31. Development of SNP markers of *starch synthase IIa* (*alk*) and haplotype distribution in Rice Core Collections

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The aim of this study was to develop single nucleotide polymorphism (SNP) markers of *starch synthase IIa* (*SSIIa*), and compare the distribution of *SSIIa* haplotype between Global Rice Core Collection (GRC) and Rice Core Collection of Japanese Landraces (JRC), selected by National Institute of Agrabiological Sciences (Kojima et al. 2004, Kojima et al. 2005). *SSIIa* of rice is known as the *alk* gene that controls disintegration of grain in KOH solution (Kudo 1968). Variations in *SSIIa* are important in determining eating and processing qualities of rice since these affects gelatinization and retrogradation of starch through the alteration in amylopectin structure (Okamoto et al. 2002, Umemoto et al. 2008). Therefore the distribution of functionally different *SSIIa* haplotypes may reflect people's preference to rice quality.

Four SNPs have been found in rice *SSIIa* that results in amino acid substitution. Two of them are individually responsible for the SSIIa activity (Umemoto and Aoki, 2005, Nakamura, 2005). A PCR marker for SNP4 has been developed by Bao et al. (2006). We developed SNP markers for the four SNPs using mismatch primer to determine the functional *SSIIa* haplotypes.

The primer sequences of the markers were shown in Fig.1. The markers for SNP1, SNP2, and SNP3 were dominant marker. The marker for SNP4 was designed as co-dominant by adding poly-T tail to one of the forward primers. The PCRs for SNP1, SNP3, SNP4 were performed in total volume of 10μL containing 6.6μL of sterilized distilled water, 1.0μL of 10X buffer (provided by ABI), 0.6μL of 25mM $MgCl_2$, 1.0μL of 2mM dNTPs, 0.2μL of 20 mM each of primer, 0.1μL of Taq polymerase (ABI), and 0.5μL template DNA (ca 20ng/μL). We used LA-Taq (Takara) for SNP2. The reaction mixture contained 2.6μL of sterilized distilled water, 5.0μL of 2X Buffer Ⅰ (provided by TaKaRa), 1.6μL of 2.5mM dNTPs, 0.2μL of 20mM each of primer, 0.1μL of LA-Taq, and 0.5μL of template DNA. Thermal conditions were 3min for 95℃ followed by 35 cycles of 94℃ for 30sec, 60℃ for 30sec, 72℃ for 1min. Amplified fragments were resolved in 2% agarose gels and detected after staining with ethidium bromide. Validation of the developed markers was performed by genotyping 10 cultivars; those *SSIIa* SNP genotypes have been determined previously by other method (Umemoto and Aoki 2005) (Fig. 2).

We used these SNP markers for the haplotype analysis of *SSIIa* with GRC and JRC (Table1). SSIIa coded by haplotypes 1, 2, and 3 are functional, on the other hand haplotypes 4 and 5 lacked activity (Umemoto and Aoki 2005). Among JRC, 9 out of 38 (23.7%) cultivars have functional *SSIIa* haplotype, whereas 45 out of 58 (77.7%) cultivars have functional *SSIIa* with GRC. Functional SSIIa have a role in synthesizing amylopectin rich in middle-length chains that retrograde faster and become harder texture after gelatinization (Umemoto et al. 2008). Japanese people prefer rice with soft texture even after cooked rice has been cooled for the consumption as a lunch box or rice balls, for example. This may reflect the higher ratio of cultivars with non-acitive SSIIa (haplotype 4 and 5) in JRC compared to GRC.

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SNP1(G) Forward : 5'-GCGGGTGAGGACGACTAG -3'
SNP1(C) Forward : 5'-GCGGGTGAGGACGACTAC -3'
SNP1 Reverse : 5'-CATCAAAGCGAATCAAAGCA -3'

SNP2(G) Forward : 5'-GCCTCGCTGGACTTCG -3'
SNP2(G) Reverse : 5'-ATGGCGTAGAGCTGGTTGA -3'
SNP2(A) Forward : 5'-CTCTGGGAGCTGAAGACGAC -3'
SNP2(A) Reverse : 5'-TTGCAGGCGCCGTTACT -3'

SNP3(G)Forward:5' -CGGGCTGAGGGACATCG -3'
SNP3(A)Forward:5' -CGGGCTGAGGGACATCA -3'

SNP4(C) Forward :5'-GAGAGCTGGAGGGGGC-3'
SNP4(T)Forward :5'-TTTTTTTTTTTTTTTTTTTTTTTTGAGAGCTGGAGGGGTT -3'
SNP3,4Reverse:5' -CACACAAACCGGAAGCTAAT -3'
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Fig. 1 Primer sequences of SNP markers for the four SNPs causing amino acid substitution in rice *SSIIa*.

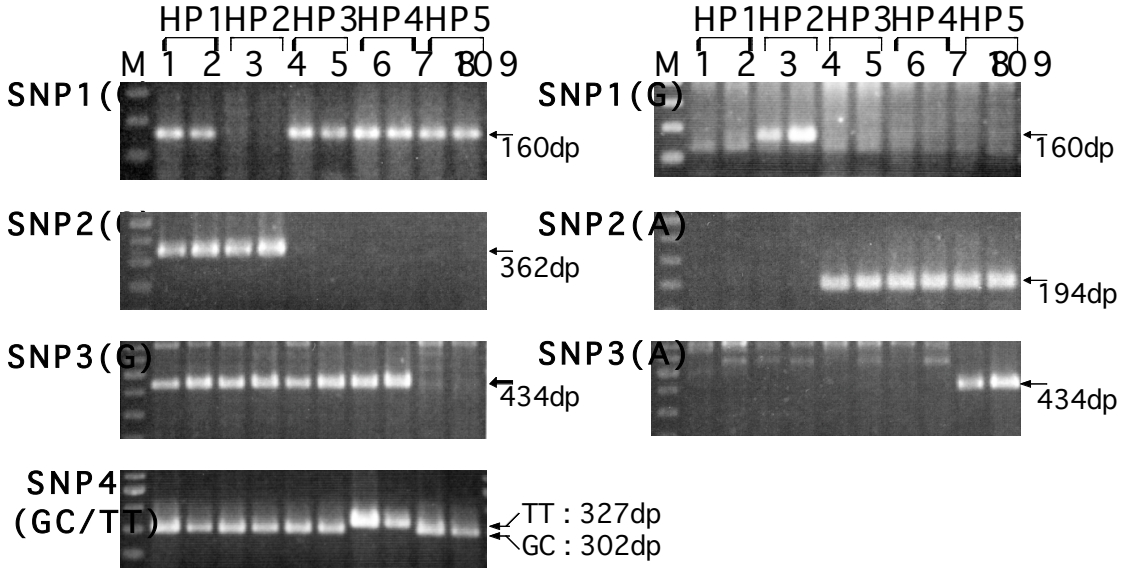

Fig. 2 Validation of *SSIIa* SNP markers with the cultivars known SNPs genotype.
M: Size marker (100bp ladder), HP1 (lane 1: IRAT109, lane 2: upland rice Kantomochi172), HP2 (lane 3: IR72, lane 4: IR64), HP3 (lane 5: Lemont, lane 6: Chiyominori), HP4 (lane 7: Aikoku, lane 8: Kamenoo), HP5 (lane 9: Taichung65, lane 10: Nipponbare). Molecular size of amplified fragments was shown at right of the each panel.

Table 1 Haplotype distribution of *SSIIa* in Global Rice Core Collection (GRC) and Rice Core Collection of Japanese Landraces (JRC).

SSIIa haplotype	SNP 1	SNP 2	SNP 3	SNP 4	SSIIa activity	GRC	JRC
HP1	C	G	G	GC	+	8	1
HP2	G	G	G	GC	+	28	3
HP3	C	A	G	GC	+	9	5
HP4	C	A	G	TT	-	5	20
HP5	C	A	A	GC	-	3	10
Total						53	39

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