

## REVIEW

# Recent Advances and Problems in Malting Barley Breeding in Japan

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### Abstract

Malting barley breeding in Japan has witnessed major changes in the past 15 years. Breeding research programs to identify agronomical and quality-related problems, including the spread of new strains of the barley yellow mosaic virus (BaYMV), the excessive degradation of malt protein, and environmental stresses such as frost damage, wet-injury and grain damages (hull-crack and ventral swelling) have been introduced with consideration to the situation of malting barley production in Japan. Further, promising high-quality breeding lines such as lipoxygenase deficient lines and those with extremely high diastatic power or low protein content have also been described.

**Discipline:** Plant breeding

**Additional key words:** barley yellow mosaic virus, diastatic power, lipoxygenase, marker assisted selection, resistance

## Introduction

A century has passed since the commercial production of 2-rowed malting barley was initiated in Japan. Along with wheat, malting barley has become a staple winter crop in Japan.

Currently, malting barley breeding is carried out in the public sector by 2 breeding teams in the Tochigi and Fukuoka prefectural agricultural experiment stations as well as 1 quality evaluation team in Tochigi, and in the private sector by the breeding teams of 2 brewing companies, namely, Sapporo Breweries Ltd. and Asahi Breweries Ltd. The breeding program in the Hokkaido Prefectural Kitami Agricultural Experiment Station for spring-sowing malting barley was closed in 2007.

Further, 4 major brewing companies all participate in the joint evaluation system for the selection of new malting barley cultivars in which all public and private breeding lines are evaluated for the quality of malt produced and agronomic performance.

During the past 15 years, significant progress has

been achieved in the field of malting barley breeding in Japan, despite the following large changes that have occurred in the circumstances surrounding barley production and breeding: (1) wide dissemination of the barley yellow mosaic virus (BaYMV) including the outbreak of new types of BaYMV infections in major cultivation areas; (2) changes in the malt quality criteria due to excessive degradation of the malt protein; and (3) instability of malting barley production, leading to reductions in the contract amount of domestic malting barley with brewing companies.

This paper presents the current situation and recent developments in malting barley breeding in Japan, with special reference to studies that have been conducted. Further, several related issues such as novel quality breeding lines and efficient breeding methods have also been described.

## Agronomic characters

The main requirements of Japanese farmers for malting barley cultivars are as follows: (1) disease resis-

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Received 7 December 2007; accepted 24 March 2008.

**Table 1. Disease reactions to barley yellow mosaic virus (BaYMV) strains and powdery mildew in Japanese major malting barley**

Cultivar	Released year	BaYMV			<i>rym</i> genotype	Powdery mildew
		type-I	type-III	type-IV		
Misato golden	1985	R	S	S	<i>rym5</i>	S
Mikamo golden	1987	R	S	S	<i>rym5</i>	S
Takaho golden	1994	R	S	S	<i>rym5</i>	R
Houshun	1998	R	S	S	<i>rym5</i>	R
Sukai golden	2000	R	R	R	<i>rym3/rym5</i>	R
Shunrei	2004	R	S	S	<i>rym5</i>	R
Sachiho golden	2005	R	R	S	<i>rym3</i>	R

R: resistant, S: susceptible.



**Fig. 1. Dominant growth of “Sachiho Golden” in BaYMV type III affected field**

Left: Amagi Nijyo (BaYMV susceptible), Middle: Mikamo Golden (*rym5*), Right: Sachiho Golden (*rym3*).



**Fig. 2. Screening of wet-injury tolerant lines in flooded-field**

Flood treatments are carried out for each 3 weeks both from the stage of second leaf emergence (December) and from the stage after the stem elongation (March).

tance, particularly against BaYMV, powdery mildew and scab; (2) high yield with early maturity, enabling the use of the double cropping system along with rice; and (3) environmental stress tolerance, particularly to frost damage, wet-injury and grain damages such as hull-crack and ventral swelling.

## 1. Disease Resistance

### (1) BaYMV resistance

In 1985, “Misato Golden”<sup>31,32</sup> was released as the first BaYMV-resistant malting barley cultivar. This cultivar and other BaYMV-resistant cultivars that were subsequently released possessed only the *rym5* allele as the resistant allele against type-I BaYMV<sup>3,22,24,25</sup>. However, with the spread of type-III BaYMV, the *rym5* cultivars became susceptible to BaYMV from the 1980s in the northern Kanto Plain<sup>15</sup> and from ca. 2000 in Kyushu Island<sup>8</sup>. “Sukai Golden”<sup>37</sup> and “Sachiho Golden”<sup>20</sup> possessing the *rym3* gene for resistance were released in 2000 and 2005, respectively; these cultivars were derived from

“Haganemugi” and conferred immunity against both type-I and type-III BaYMV (Table 1, Fig. 1). Nevertheless, immediately after the release of Sachiho Golden in Tochigi Prefecture, a new type (Ootawara type) of BaYMV that could infect it had an outbreak in Ootawara (northern district of Tochigi). By the analysis of cultivar-disease reactions using the available BaYMV-resistant cultivars, the cultivars possessing both *rym3* and *rym5*, such as Sukai Golden, were found to be resistant to Ootawara type BaYMV. Therefore, Ootawara type has the same cultivar-disease reactions with the type-IV BaYMV that had already been found in Yamaguchi Prefecture in the 1960’s and Miyagi Prefecture in 1992<sup>29</sup>. By the screening of barley genetic resources, the Pakistani cultivar “Lochink” was also identified as a novel BaYMV-resistant genetic resource after screening conducted in both BaYMV type-Ootawara and type-III affected fields.

Investigations on new BaYMV-resistant gene sources are important topics of research in both the Tochigi

and Fukuoka breeding teams, and many BaYMV-resistant genetic resources have been identified from landraces both in Japan and worldwide<sup>3,24</sup>. The loci of several resistant alleles have already been analyzed, and these alleles have been used in breeding.

Detailed molecular information on the *rym5* locus is currently available<sup>27,30,33</sup>. We confirmed its applicability in Japanese barley cultivars and several exceptions of the *rym5* tight link marker E31/M41<sup>30</sup> and have recently begun to use it in marker-assisted selection (MAS). Most breeding lines developed by the Tochigi breeding team will soon possess both *rym5* via MAS and *rym3* via selection in type-III affected fields.

## (2) Resistance to powdery mildew

As shown in Table 1, most recent cultivars that have been released after “Takaho Golden”<sup>33</sup> are resistant to powdery mildew. However, the gene(s) for powdery mildew resistance have only been identified in Japanese cultivars derived from limited pedigree cultivars such as “Mona”, “Spartan” and “Klages”<sup>4,7,23,43</sup>.

The integration of a new resistance gene into cultivars is an important task in breeding for protection against fungi race changes in powdery mildew fungi. Backcross breeding for each of 8 resistance genes, including *Mla3*, *Mla7*, *Mlg*, and *MLk*<sup>17</sup>, and MAS for *mlo* resistance gene using the om2 marker<sup>35</sup> are currently underway.

## 2. Early maturity and high yield

Early maturity and high yield along with a high plump grain percentage are the fundamental characteristics considered for malting barley breeding. A steady improvement was observed with regard to early maturity in the history of malting barley breeding until the release of “Mikamo Golden”<sup>42</sup> in 1987 by the Tochigi team and of “Houshun”<sup>4,5</sup> in 1998 by the Fukuoka team. However, recently released cultivars such as Sukai Golden and Sachi-

ho Golden have exhibited only small improvements in early maturity due to the difficulties in combining early maturity with high yield and resistance to frost damage during the stage of stem elongation in early spring.

In addition to high yield, a high percentage of plump grain is also important for farmers because brewing companies only purchase plump grain that are more than 2.5 mm thick. The plump grain percentage of newer cultivars such as Sukai Golden and Sachiho Golden is approximately 5% higher than that of Mikamo Golden (Table 2). The plump grain percentage of Mikamo Golden in the field has fluctuated between approximately 60% and 90% depending on the weather and/or field conditions. Further, in contrast to Mikamo Golden, Sukai Golden and Sachiho Golden possess superior stability even during years or in fields wherein the conditions are unfavorable for the grain filling of barley.

## 3. Tolerance to environmental stress

Due to global warming and the early stem elongation of new early maturing cultivars, the incidence of frost damage to developing spikes has been increasing in recent years, especially during an early spring following a warm winter; this increases the risk of juvenile spikes in the elongated stems encountering temperatures below the freezing point. Winter-habit barley is known to exhibit delayed stem elongation and relatively superior tolerance to frost damage. For the breeding of the winter-type cultivars, a combination of 3 alleles, namely, *vrn-H1* (formerly *sh<sub>2</sub>*), *Vrn-H2* (*Sh*) and *vrn-H3* (*sh<sub>3</sub>*), is necessary<sup>19,36,41</sup>. Among these 3 alleles, *Vrn-H2* is the key allele required for the breeding of winter-habit malting barley since most Japanese cultivars possess winter alleles at the other 2 loci. The DNA marker Zcct-Ha/b<sup>34</sup> can be used for the selection of *Vrn-H2* lines and we have already initiated intensive selection programs for *Vrn-H2* lines by using this DNA marker<sup>26</sup>.

**Table 2. Grain characteristics and malt quality characters of Japanese major malting cultivars**

Cultivar	1,000 grain weight (dm g)	Plump grain percentage (%)	Malt extract (dm %)	Total protein (dm %)	Kolbach index (%)	Diastatic power (WK/TN)	Apparent attenuation limit (%)	Total score	Wort viscosity (mPa·s)	Wort β-glucan (mg/L)
Mikamo golden	37.7	85.8	82.9	10.5	44.0	192	84.0	75.4	176	1.53
Houshun	39.3	93.0	83.8	10.2	44.1	185	85.5	76.8	114	1.54
Sukai golden	38.3	91.0	83.4	10.7	47.3	190	85.5	76.1	88	1.51
Sachiho golden	41.3	91.3	84.7	10.1	46.4	218	84.2	77.3	98	1.56
Shunrei	41.8	85.5	83.7	10.4	46.3	246	83.7	76.8	114	1.50

All data are the averages of four years (2001~2004) in the joint evaluation system.

Total score is calculated from the scores of malt extract, total protein, soluble nitrogen, Kolbach index, diastatic power, and apparent attenuation limit.

The next important characteristic for malting barley breeding is tolerance to wet-injury. Wet-injury is a major constraint to barley production in Japan since the wet-injury tolerance of barley cultivars, particularly malting cultivars, is considerably inferior to that of wheat<sup>9</sup>. Intensive researches on the wet endurance have been made by the Fukuoka team<sup>9–11</sup>. Also the Tochigi team has revealed large varietal differences among Japanese breeding lines for the degree of culm-length reduction and etiolation by the 2 times flood treatment in each 3 weeks both from the stages of second leaf unfolding and of stem elongation (Fig. 2). According to the results of this research, the etiolation index just before the stem elongation with 3 weeks flood treatment from the stage of second leaf unfolding is used as a selection criteria for wet endurance by the Tochigi team, since the etiolation index has significant correlation with the degree of culm-length reduction measured after 2 times flood treatment<sup>21</sup>.

The tolerance against the occurrence of the grain damages of hull-crack and ventral swelling is also an important character, especially in Kyushu Island. The Fukuoka team made an effective screening method and analysis against each hull-crack treatment<sup>2,6,18,38</sup> and ventral swelling<sup>1,6</sup>. Fukuoka's new cultivar "Shunrei"<sup>7,39</sup> which was released in 2005 has superiority in the tolerance against hull-crack. The hull-crack frequency of Shunrei is very low even in the early sowing cultivation that largely enhances the occurrence of hull-crack in other cultivars. The adaptability to early sowing of Shunrei is a big advantage for large-scale farmers who can extend the sowing period of malting barley.

## Quality-related characteristics

Since domestic barley is priced higher than imported barleys in Japan, high quality is indispensable for Japanese cultivars. Quality improvement has been a major breeding objective throughout the course of barley-breeding history. Here, we discuss the present status and problems related to the breeding of high-quality cultivars and describe several new materials for breeding.

### 1. Malt quality of recently released cultivars

All candidate lines for spread as new cultivars are evaluated in the joint quality evaluation system for a minimum of 7 years that includes a 2-step evaluation of each for 2 and 3 years and final full-scale evaluation for 2 years. In the full-scale evaluation, candidate lines are grown in farmers' fields and 60–90 tons of grain of each are provided for each brewing company.

The quality of all new cultivars must be identical or superior to that of the standard cultivars Mikamo Golden

and Houshun. The criteria considered in the joint evaluation system are listed in Table 3. As shown in Table 2, the newer cultivars are of high quality with regard to most malt characteristics; however, some cultivars tend to exhibit high "soluble nitrogen" (SN) and high "Kolbach index" (KI) values, as discussed below.

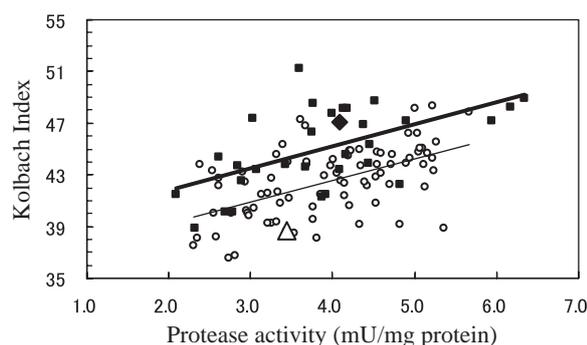
### 2. Factors related to the over degradation of protein during malting and mashing

For brewers to obtain wort that is optimal for beer production, it is necessary that barley proteins are optimally degraded to "soluble protein" (a mixture of amino acids, peptides and dissolved protein) during the malting and mashing processes. The degree of the protein degradation, which is usually evaluated in terms of SN or KI, has become the most important quality-related criterion in the joint evaluation system. Therefore, the maltsters began to critically evaluate the characteristics of the excessive protein degradation occurring in Sukai Golden. Its KI value often exceeded 50, while the optimum value lies between 40 and 45. In addition to Sukai Golden, lines exhibiting excessive protein degradation began to increase in the breeding fields; therefore, investigations to determine the factors related to excessive protein degradation were carried out extensively.

The investigations revealed that (1) high malt protease activity and (2) low  $\beta$ -glucan content in the grain exerted promotive effects on protein degradation. Analysis of 111 single-seed descendent (SSD)  $F_6$  lines derived by crossing Sukai Golden with Nishinochikara revealed that the malt protease activity was positively correlated with KI ( $r = 0.52^{**}$ ) and that in general, the KI values of lines with low  $\beta$ -glucan content ( $< 3\%$ ) were 1.5% higher than those lines with a high  $\beta$ -glucan ( $> 3\%$ ) content (Fig. 3). Reduction of the  $\beta$ -glucan content has been one of the major breeding objectives for high-quality malting barley since the  $\beta$ -glucan present in wort increases its viscosity, thus retarding the filtration process, and since the content of starch which is a major source of malt extract, can increase by reducing the  $\beta$ -glucan content.

**Table 3. Malt quality evaluation criteria in the joint evaluation system**

Characters	Target value	Full marks
Malt extract (EX)	84%	25.0
Total protein (TP)	10–11%	12.5
Soluble nitrogen (SN)	0.70–0.80%	12.5
Kolbach index (KI)	40–45%	12.5
Diastatic power (DP)	250WK/TN	25.0
Apparent attenuation limit (AAL)	88%	12.5



**Fig. 3. Influence of protease activity and grain  $\beta$ -glucan content on the degree of malt protein degradation (Kolbach index)**

◆: Sukai Golden, △: Nishinochikara,

Bold line in the figure indicates the regression of 30 low  $\beta$ -glucan lines (■,  $\beta$ -glucan content was 3.0% or less;  $y=1.69x + 38.4$ ). Thin line indicates that of 82 high  $\beta$ -glucan lines (○,  $\beta$ -glucan content was higher than 3.0%;  $y=1.66x + 35.9$ ).

Thus, to maintain the degree of malt protein degradation within the optimal range, a partial reduction in the protease activity would be necessary during the malting-barley breeding.

### 3. Promising new high-quality breeding resources for the next decade

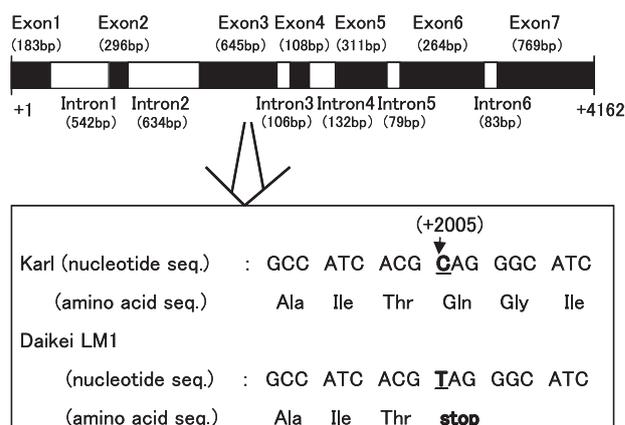
In addition to the selection of good-quality breeding lines, the Tochigi quality evaluation team has another task in the research and breeding of the high-quality materials that must be provided to the breeding teams. Several interesting breeding materials have been developed, and are now in use in practical breeding in Tochigi and Fukuoka; these have been discussed below.

#### (1) Lipoxygenase-1-deficient mutant

Lipoxygenase (LOX) catalyzes the hydroperoxidation of polyunsaturated fatty acids and serves as a precursor of beer-deteriorating substances that cause an off-flavor (cardboard flavor) and decrease the foam stability of beer. Therefore, methods for reducing LOX activity both during breeding<sup>13,14</sup> and during malting or mashing<sup>40</sup> have been extensively investigated. By performing mutation breeding, we obtained an LOX-1-deficient mutant line “Daikei LM1” from sodium azide-treated “Karl” M<sub>2</sub> lines<sup>28</sup>. This line possesses a single-nucleotide substitution that induces a stop codon in exon 3 of the *lox-1* gene, as shown in Fig. 4. Based on this single-nucleotide polymorphism (SNP) in the *lox-1* gene, the cleaved amplified polymorphic sequence (CAPS) marker has developed and is already in use for line selection<sup>26</sup>.

#### (2) Extremely high diastatic power line

Diastatic power (DP), a measure of the combined



**Fig. 4. Single nucleotide substitution in *Lox-1* gene of Daikei LM1**

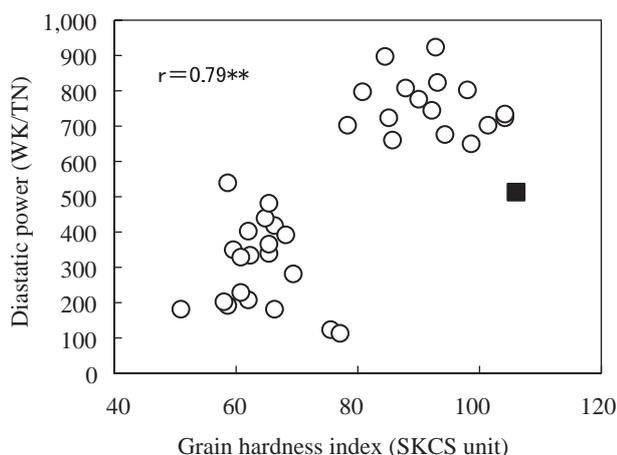
levels of amylolytic enzymes, is an important determinant of malt quality. High-DP malt is particularly desirable for “Happoushu,” which is a low-tax beer and contains a small amount of malt, in order to degrade the large amounts of starch-substituting substances.

The DP of many breeding lines derived from “Yon Rkei 1363” that possess high content of the lysine allele *lys1*<sup>12</sup> is extremely high as compared with that of 600 WK/TN, while the DP of normal cultivars is usually lower than that of 300 WK/TN. The *lys1* lines with extremely high DP can be selected in a low-labor manner based on the grain hardness index (HI), which can easily be measured within 5 minutes by using the single kernel characterization system 4100 (Perten Instruments, Stockholm, Sweden) with approximately 200 seeds. As shown in Fig. 5, lines with extremely high DP (greater than that of 600 WK/TN) also exhibit a high grain HI; therefore, we selected extremely high DP from among the offsprings derived by crossing the *lys1* parents based on high grain HI.

#### (3) Low protein content line

Protein content is an important quality parameter considered by brewing companies since it not only affects the SN and KI values but also considerably influences the fermentability, flavor and haze formation of beer. The optimal protein content ranges from 10% to 11%. However, many Japanese malting barleys have a protein content higher than 12%, despite reductions in fertilizer application by farmers.

The protein content of lines such as “Two-rowed barley parental line No.1”<sup>16</sup> and “Daikei HL138” is consistently lower than that of Mikamo Golden by approximately 1.5% to 2%. These lines with low protein content are expected to be used for high-yield and high-quality cultivation in combination with increased fertilizer appli-



**Fig. 5. Relationship between grain hardness index and diastatic power of 36 lines derived from *lys1* line "Yon Rkei 1363"**

■: Yon Rkei 1363.

cation.

## Discussion

As described above, with the introduction of Sukai Golden, followed by Sachiho Golden in the Kanto area and Houshun and Shunrei in the Kyushu area, malting barley breeding in Japan has progressed considerably in terms of both agronomics and quality. However, the demands of farmers are large and rapidly increasing, due to the critical condition of Japanese barley production, with regard to not only the natural and agronomic problems but also economic ones. As discussed above, the acquisition of tolerance to growing environmental stresses such as frost damage, wet-injury and damaged grains is a major objective of breeding strategies. Further, optimization of the protein content of barley is a primary objective for overcoming quality-related problems, which may not be resolved in the absence of combined investigations on novel cultivation methods and new cultivars. The development of new cultivars possessing the above-mentioned high-quality characteristics and improvement in the yield to a great extent are essential in order to compensate for the price difference between domestic and foreign barley.

## Acknowledgments

The research work reported in this paper was mainly supported by a grant by the designated research station system of the Ministry of Agriculture, Forestry and Fisheries of Japan.

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