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Identification of RAPD Markers Associated with Grain Weight in Rice

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ABSTRACT : This study was carried out to select randomly amplified polymorphic DNA (RAPD) markers associated with grain weight of a large-grain mutant, Hyacp 39-26-1, derived from anther culture of a rice cultivar, 'Hwayeongbyeo'. The segregation mode for grain weight in an F_2 population from a cross, 'Hwayeongbyeo/Hyacp 39-26-1', showed a nearly normal distribution. One hundred and ninety-one F_2 plants ranged from 21.8 g to 34.7 g in 1,000-grain weight with a mean of 26.8 g. Five hundred and twenty primers were used to detect the RAPD markers associated with the grain weight of the large-grain mutant. Of these primers, 54 primers showed polymorphism between 'Hwayeongbyeo' and 'Hyacp 39-26-1'. Four RAPD markers (OPB18, OPH07, OPT20, and OPX20) were significantly related to the grain weight of twenty one F_3 lines derived from the cross, 'Hwayeongbyeo/Hyacp 39-26-1'. This RAPD marker could facilitate the early and efficient selection of high-yield lines through improvement of grain weight in rice.

Keywords **:** RAPDs, Rice, Grain weight

Rice yield is the final product of the manifestation of several components, such as number of panicles per plant, number of grains per panicle, fertility and grain weight, and is a very complex trait. The dissection of such a complex trait by means of the molecular marker approach will be of great significance in helping breeders to design and combine yield components in various ways leading to enhancement of rice. The relatively high heritability estimates reported for grain length and grain width (McKenzie and Rutger 1983, Kato 1990), suggest that selection for grain size would be effective in early generation. However, it has also been noted that environmental factors may greatly influence variation in grain size (Kamijima and Watanabe 1984).

High-density molecular maps of rice have been recently constructed (Cho *et al.* 1998, Kang *et al.* 1998) and some quantitative traits have also been studied using molecular markers (Kato *et al.* 1999, Zhu *et al.* 1999). One of the main uses of the molecular maps is to locate markers linked to genes of interest. The molecular marker linked to useful genes is now possible for the breeder to conduct many rounds of selection in a year without depending on phenotypes. The recent advent of molecular markers in quantitative genetics greatly facilitates the study of complex quantitatively inherited traits and has made it possible to dissect the polygenes into individual Mendelian factors. Progress has been made in mapping and tagging many agriculturally important genes with molecular markers, which forms the foundation for marker-aided selection (MAS). Recently, RFLP and RAPD techniques have been used to identify the DNA markers associated with the resistance of blast (Hittalmani et al. 1995), bacterial blight (Zhang *et al.* 1996), gall midge (Nair *et al.* 1996), brown planthopper (Jeon *et al.* 1999, Cha *et al.* 1999), and cold tolerance (Kim *et al.* 1999) in rice.

In this paper, we report the genetic analysis of grain characteristics and the identification of RAPD markers linked to grain weight in rice. These RAPD markers were to assess the utility of MAS strategy for grain yield improvement in rice.

MATERIALS AND METHODS

Plant materials

The F_2 population used in this study was derived from a cross between a *japonica* cultivar, 'Hwayeongbyeo' and a large-grain mutant, 'Hyacp 39-26-1'. The 'Hyacp 39-26-1' was selected from the anther-derived population of a *japonica* cultivar, 'Hwayeongbyeo', which was developed by anther culture technique. This trait was normally inherited in the self-pollinated progenies. To investigate the inheritance of the large-grain characteristics, the parents, F_1, F_2, and F_3 progenies of the cross were grown in the greenhouse and field at the Experimental Farm of Kyungpook National University at Taegu, Korea.

Seeds were sown in the box nursery bed, and the 4th or 5th leaf stage of seedlings were transplanted at field under

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planting density of 30 × 15 cm with one plant per hill. The fertilizer was applied in the field at a ratio of 12-9-11 kg/10a (N-P_2O_5-K_2O). Nitrogen was applied splitly by 50% (basal)-30% (tillering stage)-20% (panicle initiation stage). Phosphorous was applied once as a basal, and potassium was applied splitly by 80% (basal)-20% (panicle initiation stage). Other cultivation management followed the conventional method in the central region of Korea.

Four grain characteristics; grain length, width, shape, and weight were examined in the F_2 population. Ten random grain-samples per plant were used for measuring grain length, grain width and grain shape (length/width ratio), and 100 random grain-samples were used for obtaining grain weight data. Length measurements were recorded to the nearest millimeter and grain weight was recorded in milligrams. The frequency distribution of grain length, grain width, grain shape (length/width ratio), and grain weight were investigated and statistically analyzed in the F_2 population.

DNA extraction and RAPD analysis

DNA was extracted from F_3 plants for 30-day-old seedlings grown in the greenhouse. Leaf material (0.3 g fresh weight) was frozen in liquid nitrogen and ground in a mortar and pestle. Total genomic DNA extraction was performed using a CTAB method (Murray and Thompson 1980). DNA was diluted to a working concentration of approximately 2 ng/μl by visual comparison to a lambda DNA standard on agarose gel.

To select of RAPD markers associated with grain weight, DNA amplification was performed on a Perkin-Elmer Gene Amp PCR System 2400 in 12 μl reactions consisting of 1.2 μl 10×buffer (10 mM Tris-HCl, pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 10 mM of dNTPs, 2.5 ng primer, 0.5U *Taq*-polymerase and 5 ng template DNA. For the polymerase chain reaction (PCR) cycling conditions, samples were first heated at 96°C for 5min before entering a 45 cycle PCR procedure of 96°C for 1min, 36°C for 1min and 72°C for 2min. A final time delay phase of 72°C for 7min was always run before an optional soak period at 4°C. Amplification products were separated by electrophoresis on 1.2% agarose gels in 1×TAE buffer (400 mM Tris base, 20 mM acetic acid, 2 mM Na_2-EDTA-H_2O), stained with ethidium bromide. DNA fragments were visualised on a UV transilluminator and photographed using polaroid film. All primers used

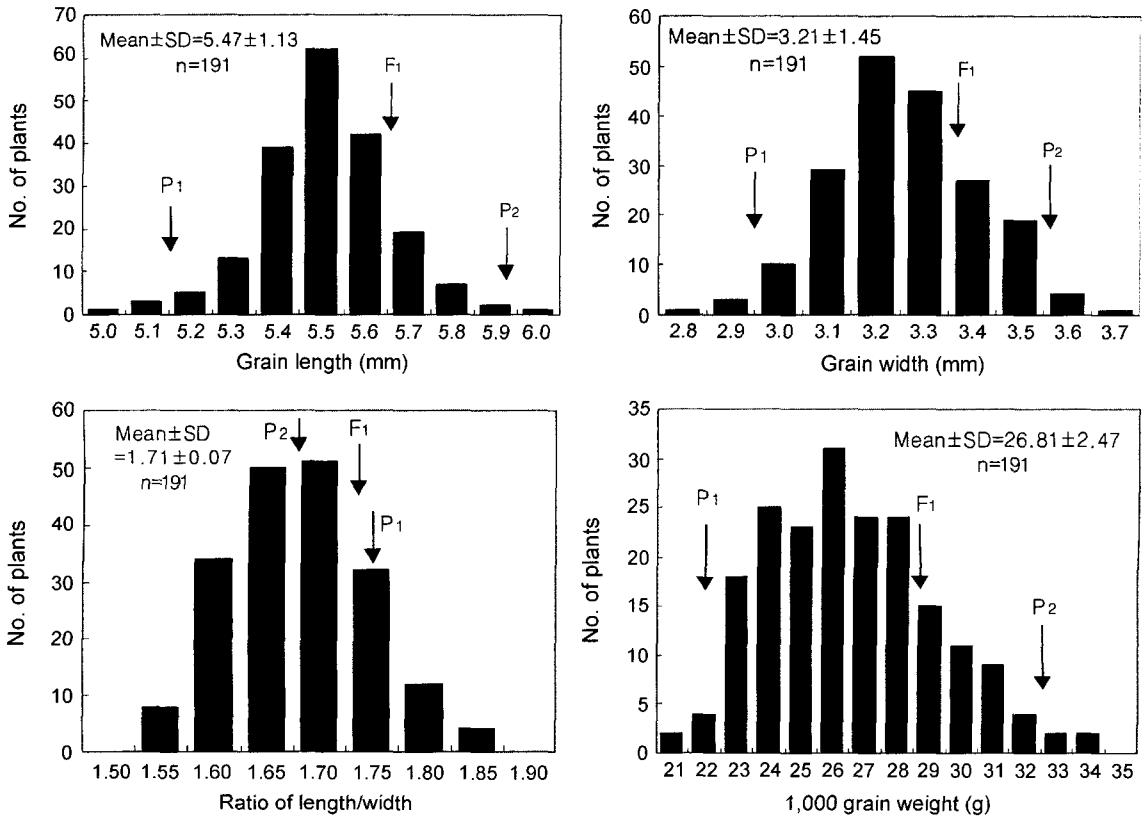

Fig. 1. Frequency distribution of grain length, width, shape (length/width ratio) and 1,000 grain weight in an F_2 population from a cross between 'Hwayeongbyeo (P_1)' and 'Hyacp 39-26-1 (P_2)'.

were 10-mer random oligonucleotide sequences purchased from Operon Technologies (Alameda, California, USA). 520 10-mer oligonucleotide primers (Operon kits A-Z) were screened for polymorphisms between the parents. Two independent PCR reactions were carried out to confirm the results when a primer detected polymorphisms between the parents. Selected polymorphic primers were used to amplify the bulked DNAs of F_3 lines with large grain (11) and small grain (10) with the parents and F_1. A t-test was carried out to find out any significant relationship between RAPD marker and grain weight.

RESULTS AND DISCUSSION

Inheritance of grain characteristics

Frequency distribution of the grain characteristics in the 191 F_2 population of 'Hwayeongbyeo/Hyacp 39-26-1' was shown in Fig. 1. Grain length showed continuous distribution with most of the F_2 lines lying between the parents. The grain length of 191 F_2 plants ranged from 5.0 mm to 6.0 mm with a mean of 5.47 mm. The mean grain length of F_1 plants was longer than the mid-parent value and was near to the longer parent 'Hyacp 39-26-1'. Frequency distribution for grain width and shape in the F_2 population were similar to that of grain length. Grain width and grain shape displayed nearly normal distribution with a range of 2.8 mm to 3.7 mm and 1.55 to 1.85, respectively. Also, 191 F_2 population showed the similar pattern for the grain weight ranging from 21.8 g to 34.7 g with a mean of 26.8 g. These patterns of frequency distribution for the grain characteristics were similar to the report by Hwang *et al.* (1984), Redona and Mackill (1998), McKenzie and Rugter (1983). Hwang *et al.* (1984) reported that frequency distribution of the grain characters in F_2 populations of the crosses between Gayabyeo and BG2, Yeongpungbyeo and TD58 showed continuous variation. Redona and Mackill (1998) reported that the frequency dis-

Table 1. Relationship between RAPD markers and grain weight of F_3 lines derived from a cross, 'Hwayeongbyeo/Hyacp 39-26-1'.

RAPD marker	Average value of grain weight in each genotype (g)		t-value
	Hwayeongbyeo allele	Hyacp 39-26-1 allele	
OPB18	25.35 ± 4.31† (10)‡	29.80 ± 3.82 (11)	2.91*
OPH07	25.38 ± 4.06 (10)	29.37 ± 4.33 (11)	2.17*
OPT20	24.30 ± 4.89 (9)	27.94 ± 4.50 (12)	2.26*
OPX20	24.73 ± 3.70 (10)	29.52 ± 4.16 (11)	2.66*

†Mean ± SD, ‡number of F_3 lines tested, *significant at 5% level.

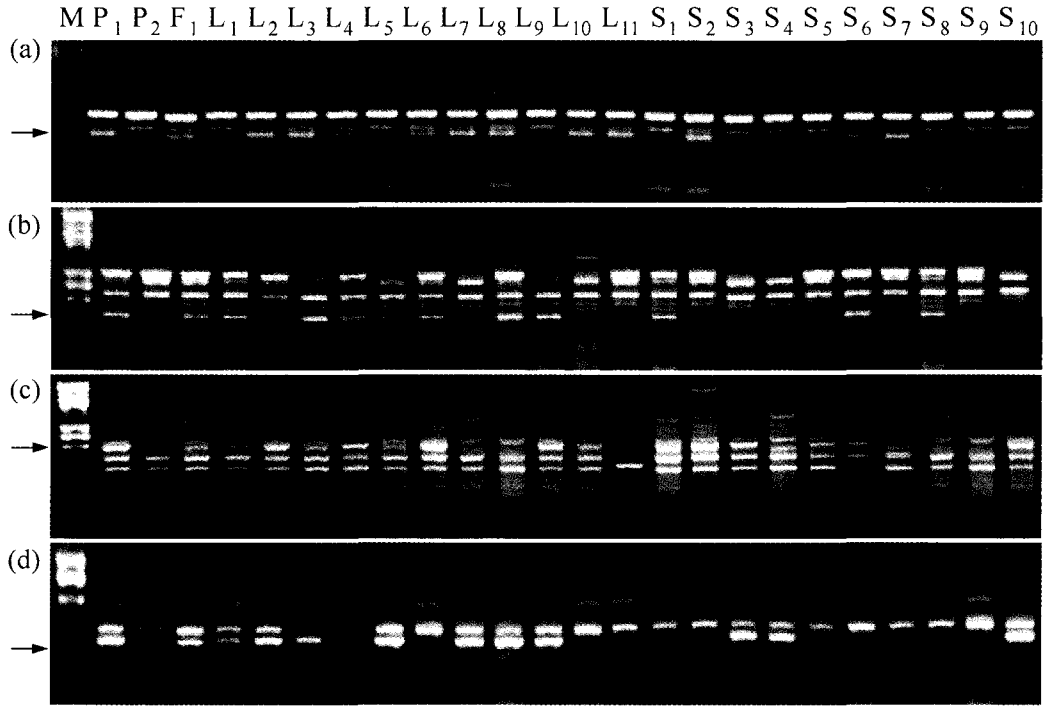

Fig. 2. RAPD polymorphisms of F_3 lines revealed after amplification with primers, OPB18 (a), OPH07 (b), OPT20 (c), and OPX20 (d). Amplification products were loaded onto a 1.2% agarose gel containing ethidium bromide. Arrows indicate the phenotype-specific RAPD fragment. M : Size marker provided by *Eco*RI and *Hind*III-digested with Lambda DNA, P_1 : 'Hyacp 39-26-1', P_2 : 'Hwayeongbyeo', $L_{1\sim11}$: F_3 lines with large grain, $S_{1\sim10}$: F_3 lines with small grain.

tribution of 1,000-grain weight in a population of 204 F_2 plants from the cross between Labelle (*japonica*) and Black Gora (*indica-aus*) was nearly normal. McKenzie and Rugter (1983) indicated that the frequency distribution of kernel width of the segregating generations were continuous, relatively symmetrical and centered between the respective parents.

Identification of RAPD markers linked to grain weight

To detect RAPD markers associated with the grain weight, the 520 oligonucleotide primers were screened between 'Hwayeongbyeo' and 'Hyacp 39-26-1'. 54 of the 520 primers yielded polymorphic fragments between 'Hwayeongbyeo' and 'Hyacp 39-26-1'. A total of 72 polymorphic bands was detected from these 54 primers, with some oligonucleotide primers revealing more than one polymorphic band. These 54 primers generated a total of 256 bands with an average of 4.78 ranging from 2 (OPT17) to 8 bands (OPH05) per polymorphic primer. Of these polymorphic bands, 35 bands were specific to the 'Hyacp 39-26-1' and 37 bands to 'Hwayeongbyeo'. Therefore, only 35 of the 72 polymorphic bands were used in the selection of RAPD markers associated with large-grain weight in rice.

Four RAPD markers (OPB18, OPH07, OPT20, and OPX20) of these 35 markers were significantly associated with the grain weight of 21 F_3 lines (Table 1), and the sizes of polymorphic bands were 850 bp, 1100 bp, 1350 bp and 1200 bp, respectively (Fig. 2). These markers showed the same result with doubled haploid (DH) lines (Table 2, Fig. 3). These 4 RAPD markers were not related with other characteristics such as culm length, panicle length and panicle per plant.

Table 2. Relationship between RAPD markers and grain weight of DH lines derived from a cross, 'Hwayeongbyeo/Hyacp 39-26-1'.

RAPD marker	Average value of grain weight in each genotype (g)		t-value
	Hwayeongbyeo allele	Hyacp 39-26-1 allele	
OPB18	25.35 ± 4.31† (10)‡	29.80 ± 3.82 (11)	2.91*
OPH07	25.38 ± 4.06 (10)	29.37 ± 4.33 (11)	2.17*
OPT20	24.30 ± 4.89 (9)	27.94 ± 4.50 (12)	2.26*
OPX20	24.73 ± 3.70 (10)	29.52 ± 4.16 (11)	2.66*

†Mean ± SD, ‡number of F_3 lines tested, *significant at 5% level.

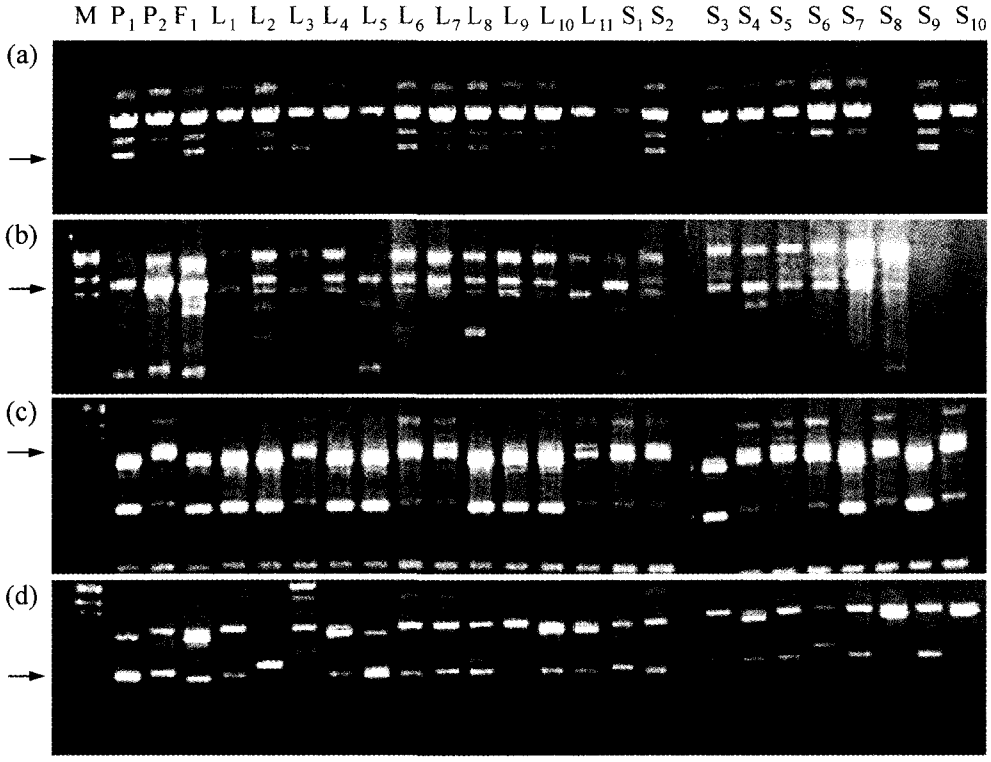

Fig. 3. RAPD polymorphisms of DH lines revealed after amplification with primers, OPB18 (a), OPH07 (b), OPT20 (c), and OPX20 (d). Amplification products were loaded onto a 1.2% agarose gel containing ethidium bromide. Arrows indicate the phenotype -specific RAPD fragment. M : Size marker provided by *EcoR*I and *Hind*III-digested with Lambda DNA, P_1 : Hyacp 39-26-1, P_2 : Hwayeongbyeo, $L_{1\sim11}$: DH lines with large grain, $S_{1\sim10}$: DH lines with small grain.

Hwang *et al.* (1984) reported that grain weight was negatively correlated with panicle length and spikelets per panicle. These RAPD markers ($OPB18_{850}$, $OPH07_{1100}$, $OPT20_{1350}$, $OPX20_{1200}$) were able to amplify phenotype-specific bands in large-grain F_3 lines derived from the cross, indicating their potential utility for MAS of selection of the large grain in rice.

The RAPD method provides a simpler, faster, safer and less expensive means for genome analysis compared with RFLP. A single, short oligonucleotide primer can amplify specific sequences of genomic DNA through PCR. The procedure is rapid, requires only small amounts of DNA, which need not be of high quality and involves no radioactivity. The RAPD markers are usually dominant because polymorphisms are detected as the presence or absence of bands. RAPD obtained by the use of random oligonucleotide primers in PCR have been extensively used as molecular markers for tagging genes. RAPD markers are useful for tagging specific genes or saturating regions sparsely populated with markers (Michelmore *et al.* 1991, Xiao *et al.* 1996).

One of the major advantages of developing markers linked to specific genes is its potential for MAS. MAS would certainly be of immense use to breeding stations involved in developing cultivars resistant to insect pests and abiotic stresses which are difficult to screen. MAS is being used as a powerful tool to increase the selection efficiency for developing new varieties with resistance to disease and insect, better quality, and higher yield potential (Nair *et al.* 1996, Cha *et al.* 1999).

In this study we selected RAPD markers associated with grain weight in rice. Use of this RAPD marker could facilitate early selection for grain weight in rice breeding program.

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