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# Grain Qualities and Their Genetic Derivation of 7 New Rice for Africa (NERICA) Varieties

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NERICA are interspecific rice varieties from crossing between the high-yielding Asian rice (*Oryza sativa* spp. *Japonica*) with locally adapted African rice (*Oryza glaberrima*). In this study, we analyzed grain qualities of 7 NERICA varieties (NERICA 1 to 7) and genetic derivation of quality-related genes. Quality analyses of NERICA grains showed that 7 NERICA varieties were clearly classified into two groups based on the difference of amylose content, and the difference influenced the pasting and physical properties of grains. Genetic analysis of the gene encoding granule-bound starch synthase I (GBSSI), which is known as a key enzyme on amylose synthesis in rice grain, revealed that varieties with higher amylose content (~29%) have the gene derived from *O. glaberrima* parent, and group 2 with lower amylose content (~22%) have the gene from *O. sativa* parent. These results indicated that the difference in amylose content as well as grain properties among 7 NERICA varieties is mainly determined by the genetic derivation of GBSSI. Further genetic analysis of starch synthesis-related genes suggested that the genetic derivation of SSIIa also influences the chain length of amylopectin in 7 NERICA varieties.

**KEYWORDS: NERICA; grain quality; genetic diversity; rice**

## INTRODUCTION

Rice is one of the most important food crops in the world and feeds over half of the global population. In Africa, two cultivated species, namely the Asian rice (*O. sativa*) and the African rice (*O. glaberrima*) are grown. *O. glaberrima* is traditionally found in West Africa but it is being increasingly replaced by *O. sativa* due to low yield-performance, high shattering, and susceptibility to lodging of most *O. glaberrima* cultivars (*1*). However, *O. sativa* cultivars are often not sufficiently adapted to various abiotic and biotic conditions in Africa. On the other hand, *O. glaberrima* has been found to have several useful traits to survive in African environment, such as weed competitiveness and tolerance to biotic and abiotic stresses (*1*).

In the early 1990s, the African Rice Center (WARDA) and its partners developed interspecific BC2 inbred lines from a cross between an upland *O. sativa tropical japonica* variety, WAB 56−104, and an *O. glaberrima* variety, CG14, as the donor parent (*2*). These new interspecific lines are called NERICA, which is an acronym for New Rice for Africa. In 2000, seven NERICA varieties (NERICA 1 to 7) derived from BC2 lines between WAB 56−104 and CG14 were released, and an additional 11 (NERICA 8 to 18) were released in 2005. These varieties combined the best traits of both parents: high yields from the Asian parent and the ability to thrive in harsh environments from the African parent. All 18 NERICA varieties are suitable for the upland rice ecology of sub-Saharan Africa.

The agronomically useful traits of NERICA varieties have been well-characterized because they themselves are selected in the breeding programs. On the other hand, the grain quality trait of NERICA (and their parental lines) had not been included in the selection criteria. Although NERICA and *O. glaberrima* grain have been well-known to have high protein (*3*) and contribute to the nutrition of African people, the other traits of grain quality of NERICA have not been sufficiently characterized in spite of their importance in nutrition, preference, and economics. For genetic analysis of grain quality, a few quality trait loci (QTL) mapping using the interspecific population of

**Table 1.** Primers Used for Genomic PCR

| gene (chromosome) | | sequence (5′ to 3′) |
|---|---|---|
| GBSSI (Chr.6) | F | CATCGTCAACGGCATGGAC |
| | R | CTTGCCCGGATACTTCTCCT |
| SSI (Chr. 6) | F | CGCATTTTACACTGAGAAGCAC |
| | R | TGCGGAGTGAACACTACCAAG |
| SSIIa (Chr. 6) | F | GTGTTCATTGACGCTCCTCTC |
| | R | ATCACAAGGACAGAGCGAGTG |
| BEI (Chr. 6) | F | ATGGTGACTGTTGTGGAGGAG |
| | R | AGCCAAGACGTGCTATGAGTG |
| SSIIIa (Chr. 8) | F | TGTGAGAGGGTATCATCCATTC |
| | R | CCGTATGAAGGGAAATCGTC |

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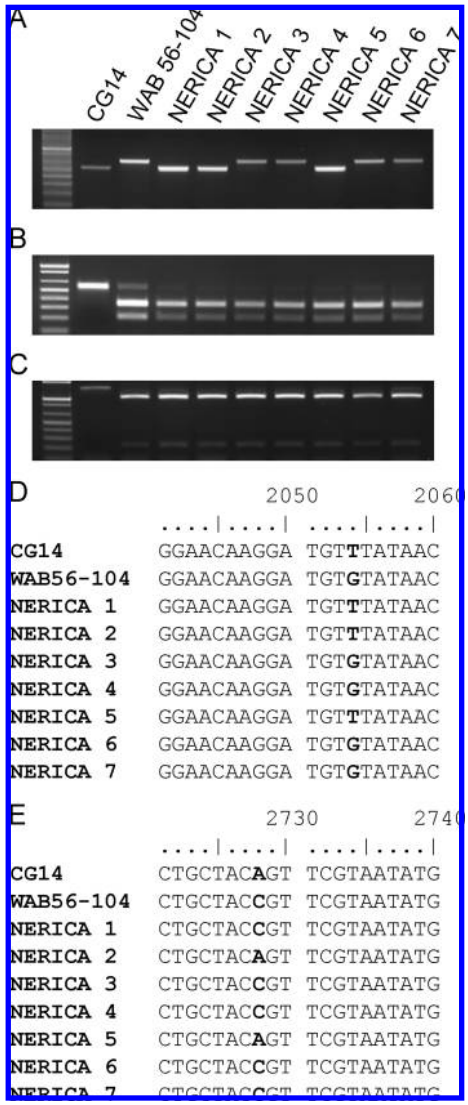


**Figure 1.** Genotyping of starch synthesis-related genes in 7 NERICA varieties. (**A**) Genomic PCR analysis of GBSSI. The genomic region around the 10th intron was amplified. The GBSSI gene in *O. glaberrima* has 139-bp deletion in the 10th intron compared to *O. sativa*. (**B**, **C**) PCR-RFLP analysis of BEI and SSIIa. PCR fragments using primers listed in **Table 1** were digested by *Nde*I and *Xho*I, respectively. (**D**, **E**) The sequence around the SNPs between CG14 and WAB 56−104 in SSI and SSIIa. PCR fragments using primers listed in **Table 1** were directly sequenced to recognize the SNP. Bold letters in the sequences indicate the SNP. Numbers above the sequences represent nucleotides from the start codon.

*O. glaberrima* and *O. sativa* were reported (*4, 5*). These reports showed that some QTLs for protein content, amylose content, and gel consistency were mapped in *O. glaberrima* allele and *O. glaberrima* may be a useful source for improving grain quality traits. However, the responsible gene for these QTLs and genetic derivation in NERICA are still unknown.

In this study, we characterized the grain quality of 7 NERICA varieties (NERICA 1 to 7) using chemical and physical analyses, and showed genetic derivation of starch synthesis-related genes to explain the difference of grain qualities among NERICA 1 to 7.

## MATERIALS AND METHODS

**Plant Materials.** Seeds of seven NERICA varieties (NERICA 1 to 7) and their parental lines, *O. glaberrima*, CG14, and *O. sativa tropical*

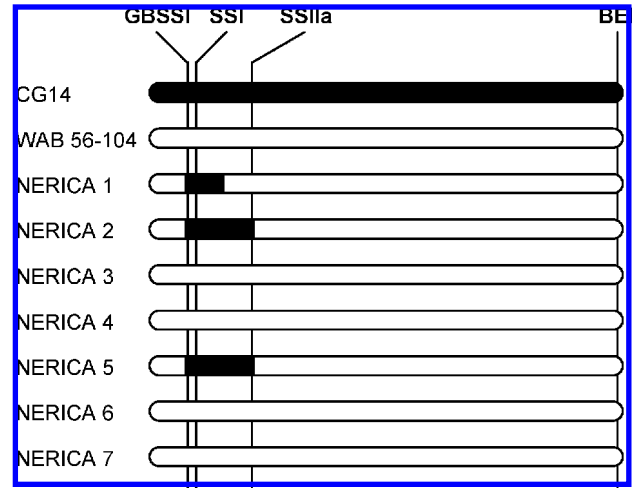


**Figure 2.** Graphical genotype of starch-related genes in chromosome 6. Black bars indicate the genomic region of CG14, and white bars indicate WAB 56−104.

*Japonica*, WAB 56−104, were used. NERICA cultivars were grown on a paddy field of the Japan International Cooperation Agency (JICA) Tsukuba, Japan in 2006. Seeds of CG14 and WAB 56−104 were provided from Dr. H. Watanabe (Recs International Inc.).

The rice samples were milled to the milling yield of 90% by a grain testing mill (TM-05, Satake, Tokyo, Japan). For chemical, pasting analysis, and isolation of DNA, the milled rice was ground with a Cyclone Sample Mill (UDY Corporation, Fort Collins, USA) to obtain rice flour.

**Chemical Analyses.** Amylose content was determined by iodine absorbance with the Juliano method (*6*). Nitrogen content was determined by a nitrogen analyzer (Leco FP-528, LECO, USA) based on the combustion method (modified Dumas method). Protein content was obtained from nitrogen by multiplying it with a mitorogen−protein conversion factor of 5.95.

**Pasting Analysis.** Pasting properties of the rice flour were determined by Rapid Visco Analyzer (RVA) (RVA-3D, Newport Scientific, Sydney, Australia) by the method of Toyoshima et al. (*7*). The properties were expressed as the peak viscosity, i.e. the maximum viscosity, breakdown viscosity, i.e. the difference between the peak viscosity and the minimum viscosity, and setback viscosity, i.e. the difference between the minimum viscosity and the final viscosity.

**Physical Analysis of Cooked Rice Grain.** Polished rice was wiped with gauze to remove polishing residue. Ten grams of the rice sample were steeped in 16 g of distilled water for 1 h. After soaking, the sample was cooked by an electric rice cooker (SR-ULH18, National Co., Ltd., Tokyo, Japan). The cooked rice was cooled for 2 h. The physical properties of the cooked rice grain were determined with a Tensipresser (Myboy system, Takemoto Electric Corp., Tokyo, Japan) by the method of Okadome et al. (*8*). This method consists of low and high compression test, and provides the physical properties of hardness and stickiness in the surface layer and the overall rice grain.

**Genotyping of Starch-Related Genes.** DNA was extracted from rice flour by using SDS method. Briefly, DNA in the rice flour was extracted in SDS buffer (0.5% SDS, 200 mM Tris-HCl (pH7.5), 25 mM EDTA, 250 mM NaCl), treated twice with chloroform, and precipitated with 2-propanol. The DNA fragment of starch-related genes in CG14 and WAB 56−104 were amplified by using the extracted DNA and gene-specific primers (**Table 1**), then directly sequenced to find single nucleotide polymorphisms (SNPs) among the parental cultivars. Genotypes of 7 NERICA cultivars were determined by either PCR-RFLP (restriction fragment length polymorphism) analysis or direct sequencing; PCR-RFLP was used in the case of SNP forming a restriction enzyme site, and direct sequencing in the other cases.

**Measurement of Gelatinization Properties.** The gelatinization and swelling mode of endosperm starch in a variable concentration of urea were measured as described previously (*9*).

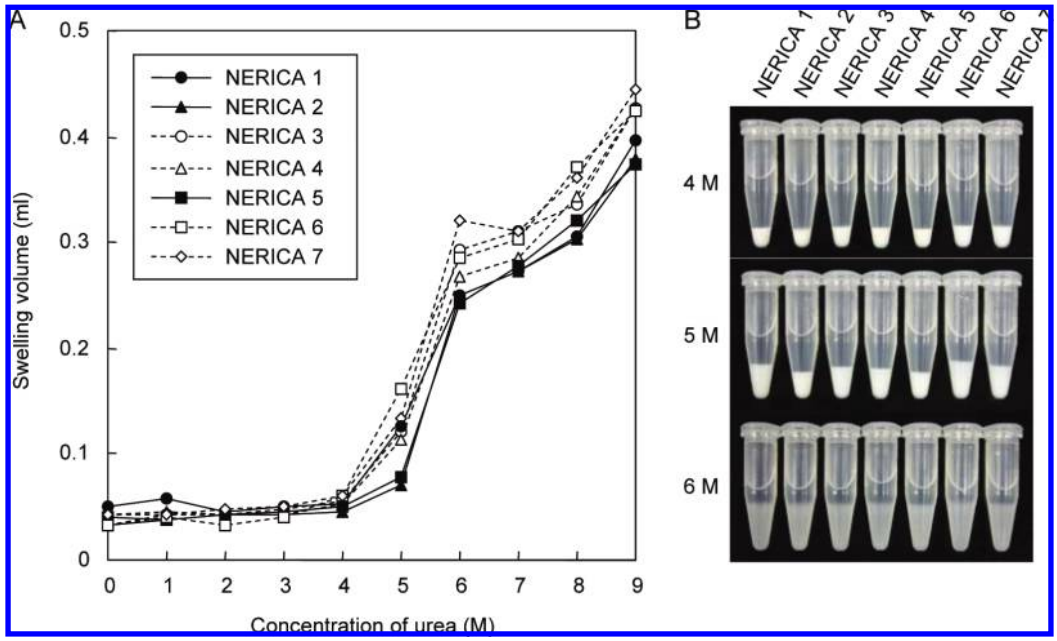


**Figure 3.** Urea gelatinization of starch from 7 NERICA varieties. (**A**) Effects of the concentration of urea on gelatinization of starch ($n = 3$). After incubation of 20 mg of rice flour in solutions of urea for 24 h, swelling was examined by measuring the volume of the swollen starch sediment. (**B**) Photographs of the swollen starch in 4, 5, and 6 M urea solution.

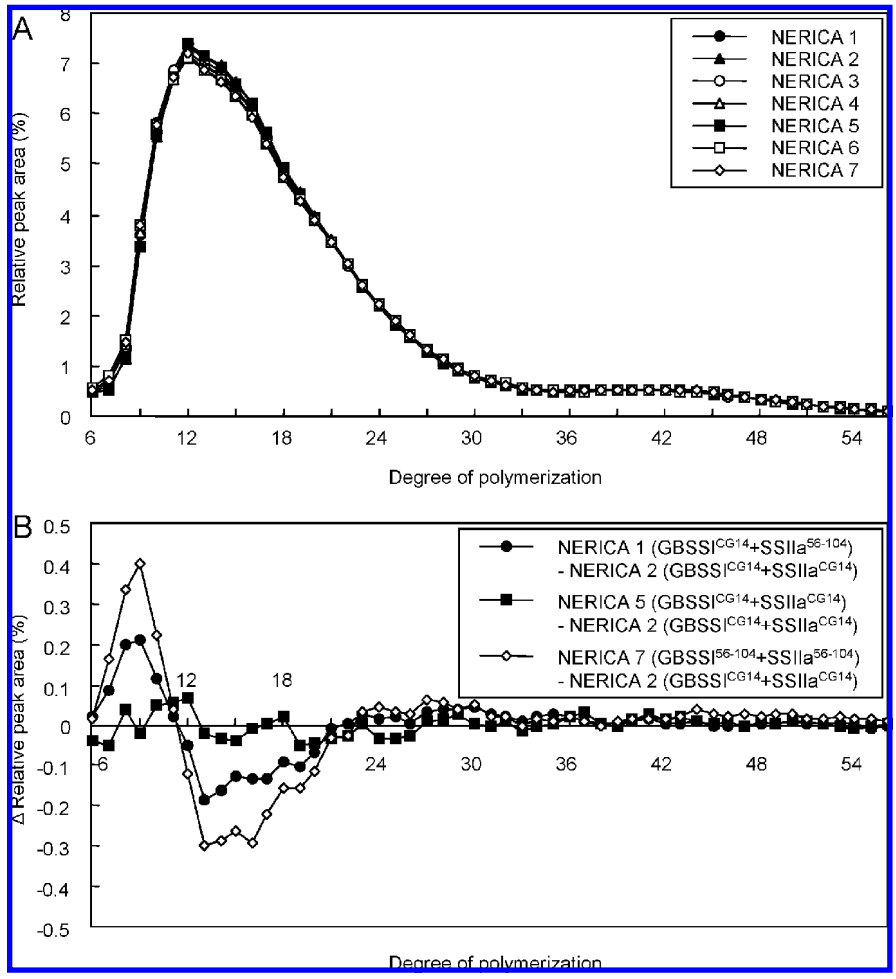


**Figure 4.** Chain length distributions of endosperm amylopectin. (**A**) Comparison of the chain length distribution of amylopectin in 7 NERICA varieties. (**B**) Differences in chain length distribution in NERICA1, NERICA 5, and NERICA 7 relative to NERICA 2.

**Measurement of Chain Length Distribution of Amylopection.** Extraction of starch from rice endosperm and the analysis of amylopection chain-length distribution were performed according to the methods of Suzuki et al. (*10*). The chain length distribution

**Table 2.** Protein and Amylose Content of 7 NERICA Varieties ($n = 3$)[a]

| name (group) | protein content (%) | amylose content (%) | amylose content (%) in "passport data"[b] |
|---|---|---|---|
| NERICA 1 (1) | 6.9ab | 28.7 a | 26.6 |
| NERICA 2 (1) | 7.2 a | 29.8 a | 26.4 |
| NERICA 3 (2) | 6.9ab | 22.3bc | 23.8 |
| NERICA 4 (2) | 6.8bc | 21.9 b | 23.8 |
| NERICA 5 (1) | 6.9ab | 28.9 a | NA[c] |
| NERICA 6 (2) | 6.8bc | 24.2 d | 24.5 |
| NERICA 7 (2) | 6.5 c | 23.3cd | 27.8 |

[a] Different letters indicate significant differences at the 1% level. [b] Amylose content of NERICA in "passport data" was extracted from WARDA's website (http://www.warda.org/publications/NERICA%201-8%20passport%20data.pdf). [c] Not available.

**Table 3.** Pasting Properties of 7 NERICA by RVA ($n = 3$)[a]

| name (group) | peak viscosity (RVU) | min viscosity (RVU) | final viscosity (RVU) | breakdown (RVU) | setback (RVU) | gelatinization temp (°C) |
|---|---|---|---|---|---|---|
| NERICA 1 (1) | 258.1 a | 177.2 a | 319.8 a | 80.9 a | 142.6ab | 78.7 a |
| NERICA 2 (1) | 268.2 a | 160.6 b | 313.7ab | 107.6 b | 158.1 c | 80.2 b |
| NERICA 3 (2) | 310.9 b | 172.0ac | 308.8 b | 138.9cd | 136.8 a | 79.1ac |
| NERICA 4 (2) | 307.5 b | 178.2 a | 314.8ab | 129.3ce | 136.6 a | 79.4cd |
| NERICA 5 (1) | 286.6 d | 167.6bc | 322.3 a | 119.0be | 154.7 c | 79.8bd |
| NERICA 6 (2) | 296.4b,d | 169.6 c | 316.0ab | 126.8ce | 146.3 b | 78.2 e |
| NERICA 7 (2) | 325.1 c | 171.5ac | 307.7 b | 153.6 d | 136.2 a | 78.7 a |

[a] Different letters indicate significant differences at the 1% level.

of amylopectins from endosperm was analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

## RESULTS

**Chemical Analyses of 7 NERICA Varieties.** NERICA varieties have been well-known to have higher protein content (*3*). In addition, some organoleptic characteristics including amylose content, milling rate, cooking qualities, and aroma of NERICA varieties (NERICA 1 to 4 and 6 to 8) were opened as "passport data" in the WARDA Web site (http://www.warda.org/publications/NERICA%201−8%20passport%20data.pdf). However, comprehensive quality analysis of NERICA has not been reported. At first, we analyzed amylose and protein content of 7 NERICA varieties, and compared them with WARDA's "passport data".

As shown in **Table 2**, all NERICA varieties had slightly high protein content (∼7%) of milled grain. On the other hand, 7 NERICA varieties could be classified into two groups based on the amylose content of grains ($p < 0.01$): group 1 (NERICA 1, 2, and 5) had higher amylose content (∼29%) and group 2 (NERICA 3, 4, 6, and 7) had lower (∼23%). This result was consistent with the amylose content in WARDA's "passport data" except for NERICA 7. Ikeda et al. (*11*) reported that the breeder seeds of NERICA were not always genetically uniform, and NERICA 7 showed the highest rate of detection of offtypes among 7 NERICA varieties. Thus, NERICA 7 seeds used in this study might not have the same genetic background as those in WARDA.

Traore et al. (*12*) reported that the amylose content of CG14 was 26.1% and that of WAB 56−104 was 21.1%. Thus, it seems that the amylose contents of groups 1 and 2 are inherited from CG14 and WAB 56−104, respectively.

**Pasting and Physical Properties of 7 NERICA Varieties.** Pasting properties of NERICA 1 to 7 by using RVA were summarized in **Table 3**. Group 1 varieties showed lower peak and breakdown viscosity and higher setback viscosity than group 2. Tan and Corke (*13*) reported that the amylose content of various rice cultivars was negatively correlated with peak viscosity and positively with setback viscosity, and protein content was negatively correlated with peak viscosity. As all 7 NERICA varieties showed the same level of protein content (**Table 1**), these results suggested that the higher amylose content of group 1 varieties influences the pasting properties of rice flour.

Physical properties of cooked rice grain of NERICA with Tensipresser were summarized in **Table 4**. Although whole grain analysis did not show a significant difference between groups 1 and 2, the analysis of the surface layer showed that group 1 had the slightly higher hardness and significantly lower stickiness ($p < 0.01$) and adhered mass ($p < 0.01$) than group 2. As amylose content had a positive correlation with surface hardness (H1) and a negative correlation with surface stickiness (−H1) and adhesiveness (L3) (*8*), physical properties of cooked grain also reflect the difference of amylose content among NERICA varieties.

**Genetic Derivation of the Gene Encoding GBSSI.** Quality analyses of NERICA grains indicated that 7 NERICA varieties are clearly classified into two groups based on the difference of amylose content, and the difference influenced the pasting and physical properties of grains. Thus, we next investigated the genetic diversity to cause the deference of amylose content between two groups. Amylose content of rice grains is mainly determined by the *Waxy* locus, which encodes GBSSI, a key enzyme in amylose synthesis (*14*). The genomic sequence of GBSSI in *O. glaberrima* has a 139-bp deletion in the 10th intron compared to the sequence of *O. sativa* (*15*). Thus, genomic PCR analysis across the 10th intron of GBSSI was conducted to reveal the genetic derivation of GBSSI in 7 NERICA varieties. As shown in **Figure 1A**, CG14 had a lower PCR fragment than WAB56−104 because of the deletion in the 10th intron. Interestingly, all group 1 varieties (NERICA 1, 2, and 5) had a lower PCR fragment derived from CG14, and group 2 (NERICA 3, 4, 6, and 7) had a higher fragment from WAB 56−104 (**Figure 1A**). This result possibly indicates that the difference of amylose content of grains would be determined by the genetic derivation of GBSSI gene.

**Genotyping of Starch Synthesis-Related Genes.** In the same groups, although all varieties showed the same tendency, some varieties had distinct characteristics in both pasting and physical analyses. For instance, NERICA 1 showed a significant low breakdown in RVA (**Table 3**) and significant high hardness of the surface layer and whole grains (**Table 4**) in group 1 varieties. As group 1 varieties had the same level of protein and amylose (**Table 2**), these characteristics of NERICA 1 may be influenced by the other factor(s).

In general, pasting and physical properties of grains were influenced by amylopectin structure as well as by the level of amylose/amylopectin and protein. Amylopectin is synthesized by the coordinated actions of starch synthase, starch branching enzyme, and starch debranching enzyme (*16*). To reveal the genetic derivation of these enzymes in 7 NERICA, we chose 4 genes encoding major enzymes for amylopectin synthesis and analyzed these genotypes in 7 NERICA varieties. Four genes were starch synthase I (SSI), SSIIa, SSIIIa, and branching enzyme I (BEI), and three genes except for SSIIIa are located on the same chromosome, chr. 6 as GBSSI (**Figure 2**).

Parts **B** and **C** of **Figure 1** showed PCR-RFLP analysis of BEI and SSIIIa. In both cases, CG14 had a single larger fragment, and WAB 56−104 had two smaller fragments derived from a digestion of the larger fragment. This analysis showed

**Table 4.** Physical Properties of Cooked Rice of NERICA by Tensipresser ($n = 30$)[a]

| | surface layer | | | | whole grain | | | | |
|---|---|---|---|---|---|---|---|---|---|
| name (group) | hardness H1 ($10^3$ dyn) | stickiness −H1 ($10^3$ dyn) | adhered mass L3 (mm) | balance degree 1 (−H1/H1) | hardness H2 ($10^6$ dyn) | stickiness −H2 ($10^6$ dyn) | adhered mass L6 (mm) | balance degree 2 (−H2/H2) | sample thickness (mm) |
| NERICA 1 (1) | 138.5 a | 0.7 a | 0.04 a | 0.006 a | 3.89 a | 0.09 a | 0.37 a | 0.025 a | 2.396 a |
| NERICA 2 (1) | 109.9 b | 0.6 a | 0.05 a | 0.007 a | 3.29 b | 0.14ab | 0.42 a | 0.049ab | 2.311ab |
| NERICA 3 (2) | 107.0 c | 5.1 b | 0.42 b | 0.049 b | 3.10 b | 0.23bc | 0.83 b | 0.076bc | 2.330 a |
| NERICA 4 (2) | 100.0 a | 5.7 b | 0.48 b | 0.058 b | 2.91 b | 0.25cd | 0.71abc | 0.091bc | 2.394 a |
| NERICA 5 (1) | 121.9 d | 0.5 a | 0.01 a | 0.004 a | 3.26 b | 0.14ab | 0.47ac | 0.049ab | 2.334 a |
| NERICA 6 (2) | 107.8 e | 6.6 b | 0.59 b | 0.068 b | 3.02 b | 0.30cd | 0.80bc | 0.105 c | 2.142 b |
| NERICA 7 (2) | 107.5 f | 6.7 b | 0.57 b | 0.062 b | 3.27 b | 0.33 d | 0.84 b | 0.102 c | 2.397 a |

[a] Different letters indicate significant differences at the 1% level.

all 7 NERICA varieties had genes of BEI and SSIIa from WAB 56−104, possibly due to the back-cross-breeding of NERICA (**Figure 1B**,**C**). **Figure 1D**,**E** showed sequences around the SNPs used for genotyping of SSI and SSIIa. Because SSI is very closely located by the GBSSI (**Figure 2**), the genetic derivation of SSI was the same as that of GBSSI, while in the genotyping of SSIIa, NERICA 2 and 5 had the gene from CG14, but NERICA 1 had the gene from WAB 56−104 (**Figures 1E** and **2**). These results indicated that group 2 varieties had WAB 56−104-type GBSSI, SSI, and SSIIa, and group 1 varieties had CG14-type GBSSI, SSI, and SSIIa except for NERICA 1, which had CG14-type GBSSI and SSI and WAB 56−104-type SSIIa.

**Gelatinization Analysis and Chain Length Distribution of 7 NERICA Varieties.** SSIIa has an important role for determining amylopectin structure and its properties (*17, 18*). Thus we next investigated the gelatinization properties of starch and chain length distribution of 7 NERICA varieties to reveal the influence of the genetic diversity of SSIIa. To examine the effects of the concentration of urea on the gelatinization of starch, we incubated rice flour with the solution of urea at concentrations from 0 to 9 M (**Figure 3**). The starch from all NERICA varieties started to be gelatinized in urea solution between 4 and 5 M. However, the extent of swelling at a concentration of 5 M urea was more significant in the case of varieties with WAB 56−104-type SSIIa (NERICA 1, 3, 4, 6, and 7) than in that of varieties with CG14-type SSIIa (NERICA 2 and 5). This result indicated that the genetic derivation of SSIIa influenced the gelatinization properties of starch possibly due to the difference of amylopectin structure.

To directly compare the structural differences in endosperm amylopectin of NERICA, we analyzed the distribution of the chain length of amylopectin by HPAEC-HAD (**Figure 4A**). **Figure 4B** compared the difference of the chain length distribution of amylopectin from NERICA 2 (genotype: $GBSSI^{CG14}$+$SSI^{CG14}$+$SSIIa^{CG14}$) with those from NERICA 1 ($GBSSI^{CG14}$+$SSI^{CG14}$+$SSIIa^{56-104}$), NERICA 5 ($GBSSI^{CG14}$+$SSI^{CG14}$+$SSIIa^{CG14}$) and NERICA 7 ($GBSSI^{56-104}$+$SSI^{56-104}$+$SSIIa^{56-104}$). There was no significant differences in amylopectin between the same genotypic varieties NERICA 2 and 5. In contrast, amylopectin in NERICA 7 was enriched in short chains with DP 7 to 11, and depleted in intermediate-sized chains with DP 12 to 20 compared to NERICA 2. The other varieties of group 2, which had the same genotype as NERICA 7, showed similar patterns with NERICA 7 (data not shown). Amylopectin in NERICA 1 also showed a similar pattern with NERICA 7, but the extent of the difference was slightly smaller than that with NERICA 7. Because the genetic derivation of SSIIa was the only difference in the genotypic analysis between NERICA 1 and 2, these results suggested that the genetic derivation of SSIIa determines the amylopectin structures, to some extent.

## DISCUSSION

**Breeding of NERICA Based on the Grain Qualities.** During the breeding of NERICA, WARDA has adopted participatory approaches for varietal development (Farmers' Participatory Varietal Selection), which farmers' own selection criteria were collected and became feedback to the breeding program. Grain quality and taste are often listed as important criteria as well as agronomic characteristics such as short growth duration and high yield, etc. (*19*). Additionally, Lançon et al. (*20*) have conducted a survey in Nigeria and showed that consumers' major criteria for the purchasing of local rice was price (46%), taste (23%), and swelling capacity (13%). Promising varieties therefore should possess not only high agronomic performance but also high qualities including good taste.

In this study, we developed the PCR-based method to distinguish a genetic derivation of major genes for starch synthesis and suggested that the genetic derivation of genes for GBSSI and SSIIa influenced grain properties of 7 NERICA varieties. GBSSI from *O. glaberrima* CG14 would have higher activity for the synthesis of amylose than that of *O. sativa* WAB 56−104, and led to hard and nonsticky grains (**Tables 2**, **3**, and **4** and **Figure 1**). SSIIa from *O. sativa* WAB 56−104 led to synthesis of amylopectin with the enrichment of short chains of DP < 11 and depletion in intermediate-size chains of 12 < DP > 20 than that from *O. glaberrima* CG14 (**Figure 4**). The difference in amylopectin structure influenced gelatinization and swelling of endosperm starch (**Figure 3**).

For the selection of NERICA based on the grain qualities, the PCR-based method of major starch-synthesis genes, we showed in this study, is thought to be very useful. Under the selection, this method could provide a prediction of the grain quality at the early stage of breeding and developing plants, and shorten the selection period. We hope this method will contribute to the breeding of high-quality NERICA in the near future.

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