

## REVIEW

# Breeding of Waxy Barley Cultivars in the National Barley Breeding Program of Japan

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### Abstract

Waxy barley is considered a good source of dietary fiber and a convenient functional food in Japan; thus, domestic demand is rapidly increasing. However, most of the demand has been met by imports from countries, such as the USA and Canada, as domestic waxy barley production levels have remained low in Japan. To promote the domestic production of waxy barley, the National Agriculture and Food Research Organization has initiated an intensive breeding program to develop and disseminate elite waxy barley cultivars with superior agronomic traits, such as high-yield potential, high disease resistance, and high quality in terms of processing and food. Extensive planting of the newly bred cultivars raised the Japanese domestic self-sufficiency rate of waxy barley from 3 to 49% in 2016 to 2021, respectively.

**Discipline:** Crop Science

**Additional key words:**  $\beta$ -glucan, dietary fiber, domestic supply, functional food, pearling quality

## Introduction

Barley is a staple food in the eastern part of Asia. In Japan, barley is used in a variety of ways, e.g., as a rice extender and in traditional fermented foods and spirits, such as “miso,” “shochu,” and roasted barley tea. Barley is also considered a healthy food in other parts of the world owing to its usefulness as a source of dietary fiber (Baik & Ullrich 2008). The level of  $\beta$ -glucan, a soluble dietary fiber, is higher in barley grains than that in other cereals (Bamforth 1982).  $\beta$ -glucan is especially important as a functional component of barley that reduces serum cholesterol and insulin responses in the blood (Klopfenstein & Hosney 1987, Newman et al. 1989, Yokoyama et al. 1997). The health benefits of  $\beta$ -glucan in barley have been officially confirmed in the USA (The U.S. Food and Drug Administration 2006), the EU (The European Food Safety Authority 2009), Korea (Korean Ministry of Food and Drug Safety 2010), Canada (Health

Canada 2012), and Australia and New Zealand (The Board of Food Standards Australia New Zealand 2013). Waxy barley grains are particularly rich in  $\beta$ -glucan (Ullrich et al. 1986); hence, countries such as the USA and Canada have started breeding waxy barley with the aim of expanding its usage in food (Bhatty 1999). Additionally, the demand for waxy barley has rapidly increased in Japan, particularly due to the public's exposure to health-related information from the media, e.g., on TV and in newspaper and magazine articles. However, most of the demand for waxy barley in Japan has been met by imports from other countries, e.g., the USA and Canada, due to the overwhelming shortage of Japanese production. To meet the increasing demand and extensively expand the domestic production of waxy barley in Japan, the National Agriculture and Food Research Organization (NARO) began breeding elite waxy barley cultivars in the national barley breeding program. In this review, the author describes the

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recent developments in waxy barley research and breeding in Japan.

### Quality properties of waxy barley

The starch of waxy barley consists mainly of amylopectin, which easily gelatinizes at low temperature and exhibits a higher viscosity than that of the normal barley starch (Eslick 1981, Vasanthan & Bhatti 1996). These starch properties give cooked waxy barley grains a soft and sticky texture conversely to the firm and dry texture of cooked normal-starch barley grains (Yoshikawa et al. 1998). The texture of cooked waxy barley is preferred by most Japanese over that of cooked normal-starch barley (Fig. 1). Two types of waxy alleles exist in waxy barley cultivars: low-amylose (2.4%-10%) and amylose-free (Ishikawa et al. 1994). Indigenous waxy cultivars have low-amylose content (Ishikawa et al. 1994) and invariably exhibit approximately 400bp deletion in part of the promoter and 5'-untranslated region of granule-bound starch synthase I (GBSS I) (Domon et al. 2002a, 2002b; Patron et al. 2002). Previous studies have indicated that the waxy alleles in GBSS I have a common origin (Domon et al. 2002a, Patron et al. 2002).

Amylose-free mutants were artificially introduced in Japan (Ishikawa et al. 1994) and the USA (Danielson et al. 1996). The amylose-free and the low-amylose alleles have single nucleotide polymorphisms (SNPs) that introduce a stop codon and an amino acid substitution in the GBSS I protein, respectively (Domon et al. 2002b, Patron et al. 2002). The grains of low-amylose cultivars exhibit a heterogeneous distribution of amylose that is localized in the outer part of the grain (Patron et al. 2002, Yanagisawa et al. 2006). Comparing the starch of the low-amylose cultivar with that of the amylose-free cultivar, the latter exhibits a lower pasting temperature and retrogradation tendency (Yanagisawa et al. 2006); compared with low-amylose isotypes, amylose-free isotypes exhibit higher  $\beta$ -glucan content and peeled whiteness (Tonooka 2010a). Thus, the amylose-free genotype is preferred over the low-amylose genotype for food and processing applications.

Waxy barley has an opaque endosperm, and the pearled grains exhibit higher whiteness than that of normal-starch barley regardless of growing conditions (Tonooka et al. 2010a). Floury grains are preferred for the barley pearling process because their pearling time is shorter and they exhibit a higher pearled whiteness than

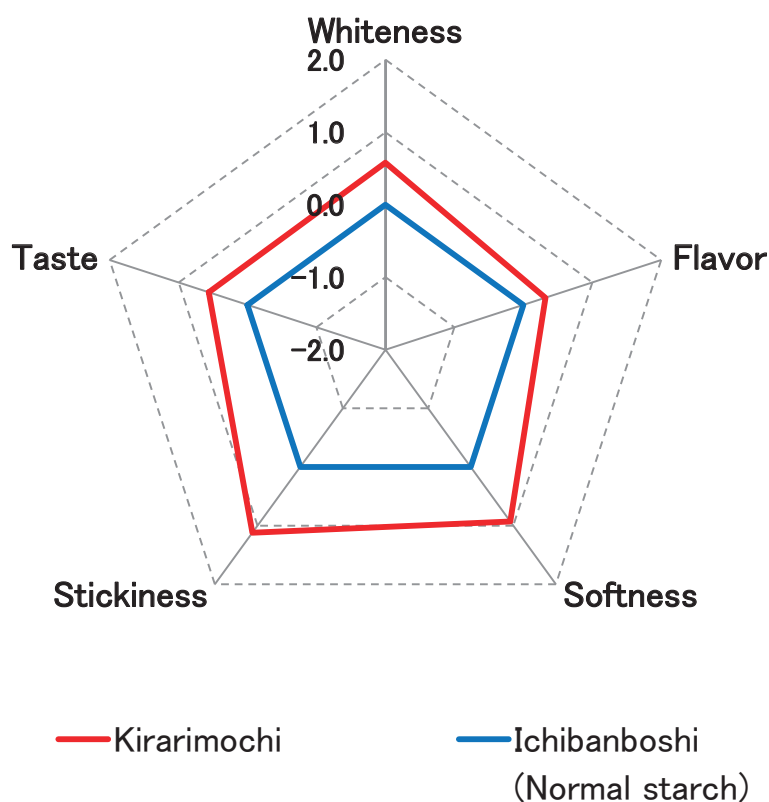


Fig. 1. Eating quality evaluation of Kirarimochi as a rice extender  
This figure was reproduced from Yanagisawa et al. (2011).

that of vitreous grains; therefore, barley processors seek to minimize the number of vitreous grains. The desirable vitreous grain rate (<40% and <50% for six-rowed hulled barley and hull-less barley, respectively) and pearled whiteness (>43%) and the acceptable vitreous grain rate (40%-50% and 50%-60% for six-rowed hulled barley and hull-less barley, respectively) and pearled whiteness (40%-43%) are set by the barley quality control standard established by the Ministry of Agriculture, Forestry and Fisheries. The barley product subsidy provided by the government to farmers depends on these values in quality control evaluations. Waxy barley grains require a longer pearling time despite their floury texture; however, the quality standards are easier to achieve with waxy barley than with normal-starch barley (Tonooka et al. 2010a). Indeed, waxy barley maintains a low vitreous grain rate despite the use of high-nitrogen topdressing to increase yield, whereas normal-starch barley exhibits a high vitreous grain rate when high-nitrogen topdressing is applied (Shimazaki & Seki 2020).

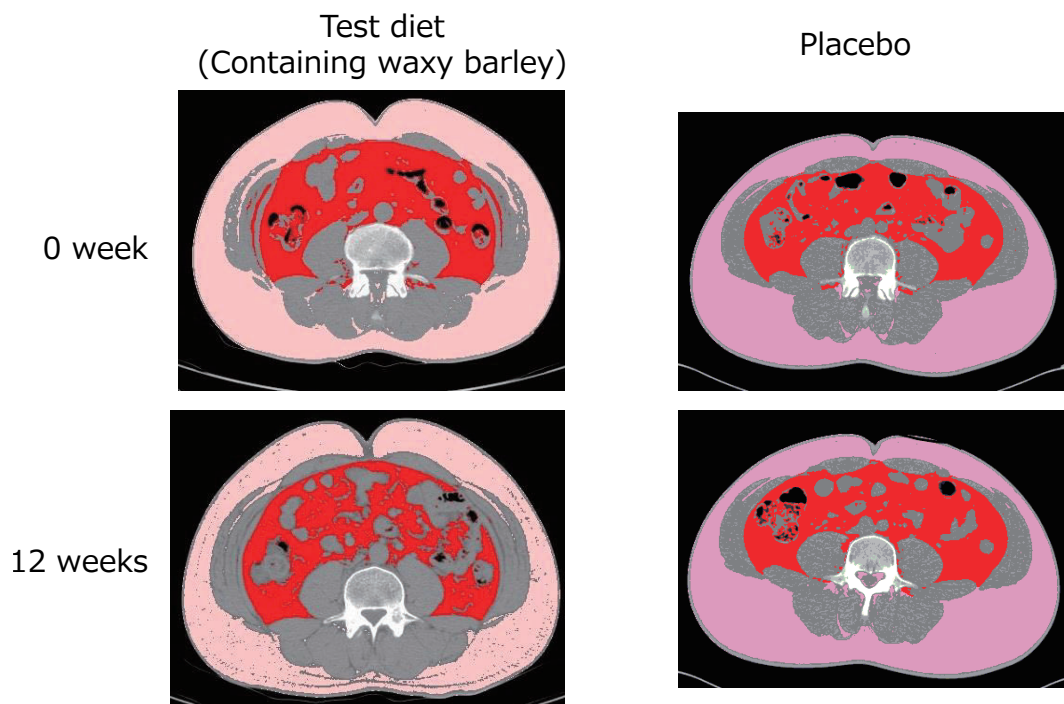
### Health benefits of waxy barley

The  $\beta$ -glucan level in the grains of waxy barley is higher than that in the grains of normal-starch barley (Ullrich et al. 1986); therefore, waxy barley is superior to

normal-starch barley as a functional food, and interest in waxy barley has increased in Japan owing to the public's awareness of its health benefits. The health functionality of waxy barley was verified by the NARO in collaboration with universities and hospitals in a research project on the health functionality of agricultural products conducted in 2013-2015. In this research, the intake of waxy barley was found to prevent elevated blood glucose levels after a meal and stabilize the daily blood glucose level (Aoe et al. 2018, Fujitani 2020). The daily intake of waxy barley was also found to improve the constipation of older Japanese (Taniguchi et al. 2017) and reduce the visceral fat level of Japanese with visceral fat obesity (Fig. 2; Aoe et al. 2017).

### Breeding of waxy barley in Japan

Waxy barley is native to East Asia (Banks et al. 1970). In Japan, some waxy landraces were formerly cultivated in limited areas of the coastal parts of the Seto Inland Sea in the Chugoku and Shikoku regions and the northern Kyushu region (Kashiwada 1930). However, these cultivars were found to have inferior agronomic traits, e.g., late maturity, long culm, low-yield, and poor resistance to lodging and diseases, including barley yellow mosaic virus and powdery mildew (The Institute



**Fig. 2.** Changes of in the area of visceral fat according to the daily intake of waxy barley for 12 weeks  
Red area shows visceral fat.

This figure was provided by Prof. S. Aoe of Otsuma Woman's University.

for Agricultural and Biological Sciences, Okayama University 1983, Ishikawa & Itoh 1997). The breeding of waxy barley was conducted in a breeding station in the former Chugoku National Agricultural Experiment Station in the 1950s, and such breeding restarted in 1987 in the former Shikoku National Agricultural Experiment Station; after which, other national and prefectural breeding stations also began breeding (Takahashi & Yasuda 1995, Ishikawa & Itoh 1997). However, these efforts had resulted to the release of only few cultivars in limited cultivation areas owing to the poor demand for waxy barley before the 2000s. Conversely, the breeding of waxy barley in the USA and Canada was accelerated in the 1980s (Bhatty 1995) in response to increased interest in the health benefits of  $\beta$ -glucan. From the 2000s, the breeding of waxy barley in Japan also accelerated in response to increased interest in waxy barley in other countries.

### Cultivars released by NARO in the national barley breeding program

To date, nine cultivars have been bred by NARO in the national barley breeding program (Table 1). These cultivars are amylose-free with the exception of Daishimochi and Mochishizuka. Representatives of these whole and pearled grain cultivars are shown in Figure 3. Except for Mochishizuka, the bred cultivars exhibit significantly higher  $\beta$ -glucan content than that of normal-starch cultivars (Fig. 4). Kusumochi Nijo and Kinumochi Nijo are two-rowed hulled cultivars with high-yield potential and resistance to disease that are suitable for cultivation in the Kyushu region (Sugita et al. 2017). Haneumamochi, Mochishizuka, and Kihadamochi are six-rowed hulled cultivars suitable for cultivation in the eastern part of Japan. Haneumamochi is an artificial waxy mutant derived from Fiber Snow (Aoki et al. 2017,

Seki et al. 2018), a commonly used normal-starch cultivar. Kihadamochi is a high-yield waxy cultivar bred using a foreign high  $\beta$ -glucan waxy germplasm; it is adaptable to cultivation in the Kanto and Tokai regions (Tonooka et al. 2022). Mochishizuka has a purple lemma and pericarp; thus, it is easily distinguished from other cultivars. Daishimochi, Kirarimochi, Waxy Fiber, and Fukumi Fiber are hull-less cultivars that are adaptable to cultivation in the west of the Kanto Region. Daishimochi was the first waxy cultivar bred by NARO to be released (Doi et al. 1999) and is now cultivated in many areas as a local product for local consumption (Saito & Yanagisawa 2009). Daishimochi also has a purple lemma and pericarp, which are characteristic of Japanese waxy landraces. Barley grains develop a brown color after-cooking, and this discoloration reduces consumer acceptance of barley. The discoloration is caused by the oxidation of catechin and proanthocyanidins contained in barley grains (Kohyama et al. 2001). Kirarimochi, Fukumi Fiber, and Kinumochi Nijo are proanthocyanidin-free cultivars that do not exhibit after-cooking discoloration (Fig. 5; Yanagisawa et al. 2011, Sugita et al. 2019); thus, these cultivars are expected to increase consumer acceptance of barley-based foods. Waxy Fiber and Fukumi Fiber exhibited significantly higher  $\beta$ -glucan content (>10%) than that of foreign waxy cultivars with high  $\beta$ -glucan levels, e.g., Azhul and CDC-Fibar (Fig. 4). Waxy Fiber has a mutant gene, *lys5.h*, in a plastidial ADP-glucose transporter that plays an important role in starch synthesis. Fukumi Fiber has a combination of *wax* (waxy) and *amol* (high amylose) genes that increases its  $\beta$ -glucan content (Fujita et al. 1999). Isoforms that carry *lys5.h* and *wax + amol* markedly exhibit reduced starch content compared with that of normal isoforms; this isoform also possesses a shrunken endosperm (Tonooka et al. 2010b). The effect of *lys5.h* and *wax + amol* on increasing  $\beta$ -glucan content is assumed to be due to compensation

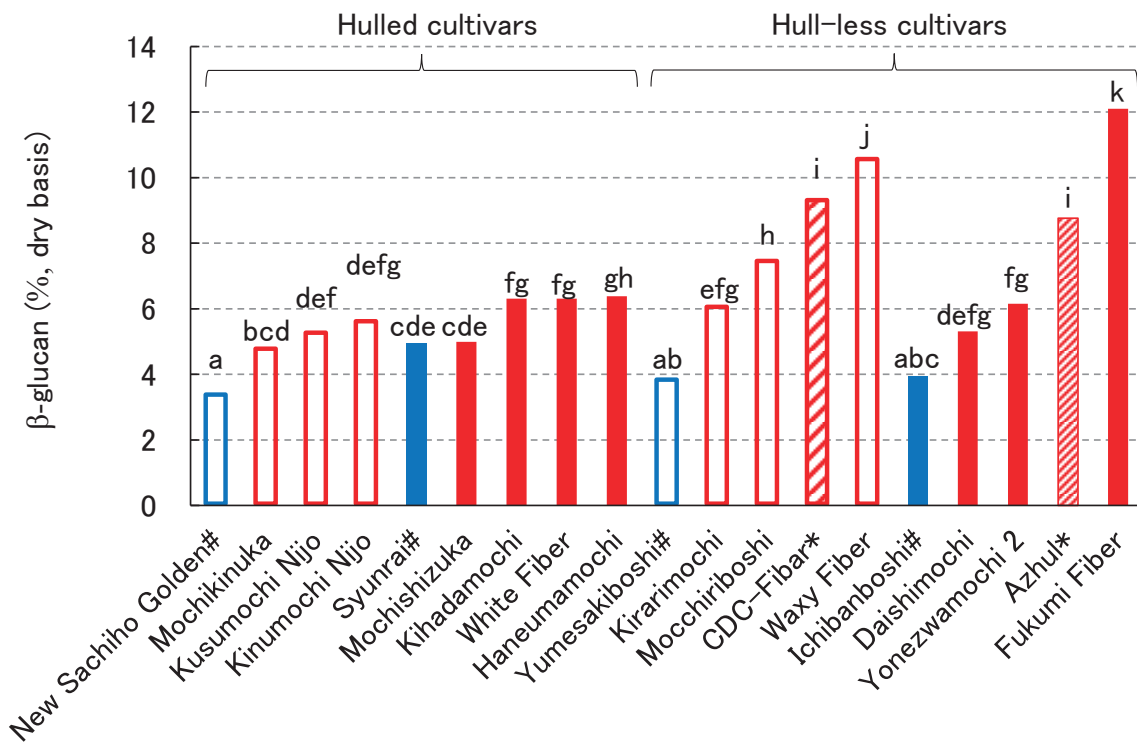
**Table 1. Waxy barley cultivars bred by NARO**

Cultivar	Release year	Hulled/Hull-less	Row type	Amylose type	Quality characteristics	Suitable area for cultivation
Daishimochi	1996	Hull-less	Six	Low-amylose	Purple lemma and pericarp	West of Kanto region
Kirarimochi	2009	Hull-less	Two	Amylose-free	Proanthocyanidin-free	West of Kanto region
Waxy Fiber	2014	Hull-less	Two	Amylose-free	High $\beta$ -glucan	West of Kanto region
Haneumamochi	2016	Hulled	Six	Amylose-free		Hokuriku and Tohoku regions
Kusumochi Nijo	2017	Hulled	Two	Amylose-free		Kyushu region
Mochishizuka	2018	Hulled	Six	Low-amylose	Purple lemma and pericarp	Tohoku region
Kihadamochi	2018	Hulled	Six	Amylose-free		Kanto and Tokai regions
Fukumi Fiber	2018	Hull-less	Six	Amylose-free	Proanthocyanidin-free, high $\beta$ -glucan	West of Kanto region
Kinumochi Nijo	2020	Hulled	Two	Amylose-free	Proanthocyanidin-free	Kyushu region



**Fig. 3. Whole and pearled grains of waxy barley cultivars bred by NARO**

A: Kusumochi Nijo; B: Kinumochi Nijo; C: Haneumamochi; D: Mochishizuka; E: Kihadamochi; F: Kirarimochi; G: Waxy Fiber; H: Daishimochi; I: Fukumi Fiber; Upper panels: whole grains; lower panels: pearled grains



**Fig. 4. β-glucan content of whole grains of waxy barley cultivars bred in Japan**

Mean values from the 2020/21 and 2021/22 seasons according to the Institute of Crop Science, NARO.

Open boxes and filled boxes show two- and six-rowed cultivars, respectively.

# Normal-starch and \* foreign cultivars are shown as blue boxes and by oblique lines, respectively.

Different letters indicate significant differences between mean values at the 5% level according to Tukey's test.

for a decrease in starch synthesis (Munck et al. 2004). Waxy Fiber and Fukumi Fiber possess harder grains and exhibit relatively low pearled whiteness; thus, their pearling performance and quality are reduced (Yanagisawa et al. 2016, Sugita et al. 2019). Therefore, these cultivars are suitable for use as barley flour-based functional foods rather than traditional rice extenders.

### Other cultivars bred in Japan

Other cultivars bred by prefectural agricultural experiment stations, a private company, and a diligent farmer are listed in Table 2. Setsugenmochi and White Fiber are six-rowed hulled cultivars bred by the Nagano Prefectural Agricultural Experiment Station in 1999 and 2016, respectively (Ushiyama et al. 2002, Maejima 2018). White Fiber is cultivated in Tosan and the southern part of the Tohoku region. Mochikinuka is a two-rowed hulled

cultivar bred by the Tochigi Prefectural Agricultural Experiment Station in 2017 (Yamaguchi et al. 2019) and is now cultivated only in Tochigi Prefecture. Mochikinuka is a proanthocyanidin-free cultivar that exhibits no after-cooking discoloration, lacks lipoxygenase (which catalyzes lipid oxidation), and has a lower offensive flavor and taste that is peculiar to barley (Yamaguchi et al. 2019). Agurimochi, Mocchiriboshi, and Fukei 1103 were bred by Sapporo Breweries Limited. in 1997, 2009, and 2015, respectively. Mocchiriboshi is cultivated only in the Saitama Prefecture. Fukei 1103 was cultivated in Hokkaido Prefecture as a spring barley. Yonezawamochi and Yonezawamochi 2 were bred by a diligent farmer, Mr. H. Yonezawa, using a Japanese waxy landrace as a breeding material (Yonezawa 1987). Yonezawamochi 2 has been cultivated for 36 years in a part of Hyogo Prefecture as a special product in the district (Tahata 1998). To date, Yonezawamochi, Agurimochi, Setsugenmochi, and Fukei 1103 have not been cultivated.



**Fig. 5. Boiled pearled grains incubated at 70°C for 18 h after boiling**

Left: Kirarimochi; Right: Ichibanboshi (Normal cultivar)  
This figure was provided by Dr. A. Takahashi of the Institute of Crop Science, NARO.

### Current production of waxy barley in Japan

Compared with imported waxy barley, the waxy barley cultivars bred in Japan have higher pearled whiteness and narrower crease (Fig. 6). In 2021, the domestic production of waxy barley cultivars bred by the NARO and other organizations led to 9,133 and 5,821 tons of produce, respectively. The domestic production of waxy barley has rapidly increased in Japan, and the extensive planting of bred cultivars raised the domestic self-sufficiency rate of waxy barley from 3 to 49% from 2016 to 2021 (Fig. 7), respectively. In Japan, waxy barley is used both as a rice extender and in convenience foods called “retort pouch foods,” cereal food products such as

**Table 2. Other waxy barley cultivars bred in Japan**

Cultivar	Release year	Hulled/Hulless	Row type	Amylose type	Suitable area for cultivation	Breeder
Yonezawamochi	1985	Hull-less	Six	Low-amylose	West of Kanto region	Mr. H. Yonezawa (A diligent farmer)
Yonezawamochi 2	1987	Hull-less	Six	Low-amylose	West of Kanto region	Mr. H. Yonezawa (A diligent farmer)
Agurimochi	1997	Hulled	Two	Low-amylose	Kanto region	Sapporo Breweries Ltd.
Setsugenmochi	1999	Hulled	Six	Low-amylose	Kanto and Tosan regions	Nagano Prefectural Agricultural Experiment Station
Mocchiriboshi	2009	Hull-less	Two	Low-amylose	Kanto region	Sapporo Breweries Ltd.
Fukei 1103	2015	Hull-less	Two	Amylose-free	Hokkaido region	Sapporo Breweries Ltd.
White Fiber	2016	Hulled	Six	Low-amylose	Hokuriku, Tohoku and Tosan regions	Nagano Prefectural Agricultural Experiment Station
Mochikinuka	2017	Hulled	Two	Amylose-free	Kanto region	Tochigi Prefectural Agricultural Experiment Station

granola, nutritional supplements, bread, noodles, and baked confectioneries made from waxy barley flour. However, all domestic waxy barley cultivars, except those carrying *lys5.h* and *wax + amol*, exhibit lower  $\beta$ -glucan content than that of imported waxy barley (Tonooka et al. 2018). Therefore, to expand the domestic waxy barley market, waxy cultivars with higher  $\beta$ -glucan levels than those of foreign cultivars must be bred without degrading pearling quality.



Fig. 6. Pearled grains from domestic (left, cv. Kirarimochi) and imported (right) waxy barley products

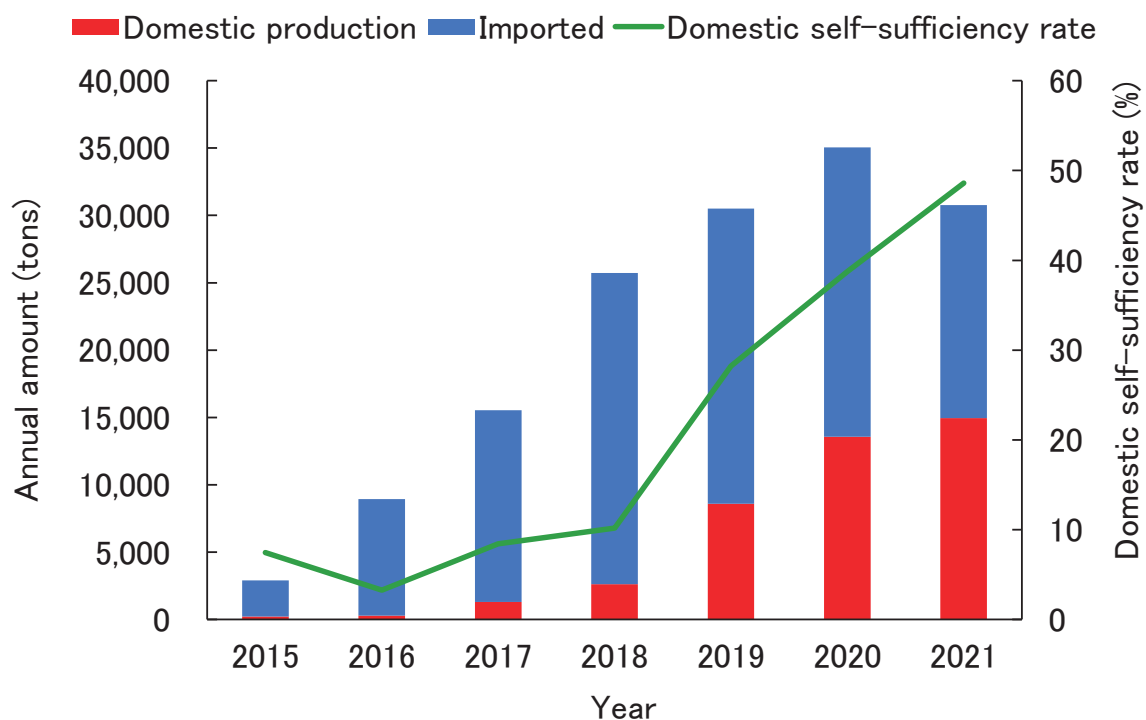


Fig. 7. Change in the domestic production and import of waxy barley in Japan from 2015 to 2021

## Conclusions

NARO has bred nine waxy barley cultivars in the national barley breeding program, and eight cultivars have been bred in other sectors. Eight of the cultivars bred by NARO and four cultivars bred by the other sectors are now cultivated in Japan. In 2021, 14,954 tons of waxy barley was produced in Japan and the domestic self-sufficiency rate was increased to 49%. Moreover, the bred waxy cultivars now have a wide range of uses in barley-based foods. Waxy cultivars with higher  $\beta$ -glucan levels and without after-cooking discoloration must be bred to expand the domestic waxy barley market.

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