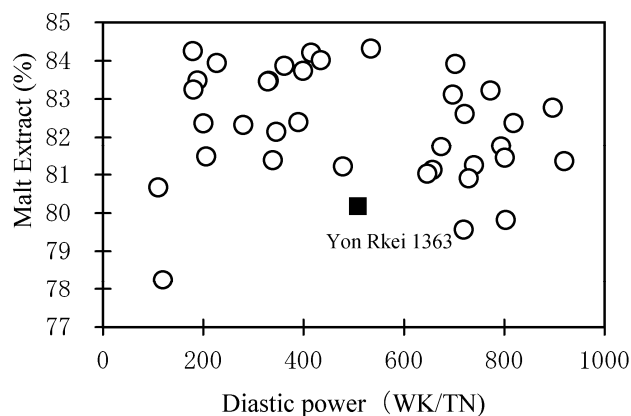


Fig. 4. Relationship between diastatic power and malt extract in 36 lines derived from *lysI* line “Yon Rkei 1363”.

From the viewpoint of malting quality, it is possible to develop a very high DP malting barley cultivar using the *lysI* allele. The lower EX of the *lysI* genotype, which may be partially due to its smaller GW, has already been improved to an acceptable level, as seen in the case of elite lines. Smaller GW of high DP lines could be overcome, since five lines among 16 high DP lines had larger GW than the average (40.2 mg) of low DP ones.

In the next stage, combination breeding to obtain high DP and large GW would be necessary for the development of high malting quality cultivars possessing the *lysI* allele.

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