JARQ 27, 102-105 (1993)

New Lines Harboring *du* Genes for Low Amylose Content in Endosperm Starch of Rice

Kazutoshi OKUNO, Tsukasa NAGAMINE[*1], Mitsunori OKA[*2], Makoto KAWASE[*3], Masumi KATSUTA, Yoshinobu EGAWA[*4] and Masahiro NAKAGAHRA

Department of Genetic Resources I, National Institute of Agrobiological Resources (Tsukuba, Ibaraki, 305 Japan)

Abstract

Two lines of rice, Tankei 1915 and 2019 with a low amylose content were registered as breeding materials in 1992. There lines were selected in the progeny following the treatment of the Japanese rice cultivars, Kinmaze and Kochihibiki with methyl-nitroso-urea (MNU). Tankei 1915 was characterized by a dull endosperm which could be distinguished from waxy and nonwaxy endosperms. The amylose content of Tankei 1915 was 9.2%, half of the content of the parental cultivar, Kinmaze. The line harbors the *du-1* gene which is responsible for lowering the amylose content and is located at a locus independent of the waxy (wx) locus on Chromosome 6. The other line, Tankei 2019, was characterized by a dull endosperm which is visually similar to the waxy endosperm. The amylose content of Tankei 2019 was 6.3%, one-third of the content of the parental cultivar, Kochihibiki. This line harbors the *du-2* gene which is responsible for the decrease of the amylose content and is located at a locus independent of the wx and *du-1* loci. The new type of endosperm starch of rice grains may contribute to the improvement of the eating quality of cooked rice and the development of new products in the food industry.

Discipline: Crop production
Additional key words: dull endosperm, grain quality, *Oryza sativa*, starch property

Introduction

Starch which is abundant in reserve tissues of plants, is a major polysaccharide consisting of amylose and amylopectin molecules. Amylose is a long linear chain primarily formed by α-1,4 glucosidic linkages with a small number of branching points. Amylopectin has a branched structure with α-1,4 and α-1,6 glucosidic linkages. Starch in the nonwaxy endosperm contains both amylose and amylopectin, whereas starch in the waxy endosperm contains only amylopectin and lacks amylose, or contains a small amount of amylose.

Waxy and nonwaxy endosperms of rice are controlled by the *wx* locus on Chromosome 6. The two different alleles, Wx^a and Wx^b, at the *wx* locus regulate differently the *Wx* gene expression which is closely related to amylose production in rice endosperms[7]. Compared to Wx^b, Wx^a markedly increases the level of starch granule-bound *Wx* protein which is the gene product of the *wx* locus as well as amylose content. Wx^a is common to wild species and Indica cultivars of rice, whereas Wx^b is widely distributed in Japonica cultivars[8].

Since the late 1970s, new starch characteristics of

Present address:
[*1] Okinawa Subtropical Station, Japan International Research Center for Agricultural Sciences (JIRCAS) (Ishigaki, Okinawa, 907 Japan)
[*2] Research Information Division, JIRCAS (Tsukuba, Ibaraki, 305 Japan)
[*3] Department of Crop Breeding, Shikoku National Agricultural Experiment Station (Zentsuji, Kagawa, 765 Japan)
[*4] Biological Resources Division, JIRCAS (Tsukuba, Ibaraki, 305 Japan)

rice endosperm have been selected in the progeny treated with radiations and chemicals[4-6,9]. A mutant expressing dull endosperm was characterized by a low content of amylose and by the absence of remarkable changes in amylopectin in endosperm starch[4]. Genetical analysis led us to conclude that the decrease in the amylose content in the dull mutant was controlled by a *du* gene which is located at a locus independent of the *wx* locus on Chromosome 6[4]. This finding indicated the existence of new genes related to amylose production in rice endosperm. Five *du* loci controlling the differences in the amylose content have been identified so far[10], suggesting that the combination of different *du* genes with the *Wx* gene could broaden the genetic variation of amylose content in endosperm starch of rice.

The amylose content in endosperm starch is considered to be one of the major determinants of eating, cooking and processing qualities of rice grains. In particular, rice breeders in Japan have centered their attention on the development of new rice cultivars with low amylose content in recent years. The authors have induced low amylose mutants by using chemical mutagens and investigated their characteristics in the following generations.

This paper reports the characteristics of low amylose lines and their use in rice breeding.

A low amylose line bearing *du-1*, Tankei 1915

A low amylose line, Tankei 1915, was bred from the progeny of the cross between dull and floury mutant lines induced by the treatment of fertilized eggs of a nonwaxy rice cultivar, Kinmaze with methyl-nitroso-urea (MNU).

The line was evaluated for several agronomic traits in a paddy field. Mature grains were gelatinized overnight in 1N potassium hydroxide at room temperature after milling. The amount of amylose was colorimetrically determined using an autoanalyzer. The amylose content was calculated on a dry weight basis. Environmental changes in the amylose content were also investigated using rice plants which were grown at nine locations from the northern to the southern part of Japan; Sendai, Joetsu (two sites), Tsukuba, Mishima, Nagoya, Kurashiki, Fukuoka and Okinawa. The amylose content was determined as mentioned above.

Rice grains of Tankei 1915 were characterized by a dull endosperm which could be distinguished from that of Kinmaze (Plate 1). The amylose content of Tankei 1915 ranged from 6.8 to 11.0% for 5 years from 1986, being 9.2% on the average (Table 1). The amylose content of Tankei 1915 was one-half of that of the parental cultivar, Kinmaze (19.2%). Based on the analysis of crossed seeds between Tankei 1915 and each of the testers with five different *du* genes, this line was found to harbor the *du-1* gene which is responsible for lowering the amylose content. The *du-1* gene expression in Tankei 1915 was markedly affected by the temperature during the

Plate 1. Rice grain of low amylose line, Tankei 1915 (left) and its parental cultivar, Kinmaze (right)

Table 1. Characteristics of low amylose lines of rice

Materials	Amylose content (%)	Heading time	Culm length (cm)	Panicle length (cm)	No. of panicles (/m^2)	Yielding ability (kg/a)
Tankei 1915	9.2	Aug. 30	93	20.7	234	45.7
Kinmaze	19.4	Aug. 30	78	21.3	405	46.3
Tankei 2019	6.3	Aug. 22	69	21.4	393	43.3
Kochihibiki	21.5	Aug. 21	71	21.1	348	47.5

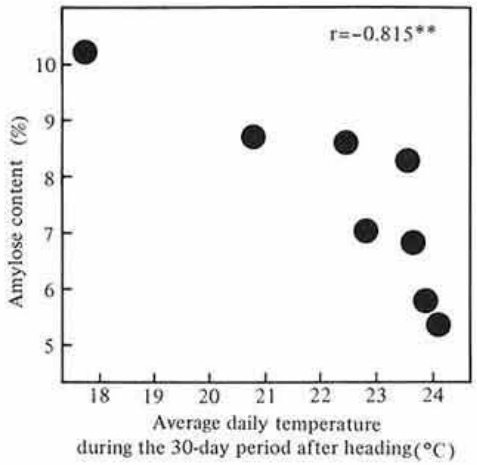

Fig. 1. Relationship between amylose content and temperature during the grain filling period in a low amylose line, Tankei 1915

grain filling period (Fig. 1), as reported in low amylose mutants with a different genetic background[1].

As shown in Table 1, there was no difference between Tankei 1915 and Kinmaze in terms of heading time and panicle length. Tankei 1915 was 15 cm taller and bore fewer panicles than Kinmaze. The yielding ability of Tankei 1915 was similar to that of Kinmaze.

Tankei 1915 was registered as "Rice Breeding Material No. 13" by the Ministry of Agriculture, Forestry and Fisheries (MAFF) in December, 1992.

A low amylose line bearing *du-2*, Tankei 2019

A low amylose line, Tankei 2019, was selected in the progeny after treatment of fertilized eggs of a nonwaxy rice cultivar, Kochihibiki with MNU. M_2 seeds on M_1 plants were observed for endosperm characteristics. Mutant lines selected for dull endosperm were genetically fixed in the following generations. One of them was named Tankei 2019. The agronomic traits and amylose content of Tankei 2019 were compared with those of the parental cultivar, Kochihibiki, as reported previously.

The grains of Tankei 2019 were characterized by a dull endosperm which was more similar to the waxy endosperm than to the nonwaxy endosperm (Plate 2). The amylose content of Tankei 2019 ranged from 5.1 to 9.0%, with a 6.3% value on the average

Plate 2. Rice grain of low amylose line, Tankei 2019 (left) and its parental cultivar, Kochihibiki (right)

(Table 1). The amylose content of this line was about one-third of that of Kochihibiki (21.5%). Allelism tests in five *du* loci indicated that Tankei 2019 carries the *du-2* gene which is located at a locus independent of the *du-1* and *wx* loci.

There were no significant differences between Tankei 2019 and Kochihibiki in terms of heading time, culm length, panicle length and number of panicles (Table 1). Tankei 2019 may become a promising semi-dwarf low amylose line, though the yielding ability of this line is slightly lower than that of Kochihibiki.

Tankei 2019 was registered as "Rice Breeding Material No. 14" by MAFF in December, 1992.

Use of low amylose lines for rice breeding

Rice is consumed almost exclusively in the form of grains in contrast to other major cereals of the world. Since the 1980s, overproduction of rice has become an important problem in Japan. In this country, waxy and nonwaxy grains are used as raw materials for the brewing, confectionery, seasoning and other industries and as staple food. The use of rice grains in the food industry is still limited, accounting for about 10% of the annual rice production. One of the major objectives in rice breeding programs is the selection of new gene donors to diversify the use of rice grains. Such approaches may enable to address the current problem of overproduction of rice in Japan.

Among the new gene donors, the *du* genes which

lower the amylose content in endosperm starch are likely to play an important role in rice breeding. The authors have developed two low amylose lines with different *du* genes. These lines are considered to be useful breeding materials for improving the eating or cooking quality and also for developing new rice products.

In general, Japanese consumers prefer more viscous cooked rice as staple food. Since there is a negative correlation between the viscosity level of cooked rice and the amylose content, rice breeders have concentrated their efforts on the breeding of cultivars with low amylose content. For example, rice grains produced in the northernmost island, Hokkaido, contain about 5% more amylose than those produced in other regions[2]. This difference depends on the effect of the temperature on the expression of the *Waxy* gene at the *wx* locus[6]. Cooler temperature during the grain filling period in Hokkaido affects considerably the increase of amylose content. Rice breeders in Hokkaido have made utmost efforts to improve the eating quality of rice grains produced thereby using the *du* genes. Recently good results have been achieved using an unknown *du* gene. The first low amylose cultivar designated as Aya was released in 1991[3].

On the other hand, low amylose lines have also been considered to be useful for diversifying the characteristics of rice grains. These lines have been tested for their quality as raw materials in the food industry. Out of them, Tankei 2019 is likely to produce a new type of rice cookies. Thus, the use of the *du* genes may enable to diversify the amylose content of starch accumulated in rice endosperm and the characteristics of rice grains.

References

1) Asaoka et al. (1989): Effects of environmental temperature at the early developmental stage of seeds on the characteristics of endosperm starches of rice (*Oryza sativa* L.). *Denpun Kagaku*, **36**, 1-8.
2) Inatsu, O. (1979): Improvement of the quality of rice grown in Hokkaido. *Denpun Kagaku*, **26**, 191-197, [In Japanese with English summary].
3) Kunihiro, Y. et al. (1993): A new paddy rice variety "Aya" with good eating quality due to low amylose content developed by anther culture breeding. *Jpn. J. Breed.*, **43**, 155-163, [In Japanese with English summary].
4) Okuno, K., Fuwa, H. & Yano, M. (1983): A new mutant gene lowering amylose content in endosperm starch of rice, *Oryza sativa* L.. *Jpn. J. Breed.*, **33**, 387-394.
5) Okuno, K. & Yano, M. (1984): New endosperm mutants modifying starch characteristics of rice, *Oryza sativa* L. *JARQ*, **18**, 73-78.
6) Okuno, K. (1985): Expression of mutant genes specifying starch synthesis in cereal grains. *Gamma Field Symposia*, **24**, 39-62.
7) Sano, Y. (1984): Differential regulation of *Waxy* gene expression in rice endosperm. *Theor. Appl. Genet.*, **64**, 467-473.
8) Sano, Y., Katsumata, M. & Okuno, K. (1986): Genetic studies of speciation in cultivated rice. 5. Inter- and intraspecific differentiation in the *Waxy* gene expression of rice. *Euphytica*, **35**, 1-9.
9) Satoh, H. & Omura, T. (1981): New endosperm mutations induced by chemical mutagens in rice, *Oryza sativa* L. *Jpn. J. Breed.*, **31**, 316-326.
10) Yano, M. et al. (1988): Chromosomal location of genes conditioning low amylose content of endosperm starches in rice, *Oryza sativa* L. *Theor. Appl. Genet.*, **76**, 183-189.

(Recieved for publication, May 26, 1993)