4. Japan

Creation of Materials for Breeding Amylose Library of Primary Rice Varieties

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4.1 Introduction

Low-amylose rice holds promise for increased use in applications such as aseptically packed rice. The Institute of Radiation Breeding has raised mutant strains of rice varieties such as Norin 8 and Reimei with various amylose mutations, and has provided many of the breeding mother plants of existing low-amylose varieties. The institute is also working on raising isogenic lines of Koshihikari that relate to the low-amylose genes of these varieties. The purpose of this research is to induce mutations by means of gamma rays and other radiation to create near isogenic lines (NILs) with amylose-content gradients of about 2% from primary rice varieties, Koshihikari and Hitomebore.

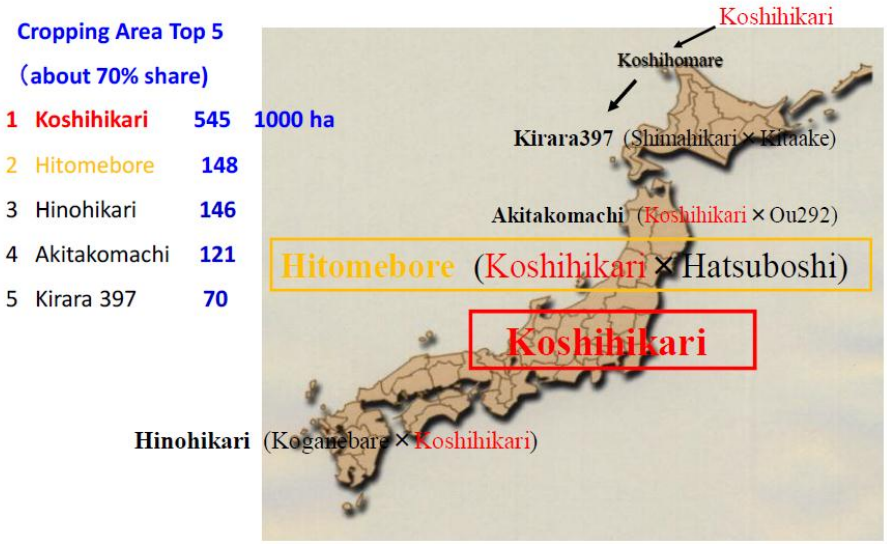

Figure 1. Main rice varieties in Japan

Figure 2. Low-amylose varieties for pre-cooked rice

4.2 Materials and Methods

(1) Creation of Koshihikari near isogenic lines (NILs) using existing amylose mutants

(2) Selection of amylose-content mutants from primary varieties such as Koshihikari and Hitomebore.

(3) Evaluation of amylose mutants

Cultivate under various conditions to evaluate the phenotype of the amylose library created in the project.

Table 1. Specific research methods and other actions taken throughout the research term

Year	Research objective	Research method	Expected achievements
2006	Raise Koshihikari NILs relating to amylose	Use existing amylose variants	Broadened amylose variants
2007-2010	Screen amylose variants from primary varieties	Use mutagens such as γ rays , ion beams and EMS treatment	Creation of amylose library
2011-2012	Evaluate amylose library	Evaluate amylose library under various cultivation conditions	Completion of amylose library

4.3 Results and Discussion

4.3.1 Creation of Amylose Library of Koshihikari

We created the amylose library consisted of mutants backcrossed with original variety, Koshihikari. Furthermore, we continued to screen amylose variants from Koshihikari. Consequently, we obtained NILs consisted of $F_{3\text{-}5}$ lines backcrossed with Koshihikari and newly selected mutants to be added to this library induced by EMS treatment and gamma-ray irradiation. We developed amylose library consisted of 29 lines. As a result, we completed raising amylose library consisted of 29 mutant strains with amylose-content gradients of about 2% (Fig.1). Amylose contents of mutants and NILs were determined by absorption spectrophotometry method in 2011. In 2012, we will try the evaluation of the response of amylose content of NILs to the temperature during grain-filling period.

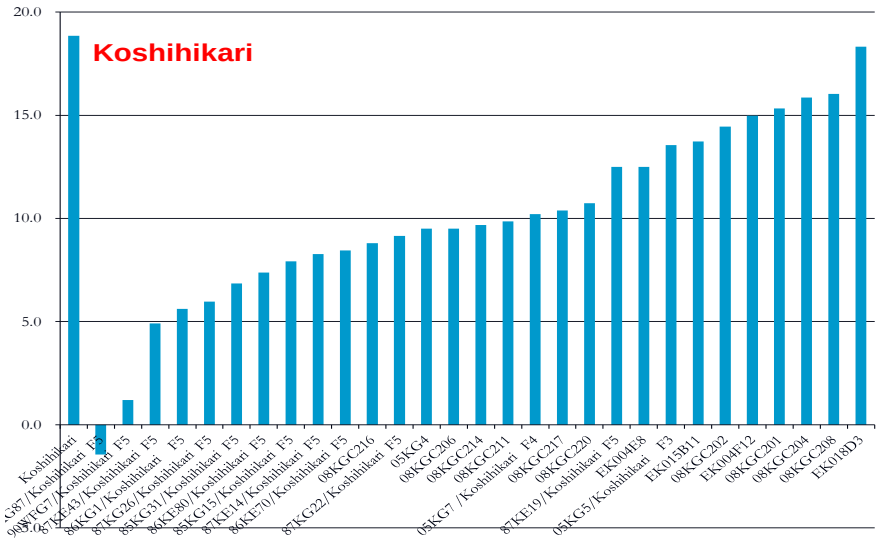

Figure 3. Koshihikari NILs relating to amylose content in 2011.

4.3.2 Selection of new mutants and raising NILs with low amylose content from Hitomebore

Hitomebore NILs were created by backcrossing with an original variety and ten mutants with waxy or low amylose contents were newly selected. The generations of these mutants are M_7 to M_{10} in 2013.

Ion beam irradiation was conducted at the AVF cyclotron in the Takasaki Advanced Radiation Research Institute of the Japan Atomic Energy Agency. The ion particles used were $^{12}C^{5+}$ (220 MeV), $^{12}C^{6+}$ (320 MeV), and $^{4}He^{2+}$ (100 MeV). Gamma-ray irradiation was conducted at the Institute of Radiation Breeding using a 10 Gy/h dose rate.

We obtained NILs consisted of 12 $F_{5\text{-}6}$ lines backcrossed with Hitomebore, No.2 variety in Japan and newly selected 10 mutants to be added to this library induced by ion beam and gamma-ray irradiation. In culm length, some lines of NILs were shorter than original variety in spite of backcrossed by Hitomebore. Amylose contents of mutants and NILs were determined by absorption spectrophotometry method in 2011.

Amylose content (%)

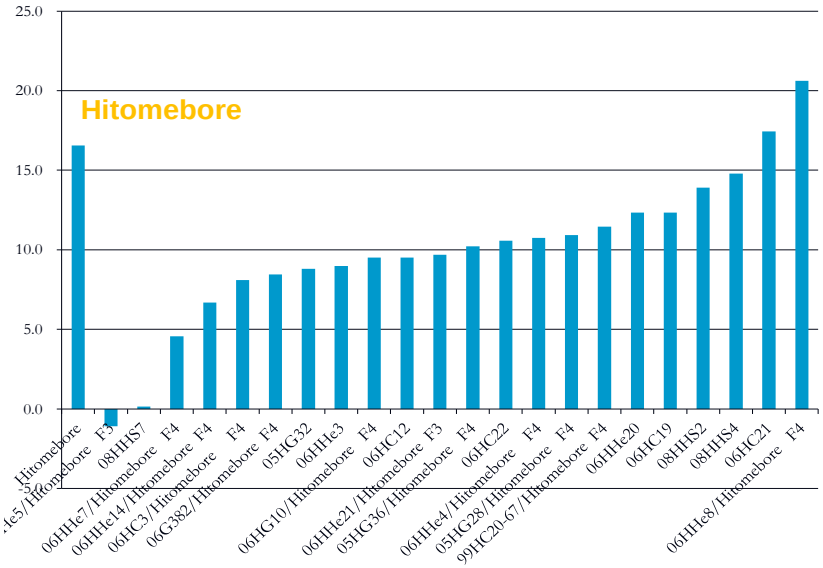

Figure 4. Hitomebore NILs relating to amylose content in 2011.

4.3.3 Analysis of temperature response of low amylose mutant strains

The mutant strains developed from parental Koshihikari were cultivated a in a double cropping system (planted on May 16 and transplanted on June 14 in 2012) with various grain-filling temperatures. The fully matured grains were evaluated for endosperm amylose content.

In general, the temperature in August of 2012 was higher than that of 2011, therefore, amylose content of mutant strains were relatively lower. As shown in Fig.5, the strains with relatively low amylose content (around 5%) were stable throughout the range of tested temperatures. On the other hand, the strains with an amylose content around 10% were classified into two types of stable and sharp response to the temperature. There are mutant strains that have an amylose content of about 12%, which is less than that of standard non-glutinous varieties. Some of them have a relatively

stable temperature response and they could be promising candidates for the further breeding material.

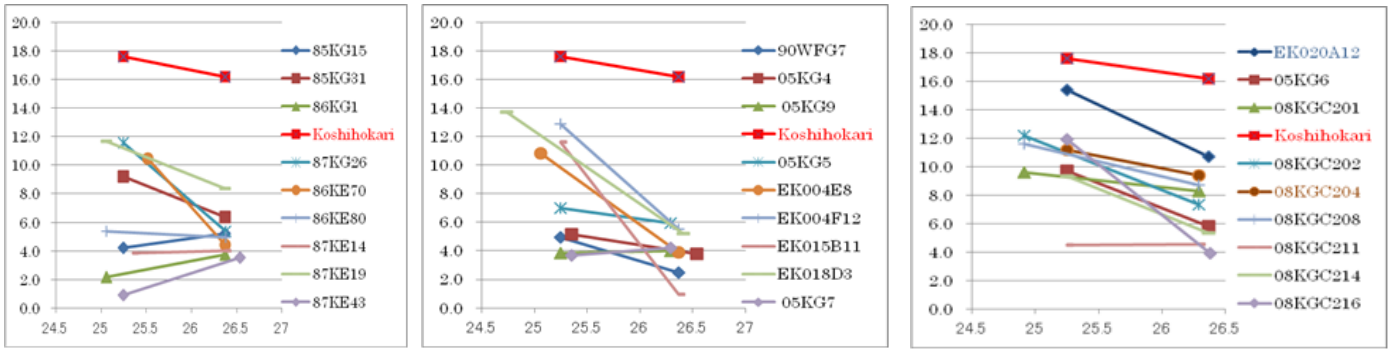

Figure 5. Response of amylose content to the temperature in Koshihikari NILs in 2012.
(vertical scale: amylose content %, horizontal scale: mean temperature during 20 days after heading)

4.4 Conclusion

We completed amylose library consisted of Koshihikari and Hitomebore NILs with amylose-content gradients of about 2%. They will be used for the genetic analysis in the new MAFF project from 2013. We also analyzed the temperature response of low amylose mutant strains.