JARQ 59 (4), 253-260 (2025) https://www.jircas.go.jp

Hanaemaki and Sansanmaru: Low Amylose Content Rice Cultivars Suitable for Direct Seeding Cultivation in Hokkaido

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Abstract

Hanaemaki and Sansanmaru are rice cultivars suitable for direct seeding cultivation in Hokkaido, because they show high levels of lodging resistance and grain yield. The grain amylose contents of Hanaemaki and Sansanmaru are approximately 10% and 16%, respectively, which are lower than those of other cultivars for direct seeding cultivation. Because of their lower levels of amylose content, these cultivars showed higher eating quality of cooked rice in the sensory test compared to other cultivars. Thus, the low amylose content trait may be important for improving the eating quality of rice cultivars used in direct seeding cultivation in Hokkaido, as is the case with cultivars in transplanting cultivation.

Discipline: Crop Science
Additional key words: rice breeding, eating quality of cooked rice

Introduction

In Japan, rice has been grown mostly by using transplanting cultivation. However, direct seeding could be an alternative cultivation method because it reduces cultivation costs and labor (Sasaki et al. 2005, Yasumoto et al. 2017). According to a report by the Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF), direct seeding cultivation reduces working hours and cultivation costs by approximately 20% and 10%, respectively, compared to transplanting cultivation (MAFF 2008). Recently, in Japan, the number of large-scale farmers has rapidly increased, along with the reduction of the number of farmers and the accumulation of farming land by core farmers (Hiraishi 2009). Nihei (1991) reported that approximately 20 ha is the upper

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Received 28 August 2024; accepted 10 February 2025; J-STAGE Advanced Epub 30 July 2025.
https://doi.org/10.6090/jarq.24S08

limit of acreage for family rice farming in Hokkaido when only transplanting cultivation is used. Large-scale rice farmers have tended to introduce direct seeding (Nakamura & Koshiyama 2018, Sasaki et al. 2005) to not only reduce costs but also efficiently manage their paddy fields. On the other hand, improving the eating quality of rice has been a challenge in Hokkaido, with recent cultivars having attained higher levels (Fujino 2019). Cultivars with good eating quality are medium-maturing cultivars (Yoshimura 2002); in this regard, they are not suitable for direct seeding cultivation except for some warm areas in Hokkaido, where early maturity is required for direct seeding cultivation due to the short growing period (Sato et al. 2006). Therefore, the eating quality of cultivars suitable for direct seeding is low compared to that of medium-maturing cultivars. Improvements in the eating quality of medium-maturing cultivars have been achieved by conferring low-amylose content characteristics ascribed to low-amylose genes and QTLs, such as *Wx1-1*, *qAC9.3*, or *du* (Ando et al. 2010, Kunihiro et al. 1993). It is expected that introducing low-amylose genes could also be effective in improving the eating quality of early-maturing cultivars. Based on this concept, the Hokkaido Agricultural Research Center (HARC), NARO, developed the Hanaemaki cultivar with a low amylose trait (dull) derived from NM391 (a mutant of Nihonmasari) and Sansanmaru harboring *Wx1-1*. In this paper, we report the breeding process and agronomic traits of these two cultivars.

Experimental methods

1. Growth conditions for direct seeding

(1) Direct seeding cultivation

The seeding dates were May 14th (five-year average) for Hanaemaki and May 15th (four-year average) for Sansanmaru. They were sown by seed-tape in a submerged field: one plot consisted of four rows with a length of 4 m with a row spacing of 0.3 m, and the seeding density was 400 seeds per m^2 for Hanaemaki, with a row spacing of 0.2 m, and the seed density was 250 seeds for Sansanmaru. They were arranged as a randomized block design with two replicates. Plants were harvested from plots with a size of 1.7 m^2 for Hanaemaki or 1.6 m^2 for Sansanmaru.

(2) Transplanting cultivation

The transplanting dates were May 21st (four-year average) for both Hanaemaki and Sansanmaru. One plot consisted of three rows with a length of 2 m, a row spacing of 0.33 m, and a plant spacing of 0.125 m. They were arranged as a randomized block design with two replicates. Plants were harvested from plots with a size of 2 m^2 for both cultivars.

2. Sensory test of eating quality

Stickiness, hardness, and overall evaluation, which are considered to be related to the amylose content, were evaluated. The test was conducted during experimental periods of 1998 and 2000-2002 for Hanaemaki and 2014-2017 for Sansanmaru in Sapporo. Each trait was scored (−3 to +3) by comparison with a reference cultivar, where the score of the reference was rated 0. The average number of judges for the experimental periods was 19 for Hanaemaki and 22 for Sansanmaru.

3. QTL analysis for low amylose content of Hanaemaki

Two hundred F_2 individual plants obtained from a cross of Hatsushizuku and Hanaemaki grown in a greenhouse were provided for the analysis. Based on the amylose contents of the F_3 grains produced on each F_2 plant, 29 genotypes with extremely low values, 49 genotypes with extremely high values, and 16 genotypes with intermediate values were subjected to selective genotyping analysis (Yoo et al. 2007). Linkage map construction and QTL analysis were conducted by using the MAPL package program (Ukai et al. 1995).

Breeding process

1. Hanaemaki

Hanaemaki was derived from a cross between the F_1 of Dohoku 53/Norin-PL11 and Kuiku139 (later Yukimaru) (Fig.1(a)). Dohoku 53 is a breeding line that harbors a dull gene inherited from NM391 through Eikei 84271 (Tanno et al. 1997). Norin-PL11 is a parental line with extreme tolerance to cooling injuries at the booting stage (Murai et al. 2003). Kuiku 139, which was later registered as Yukimaru, is an early-maturing line used for direct seeding cultivation. Crossing was performed to combine these traits in 1992 at HARC, Sapporo. Following the F_2 bulk nursery, the pedigree method was employed to advance their generation. After completing yield and local adaptability trials, Hanaemaki was registered in 2006.

2. Sansanmaru

Sansanmaru was derived from a cross between Satsukei 08037 and Satsukei 06007 (Fig. 1(b)). Satsukei 08037 and Satsukei 06007 are breeding lines: the former is characterized by early maturity, short stature, and good eating quality ascribed to *qAC9.3*; the latter is characterized by early maturity, short stature, and good eating quality ascribed to *Wx1-1*. Crossing was conducted

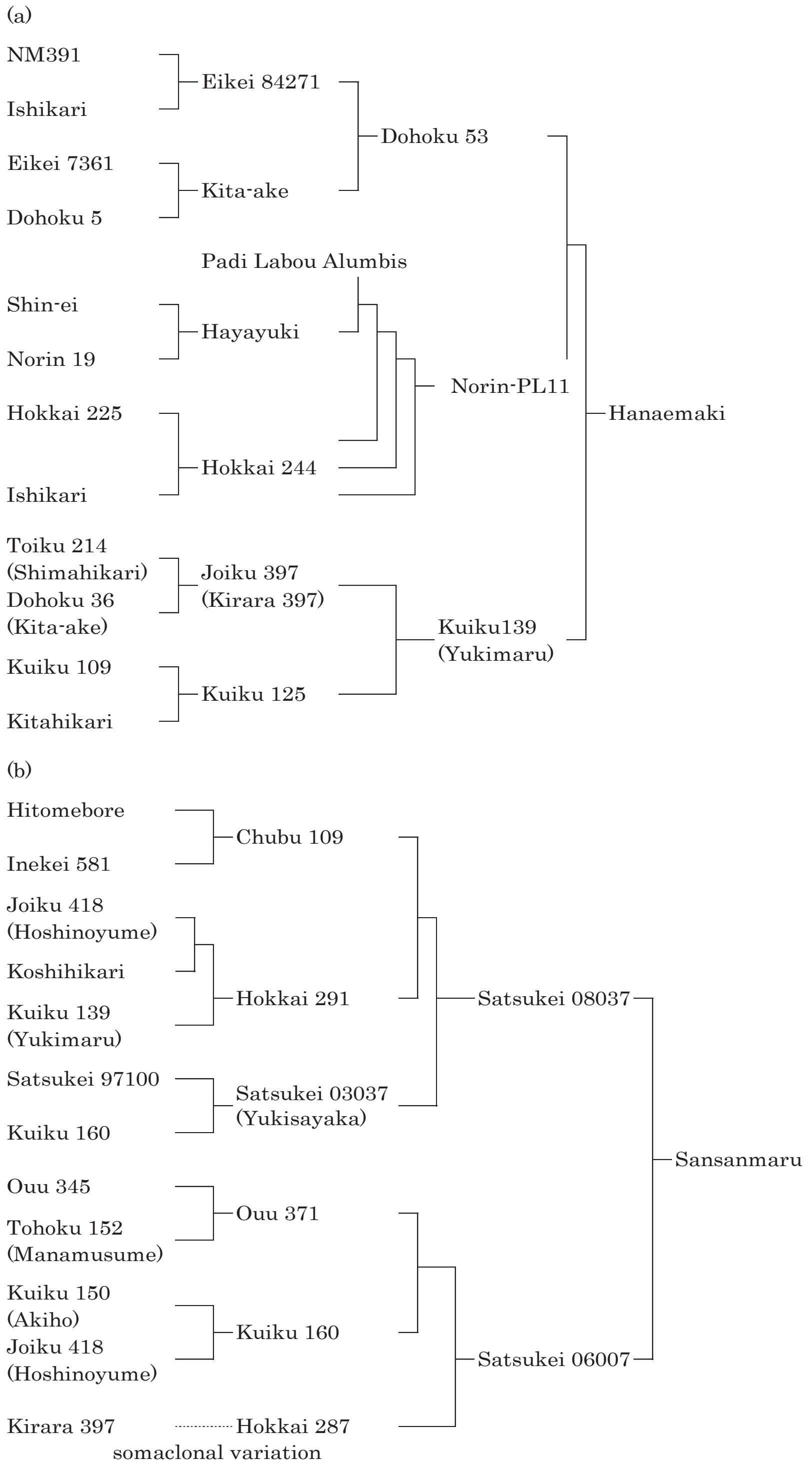

Fig. 1. Genealogy of Hanaemaki (a) and Sansanmaru (b)

in 2008 to combine these traits at the Tropical Agriculture Research Front (TARF), Japan International Research Center for Agricultural Sciences (JIRCAS), Ishigaki, Japan. F_1 plants were grown at HARC, Sapporo, in 2009, followed by the growth of F_2 and F_3 bulk populations at TARF in 2010. From 2011, their descendants were advanced in Sapporo according to the pedigree method. After completing yield and local adaptability trials, Sansanmaru was registered in 2022.

Major agronomic characters

1. Hanaemaki

Table 1 shows a comparison of agronomic traits on direct seeding cultivation between Hanaemaki and Yukimaru (a major cultivar for direct seeding cultivation around the time Hanaemaki was developed). Compared to Yukimaru, Hanaemaki showed significantly lower seedling emergence (57%), which is not considered to be a significant problem in practical use; the maturation date was not significantly different; the culm length was significantly longer by 5 cm, however, the lodging index was not significantly different; the brown rice weight was similar in spite of significantly lower values of the 1,000-kernel weight; the grain amylose content of 10.2% was significantly lower by 6.6%; the grain protein content of 6.5% was significantly lower by 0.6%.

2. Sansanmaru

Table 2 shows a comparison of agronomic traits on direct seeding cultivation between Sansanmaru and Hoshimaru (a major cultivar for direct seeding around the time Sansanmaru was developed). Compared to Hoshimaru, Sansanmaru showed similar values of seedling emergence and maturation date; the culm length of 62 cm was shorter by 6 cm, though the difference was not significant; lodging index showed similar values; the brown rice weight was significantly higher by 0.58 t/ha; the 1,000-kernel weight was significantly lower by 0.9g; the grain amylose content of 15.9% was significantly lower by 3.9%; the grain protein content of 6.0% was lower by 0.3%, though the difference was not significant.

3. Early maturing habit and amylose content of transplanted Hanaemaki and Sansanmaru

Hanaemaki and Sansanmaru were compared with Hoshinoyume and Nanatsuboshi, which were medium-maturing leading cultivars with good eating quality around the time they were developed with transplanted cultivation, respectively (Table 3). Compared to Hoshinoyume, Hanaemaki headed significantly earlier; it matured earlier by 3 days, though the difference was not significant. Compared to Nanatsuboshi, Sansanmaru headed significantly earlier; it matured significantly earlier by 11 days. Regarding the amylose content, the values of Hanaemaki (8.2%) and Sansanmaru (12%) were significantly lower than those of comparative cultivars (19.7% and 18.7%). Furthermore, the amylose content of Sansanmaru was significantly lower than that of direct seeding cultivation at the 1% level (12.0% vs

Table 1. Agronomic traits of Hanaemaki and the reference cultivar Yukimaru on direct seeding cultivation

Cultivar	Seedling emergence (%)	Heading date	Maturation date	Culm length (cm)	Panicle length (cm)	Number of panicles (/m^2)	Brown rice weight (t/ha)	Lodging index (0-5)	1,000-kernel weight (g)	Grain amylose content (%)	Grain protein content (%)
Hanaemaki	57	8.09	9.25	66	16.1	734	5.02	1.5	21.2	10.2	6.5
Yukimaru	68	8.07	9.22	61	14.3	843	4.98	1.9	21.5	18.8	7.1
t-test	*			**	*	**			*	**	*

The five-year average values during the experimental periods (1998-2002) in Sapporo are shown.
Seeding date: 5.14 (five-year average)
Seeding rate: 400 seeds/m^2
Basal dressing: N 100, P_2O_5 61, K_2O 42 kg/ha
*, **: Values are significantly different at 5% or 1% levels, respectively.

Table 2. Agronomic traits of Sansanmaru and the reference cultivar Hoshimaru on direct seeding cultivation

Cultivar	Seedling emergence (%)	Heading date	Maturation date	Culm length (cm)	Panicle length (cm)	Number of panicles (/m^2)	Brown rice weight (t/ha)	Lodging index (0-5)	1,000-kernel weight (g)	Grain amylose content (%)	Grain protein content (%)
Sansanmaru	71.3	8.05	9.14	62	15.7	688	6.37	0.2	24.1	15.9	6.0
Hoshimaru	70.1	8.05	9.14	68	15.8	714	5.79	0.2	25.2	19.8	6.3
t-test							*		*	**	

The four-year average values during the experimental period (2014-2017) in Sapporo are shown.
Seeding date: 5.15 (four-year average)
Seeding rate: 250 seeds/m^2
Basal dressing: N 100 (2014-2016) or 80 (2017), P_2O_5 85 (2014) or 97 (2015-2017), K_2O 60 (2014) or 69 (2015-2017) kg/ha
*, **: Values are significantly different at 5% or 1% levels, respectively.

15.9%), while the difference was not significant in the case of Hanaemaki (8.2% vs 10.2%). The early maturing habit and low levels of grain amylose content of both cultivars were confirmed in the transplanted cultivation as well as in the direct seeding cultivation.

Eating quality of cooked rice of Hanaemaki and Sansanmaru

Table 4 shows the eating quality of cooked rice for Hanaemaki and Sansanmaru with direct seeding cultivation as evaluated by sensory tests. Both cultivars showed significantly higher stickiness, softness, and overall evaluation compared to reference cultivars.

QTL analysis for low amylose content of Hanaemaki

The QTL analysis revealed that the locus conferring low amylose content in Hanaemaki differs from the dull genes previously reported (Yano et al. 1988), because the QTL was located on the distal part of the long arm of chromosome 6 (Table 5; Fig. 2(a)). The QTL was observed in the vicinity of the SSR markers RM7555 and RM2099, further, in the 200 individuals investigated, genotypes of EM7555, including Hanaemaki homozygote, Hatsushizuku homozygote, and heterozygote, fit phenotypes of low, high, and intermediate amylose content (Fig.2(b)), thus, these markers could be useful for selecting the low-amylose-content genotype originated from NM391.

Discussion

Hanaemaki and Sansanmaru are rice cultivars suitable for direct seeding cultivation in Hokkaido, because they show high levels of lodging resistance and grain yield (Tables 1, 2). Lodging resistance is one of the traits required for the direct seeding cultivation of rice. The high resistance of Sansanmaru can be attributed to its short culm length (Table 2). We speculate that the short stature could be the result of the accumulation of genes responsible for the short culm length of the parental breeding lines, Satsukei 08037 and Satsukei 06007.

For direct seeding of rice, seedling establishment at low temperatures is important (Ogiwara & Terashima

Table 3. Early maturing habit and amylose content of Hanaemaki and Sansanmaru on transplanting cultivation

Cultivar	Heading date	Maturation date	Amylose content (%)	Transplanting date
Hanaemaki	7.26	9.15	8.2	5.21
Hoshinoyume	8.01	9.18	19.7	
t-test	**		**	
Sansanmaru	7.21	9.05	12.0	5.21
Nanatsuboshi	7.30	9.16	18.7	
t-test	**	**	**	

The four-year average values during the experimental periods (1999-2002 for Hanaemaki and 2014-2017 for Sansanmaru) in Sapporo are shown, except for the amylose content of Hanaemaki. The value of the amylose content of Hanaemaki was obtained from 1999 to 2001.
Basal dressing: N 100 (2014-2016) or 80 (2017), P_2O_5 85 (2014), K_2O 60 (2014) kg/ha
Hoshinoyume and Nanatsuboshi are the leading varieties of medium maturing with good eating quality during the experimental periods.
**: Values are significantly different at the 1% level.

Table 4. Sensory test of cooked rice for Hanaemaki and Sansanmaru on direct seeding cultivation

Cultivar	Stickiness [a, b]	Hardness [a, b]	Overall evaluation [a, b]	Reference cultivar
Hanaemaki	1.80*	–1.60**	0.53*	Hoshinoyume
Sansanmaru	0.70**	–0.57*	0.22*	Nanatsuboshi

[a] Each trait was scored (–3 to +3) by comparing it with the reference cultivar, where the score of the reference was rated 0.
[b] The four-year average values during the experimental periods (1998 and 2000-2002 for Hanaemaki and 2014-2017 for Sansanmaru) in Sapporo are shown.
The average number of judges during the experimental period was 19 for Hanaemaki and 22 for Sansanmaru.
*, **: Values are significantly different from the references at 5% or 1% levels, respectively.

Table 5. QTL analysis for low amylose content of Hanaemaki

Map interval	Chromosome	LOD	R^2 (%)
RM7555-RM20669 [a]	6L	40.96	78

[a] SSR marker (International Rice Genome Sequencing Project & Sasaki 2005)

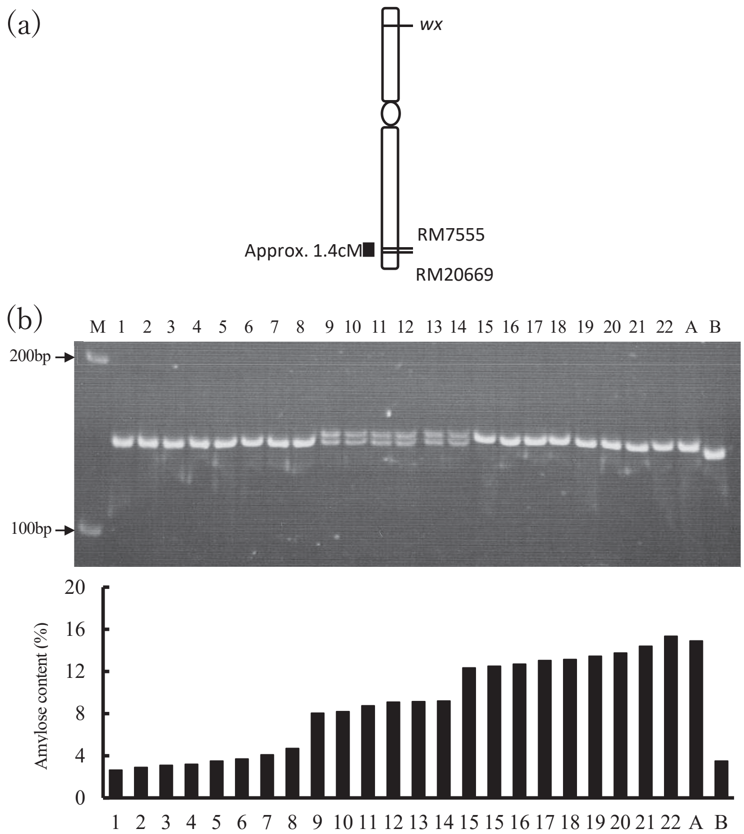

Fig. 2. Location of the QTL on Chromosome 6 associated with the low amylose content trait of Hanaemaki (a) and Comparison of the RM7555 genotypes and amylose contents in the Hatsushizuku/Hanaemaki F_2 population (b)

M: molecular marker; 1-22: F_2 individuals; A: Hatsushizuku; B: Hanaemaki

2001), especially in Hokkaido. Recently, Emimaru, a new rice cultivar with good seedling establishment, has been developed (Kiuchi et al. 2023). Compared to Emimaru, the seedling emergence of Hanaemaki and Sansanmaru was insufficient; therefore, the combination of low amylose content and good seedling establishment will be the next step for rice breeding for direct seeding cultivation in future breeding.

The scores for total evaluation, stickiness, and softness of cooked rice of Hanaemaki and Sansanmaru were significantly higher than those of the reference cultivars (Table 4). Furthermore, the amylose contents of both cultivars were lower than those of the reference cultivars. It could be inferred that the lower levels of amylose content contributed to the improvement of eating quality in the direct seeding cultivation in Hokkaido, as is the case with cultivars in transplanting cultivation. The degree of acceptance of low-amylose cultivars by consumers varies depending on the amylose content. Low-amylose cultivars like Aya, having an amylose content of around 10%, are not always accepted by consumers, because some feel the cooked rice of such cultivars is too sticky (Kunihiro et al 1993). The amylose content of Hanaemaki is also around 10%, so it is not thought to be always accepted by consumers. On the other hand, the stickiness of Sansanmaru, which harbors

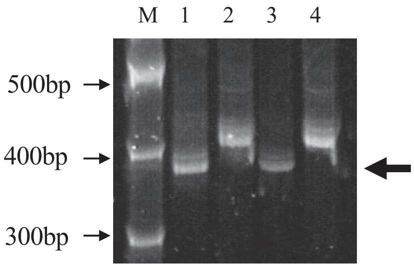

Fig. 3. PCR assay for *Wx1-1* in Sansanmaru with Wx-U1L3 primer (Ando et al. 2010)
The specific *Wx1-1* band is denoted by a thick arrow. M: molecular marker; 1: Sansanmaru; 2: Hoshimaru; 3: Oborozuki; 4: Nanatsuboshi

Wx-1 (Fig. 3), is thought to be generally accepted by consumers because of its moderately low amylose content (approximately 16 %). Regarding Sansanmaru, the effect of temperature during the ripening stage on amylose content should be taken into consideration. The amylose content of rice grains decreases at higher temperatures during the ripening stage (Resurreccion et al. 1977, Tateyama et al. 2005). In the case of growing Sansanmaru by transplanting cultivation, heading and maturity dates were earlier by about 2 weeks compared to direct seeding cultivation because of the early maturing trait, resulting in suffering higher temperatures during ripening and having lower levels of grain amylose content (Tables 2, 3). Thus, to attain amylose content that is acceptable to the majority of consumers, it is better to grow Sansanmaru by direct seeding. Hanaemaki and Sansanmaru showed lower levels of grain protein content (Tables 1, 2), which also might contribute to their development of good eating quality, because the trait is preferable for the eating quality of rice (Inatsu 1988).

The QTL related to low amylose content in Hanaemaki, which is considered to be inherited from NM391 through Dohoku 53 and Eikei 84271, is considered to be identical to *du12* in Milyang262 (Kiswara et. al. 2014), because Milyang262 is also a progeny of NM391, and the location of *du12* is almost identical to that of Hanaemaki.

Acknowledgements

We wish to thank personnel from TARF, JIRCAS, and all technical support staff of our rice breeding group in HARC. This work was supported by a grant for “breeding and integrated research toward enhancing the consumption of domestic farm products in the food service industry: 4. rice” from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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