Natural Variation in *Alk* Locus Affects Starch Property and Cooked Rice Quality

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Abstract

Effects of natural variation in the *alk* (*starch synthase IIa*) locus of rice on the grain properties were characterised using near-isogenic lines (NILs). These NILs were developed by introducing a chromosomal segment including the *alk* locus of an alkali-tolerant cultivar Kasalath into alkali-susceptible cultivar Nipponbare genetic background by DNA-marker assisted selection. That variation in *alk* is the major factor causing the differences in chain-length distribution of amylopectin between the cultivars was confirmed. A higher ratio of short chains of amylopectin was accompanied by lower onset temperature of starch gelatinisation. These differences in physicochemical properties of starch probably affect the cooked rice quality only when the sensory tests were conducted after the samples were once cooled.

Media summary

Effects of natural variation in *alk* locus on cooked rice quality is firstly evaluated by developing and using near isogenic lines.

Key Words

Gelatinisation temperature (GT), alkali test, alkali spreading score, *Oryza sativa* L.

Introduction

The alkali disintegration property of rice grain is regarded as a parameter for evaluating rice quality since it is highly correlated to the gelatinisation temperature of the rice flour (Juliano 1998). However, precise effects of the property on cooked rice quality have not been presented. In this poster paper, we report on development of near isogenic lines for the *alk* locus, a major locus controlling the varietal difference in alkali disintegration (Kudo 1968; Umemoto et al. 2002). These lines were compared with the recurrent parent, Nipponbare in its amylopectin structure, gelatinisation property, and sensory test of cooked rice to understand how the variation in *alk* affects grain qualities.

Materials and Methods

Marker-assisted selection of near-isogenic lines for alk

From advanced backcross progeny derived from a cross between rice (*Oryza sativa* L.) cultivar Nipponbare as the recurrent parent and Kasalath as the donor parent, near isogenic lines in which the *alk* region on chromosome 6 was homozygous for Kasalath with a Nipponbare genetic background were selected with DNA markers.

Plant materials and starch preparation

Field-grown rice was harvested as each cultivar matured. Starch from endosperm was purified by washing with 0.2% SDS / 50 mM Tris-HCl (pH 7.5) and passed through a 200-mesh (ca. 75 μm) sieve.

Starch properties

Amylose content was measured basically according to the method by Juliano (1971). Chain-length distribution of amylopectin with HPAEC-PAD by Nakamura et al. (1997). Starch gelatinisation was measured by a Rapid Visco Analyzer (RVA-4, Newport Scientific, Sydney, Australia).

Sensory evaluation of cooked rice

Polished rice was cooked by a conventional rice cooker (National SR-ULH10, Kadoma, Japan), and sensory test was performed by trained staff in National Institute of Crop Science.

Results

Near-isogenic lines (NILs) for alkali disintegration

Three near isogenic plants, NILs(*Alk*), having the *alk* genotype of Kasalath, higher alkali tolerance, with the Nipponbare, less alkali tolerance, genetic background were selected. Only a segment of chromosome 6 including the *alk* locus of Nipponbare was substituted with that of Kasalath (Fig. 1A). Alkali disintegration property of NILs(*Alk*) were confirmed (Fig. 1B). As expected from the fact that the NILs have the *alk* allele of Kasalath, the NILs were more alkali-tolerant than Nipponbare.

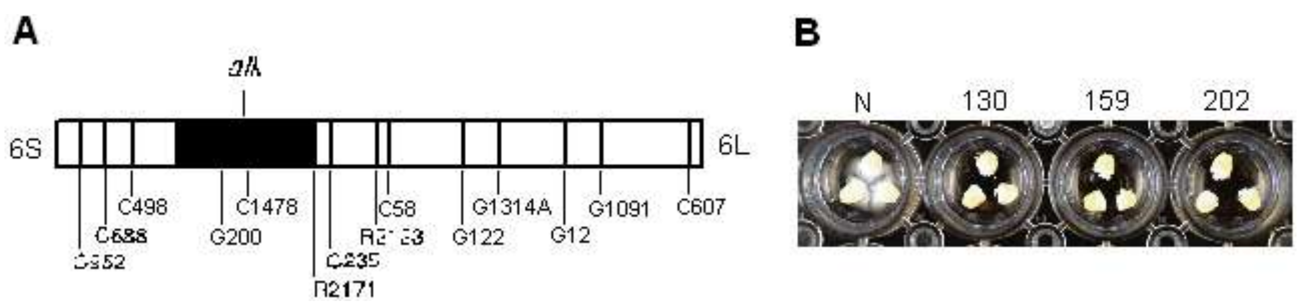

Fig. 1. (A) Graphical genotype of near-isogenic lines (NILs) having a chromosomal segment of Kasalath including the *alk* (starch synthase IIa) locus on chromosome 6, with a Nipponbare genetic background. The chromosomal segment of Kasalath is shown in solid rectangle. Positions of DNA markers used for the selection are shown below the bar. (B) Alkali disintegration property with 1.5% KOH. N; Nipponbare, 130; NIL(*Alk*)-130, 159; NIL(*Alk*)-159, 202; NIL(*Alk*)-202.

Chain-length distribution of amylopectin and gelatinisation of starch

The chain length distribution of amylopectin in endosperm was compared for one of the NILs(*Alk*), Kasalath, and Nipponbare (Fig. 2A). The NIL clearly had fewer chains of DP 7 to 11 and more chains of DP 13 to 23 than Nipponbare, while NIL and Kasalath had almost identical profiles. This result suggests that differences in chain length distribution of amylopectin between Nipponbare and Kasalath are determined by the substituted chromosomal region including the *alk* locus. Gelatinisation of starch provides useful information for determining the quality of cooked rice (Juliano 1998). Viscosity profiles measured by RVA between NIL and Nipponbare clearly differed especially in that starch from Nipponbare showed lower onset temperature, lower peak viscosity, slower increase from onset to peak viscosity, and lower final viscosity, while no clear difference was observed in hot paste viscosity (Fig. 2B).

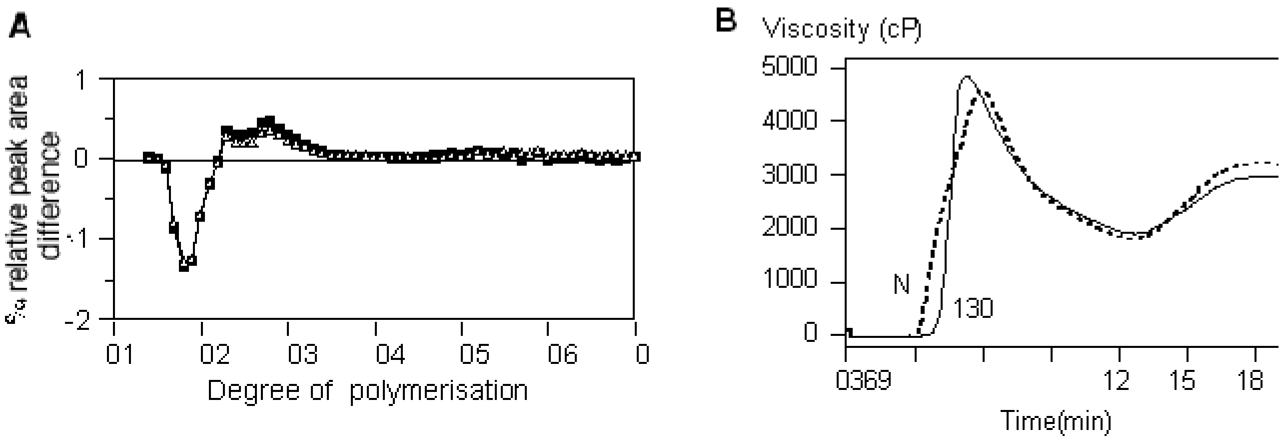

Fig. 2. (A) Chain length distribution of amylopectin. Isoamylase debranched starch was analysed by HPAEC-PAD. Difference plots of the peak area percentage for NIL(*Alk*)-130 (solid squares) and Kasalath (open triangles). The percentage of each peak for NIL(*Alk*)-130 or Kasalath was subtracted from that of Nipponbare. (B) Viscosity analysed by a Rapid Visco Analyzer. N; Nipponbare, 130; NIL(*Alk*)-130.

Sensory evaluation of cooked rice

Cooked rice qualities were compared between the NIL(*Alk*) and Nipponbare just after cooking and after the samples were kept 16h at 5?C and sensory tests conducted after samples reached room temperature (Fig. 3). The firmness and overall preference score for NIL(*Alk*) did not largely differ from those of Nipponbare just after cooking. However, NIL(*Alk*) was judged by Japanese tasters to have a very firm texture, and a very poor overall preference after cooling,

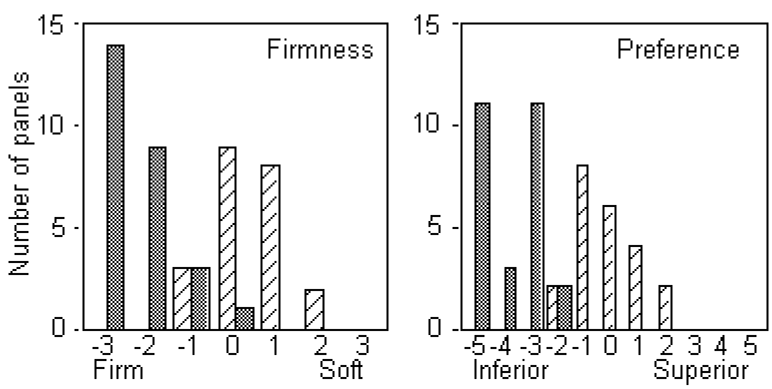

Fig. 3. Sensory evaluation of cooked NIL(*Alk*) rice. Nipponbare as a standard (=0). Sensory tests were conducted just after rice has been cooked (hatched bars), and after samples had been kept 16h at 5?C (shaded bars).

Amylose content vs amylopectin chain length

Besides the chain-length distribution of amylopectin, amylose content is the key factor affecting cooked rice quality. We explored both characters with 55 non-waxy cultivars in addition to NIL(*Alk*), Nipponbare and Kasalath (Fig. 4). Chain-length distribution of amylopectin was clearly divided into two types (Umemoto et al. 2002, Nakamura et al. 2002). Amylose content also mainly classified into two groups in this study, one around 16% to 22% and the other around 25% to 32%. It appeared that the only a limited number of cultivars had lower ratio of short chain in amylopectin with lower amylose content like NIL(*Alk*) (Fig. 4).

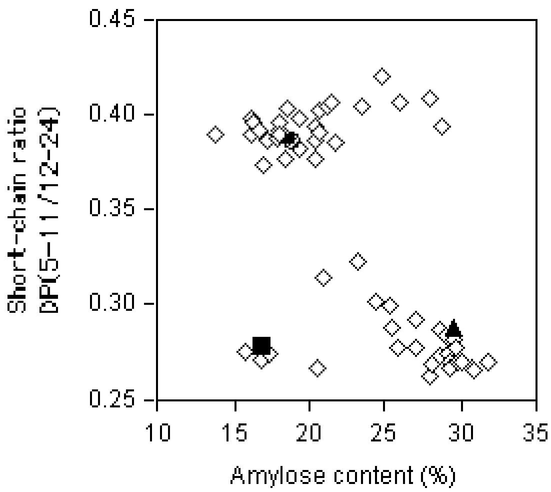

Fig. 4. Correlation between amylose content in polished rice and ratio of short chain in amylopectin. Fifty-five non-waxy cultivars (open diamond), NIL(*Alk*) (closed square), Nipponbare (closed circle), and Kasalath (closed triangle) were investigated.

Conclusion

NILs for alkali disintegration property of rice grain were developed and used for characterising the property accurately. It was confirmed that the *alk* is the major locus determining the differences in chain-length distribution of amylopectin observed between Nipponbare and Kasalath. The cooked rice quality of the NIL once cooled was quite inferior when compared to Nipponbare for the Japanese testers. This is probably attributed to the differences in amylopectin chain length through the changes in physicochemical characters of the starch. Effects of variation in alkali disintegration on cooked rice quality have not been clearly shown. This is probably because that there are few cultivars with alkali tolerance with low amylose content. With the high amylose background, effects of differences in *alk* on cooked rice quality might be masked and cannot be evaluated properly.

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