

## Genetic Analysis for Rice Grain Properties Using a Doubled Haploid Population

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**ABSTRACT** Demand for high quality rice has always been a major factor in the international rice marketing. In the present study, doubled haploid (DH) population derived from anther culture of a *Tongil/japonica* hybrid was used for genetic analysis of rice grain quality. The average values of DH lines for grain weight, grain length and the ratio of grain length to width were near the mid-parent value. More than 40% DH lines showed transgressive segregation for grain weight, length, amylose and lipid content, but less than 10% DH lines observed on ratio of length to width and grain thickness were transgressive segregation. Correlation analysis between appearance qualities and physicochemical characters indicated that grain width and grain thickness both significantly and negatively correlated to protein and lipid content. A highly significant negative correlation between protein content and amylose content was observed.

**Keywords** : appearance properties, genetic analysis, physicochemical characters, rice quality

**Rice** is one of the major staple cereal foods, feeding more than half the world population. Its higher yield and better taste are two major subjects for many breeding programs. Knowledge accumulated in the past decades indicates that both grain yield and quality are controlled by quantitative trait loci (QTLs) showing continuous phenotypic variation in rice progeny (Yano & Sasaki, 1997; He *et al.*, 1999). Nutritional quality, appearance quality, cooking and eating quality are prevalently mentioned as evaluating indicators of rice grain quality. The grain appearance, consisting of grain weight, length, width, shape and thickness, without doubt plays an important role in the international marketing for consumers or sellers. In addition, nutrition quality associated with amylose, protein and lipid content

has increasingly become one of the main breeding objectives. As rice grain quality is universally considered more complicated inheritance conditioned not only by the triploid endosperm genotype, but also by the diploid maternal genotype and any additional possible cytoplasmic differences (Xu *et al.*, 1995; He *et al.*, 1999). By way of proving the viewpoints above-mentioned, the genetic models including direct seeds, maternal and cytoplasm models had been studied at the end of last century. However, with molecular marker-based analysis and linkage map construction applied for rice quality breeding program in recent years, QTL detection of quality as a combination of traditional genetics and biotechnology, had been widely studied using populations such as F<sub>2</sub>'s, recombinant inbred lines (RILs) and doubled haploid lines (DH).

In this study, the appearance qualities and physicochemical traits of 120 lines derived from DH population had been evaluated to depict mean and frequency distribution of the traits, and to analyze phenotypic genetics and coefficients among traits. The results may be employed in QTL analysis and construction of linkage map for those traits in the future.

### MATERIALS AND METHODS

A total of 120 doubled haploid (DH) lines that were developed by anther culture from the F<sub>1</sub> hybrid of a cross between 'Samgangbyeo', a *Tongil* variety, and 'Nagdongbyeo', a *japonica* variety were incorporated in this research. For each DH line, 40 plants were grown in two rows, with parent plants being grown between every ten DH lines as the control in the experimental field of Kyungpook National University, at Gunwie, Korea. Using a spacing pattern of 15 cm between plants and 30 cm between rows, about

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30-day-old seedlings of each DH line were grown in the summer rice growing season of 2004 and 2005. Field management and fertilizer employing followed the normal agricultural practice through the whole rice growth stage.

### Rice grain properties evaluation

The harvested paddy rice grains of each DH line or parent were dried and then stored at room temperature for at least 3 months before analysis. After paddy rice de-hulling, appearance properties and physicochemical characteristics were evaluated separately.

### Appearance properties

Grain length, width and thickness of three repeats with 10 brown grains as one repeat for each line were determined using vernier caliper.

### Physicochemical characteristics

NIR spectrophotometer (Foss 6500) was used to determine amylose, lipid and protein content of three repeats for each DH line.

## RESULTS

### Statistical analysis

Standard deviation, average deviation, variance, range and coefficient of variation were evaluated for grain appearance

and physicochemical characters of 120 DH lines (Table 1). Slight variations of all traits were observed, indicating that 120 DH lines possessed good central tendency and representative averages. Skewness and Kurtosis were detected as normal distribution test suggesting that all five grain appearance traits and amylose content segregated continuously and approximately fit normal distributions with absolute values less than 1.0, but those of protein and lipid content with absolute values more than 1.0 implicated unremarkable normality distribution.

### Phenotypic analysis of grain appearance

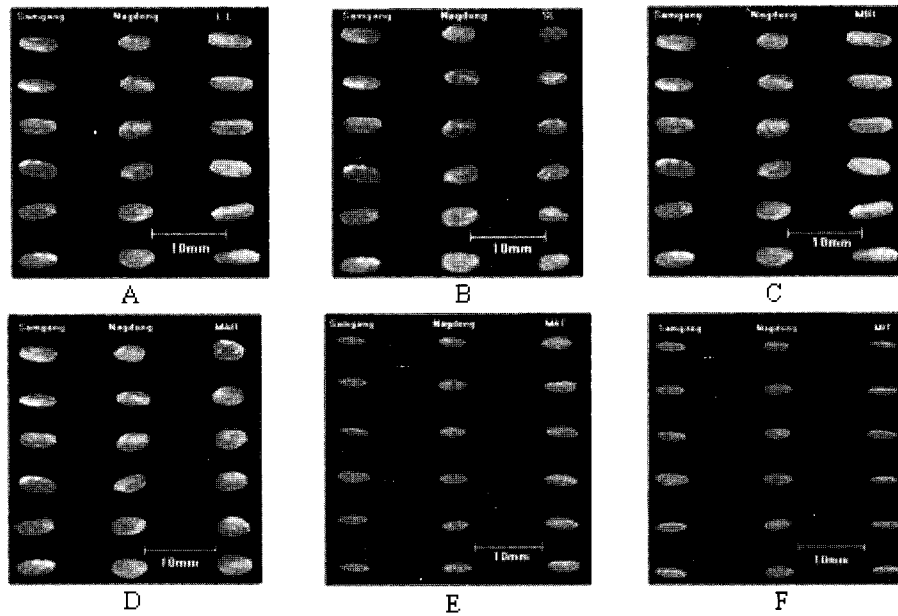
As shown in Fig. 1 and Table 2, significant differences were observed on all five traits between two parents ( $p < 0.001$ ). The analyses of variance indicated highly significant differences for all traits among DH lines ( $p < 0.0001$ ). As compared with 'Samgangbyeo', 'Nagdongbyeo' was associated with a higher grain weight, a shorter grain length, a wider grain width, a lower ratio of grain length to width and a bigger thickness. The means of two parents for the grain appearance were higher than that of DH population, except for that of grain thickness.

The phenotypic frequency distributions for grain appearance were shown on Fig. 2 (1)-(5). More than 40% of the DH lines showed transgressive segregations for grain weight and length with a range from 12.60 to 24.90 and from 4.76 to 5.87 (Table 2), respectively. The mean values of grain

**Table 1.** Statistics parameters evaluation and normal distribution test for grain appearance and physicochemical characteristics of 120 DH lines from a cross 'Samgangbyeo' and 'Nagdongbyeo'

Statistics parameters	Standard Deviation	Average Deviation	Variance	CV (%)	Range	$h_B^{2†}$ (%)	Skewness	Kurtosis
Grain weight	2.294	1.75	5.264	12.71	12.33	86.1	0.259	0.229
Grain length	0.238	0.20	0.057	4.52	1.11	88.6	-0.017	-0.455
Grain width	0.187	0.15	0.035	6.95	0.87	98.7	0.536	-0.227
Ratio of length to width	0.154	0.12	0.024	7.79	0.81	83.7	0.180	0.173
Grain thickness	0.093	0.08	0.009	4.76	0.51	75.0	0.083	-0.136
Protein content	0.875	0.65	0.766	10.90	5.13	95.0	1.175	2.553
Lipid content	0.219	0.17	0.048	10.45	1.39	82.8	0.886	2.326
Amylose content	0.976	0.80	0.953	4.72	4.74	95.5	-0.435	-0.066

<sup>†</sup>Indicated broad-sense heritability calculated as :  $h_B^2 = \delta_g^2 / (\delta_g^2 + \delta_e^2 / n) \times 100\%$ , where  $\delta_g^2$  is genetic variance,  $\delta_e^2$  is error variance and  $n$  is the number of replicates of the trial (Tan *et al.*, 2001; Hu *et al.*, 2004).



**Fig. 1.** The comparison on the grain length, grain shape and thickness between DH lines and parents. A and B showed comparison on grain length, but C and D on the ratio of length to width. E and F indicated comparison on grain thickness. The third row of each figure was DH line.

Note. LL : The longest length; SL : The shortest length; MAR : The maximal ratio of length to width; MIR : The minimal ratio of length to width; MAT : The maximal thickness; MIT : The minimal thickness.

**Table 2.** The phenotypic performance of the DH population and its parents for eight quality traits

Quality traits	Parents			DH population	
	Samgangbyeo	Nagdongbyeo	Means	Range	Means
Grain weight (g)	17.73±0.006	20.77±0.021	19.25±0.014	12.60-24.90	18.06±0.210
Grain length (cm)	5.64±0.032	5.20±0.031	5.42±0.031	4.76-5.87	5.28±0.022
Grain width (cm)	2.50±0.019	2.94±0.012	2.72±0.016	2.26-3.13	2.69±0.017
Ratio of length to width	2.25±0.021	1.77±0.011	2.01±0.016	1.65-2.46	1.97±0.014
Grain thickness (cm)	1.83±0.015	2.03±0.014	1.93±0.015	1.71-2.22	1.95±0.009
Protein content (%)	8.04±0.048	6.96±0.040	7.50±0.044	6.29-11.47	8.03±0.080
Lipid content (%)	2.07±0.015	2.03±0.040	2.05±0.025	1.67-3.06	2.10±0.020
Amylose content (%)	21.62±0.082	21.29±0.068	21.45±0.076	18.03-22.77	20.69±0.089

weight, length and width were just close to the value of one parent, 'Samgangbyeo' or 'Nagdongbyeo', indicating that these three traits are controlled by at least two non-allelic QTLs, respectively. The distribution of ratio of length to width and grain thickness ranged from 1.65 to 2.46 and from 1.71 to 2.22 with mean values 1.97 and 1.95 in 120 DH lines, though less than 10% lines of both two traits showed transgressive segregations.

#### Phenotypic analysis of grain physicochemical characteristics

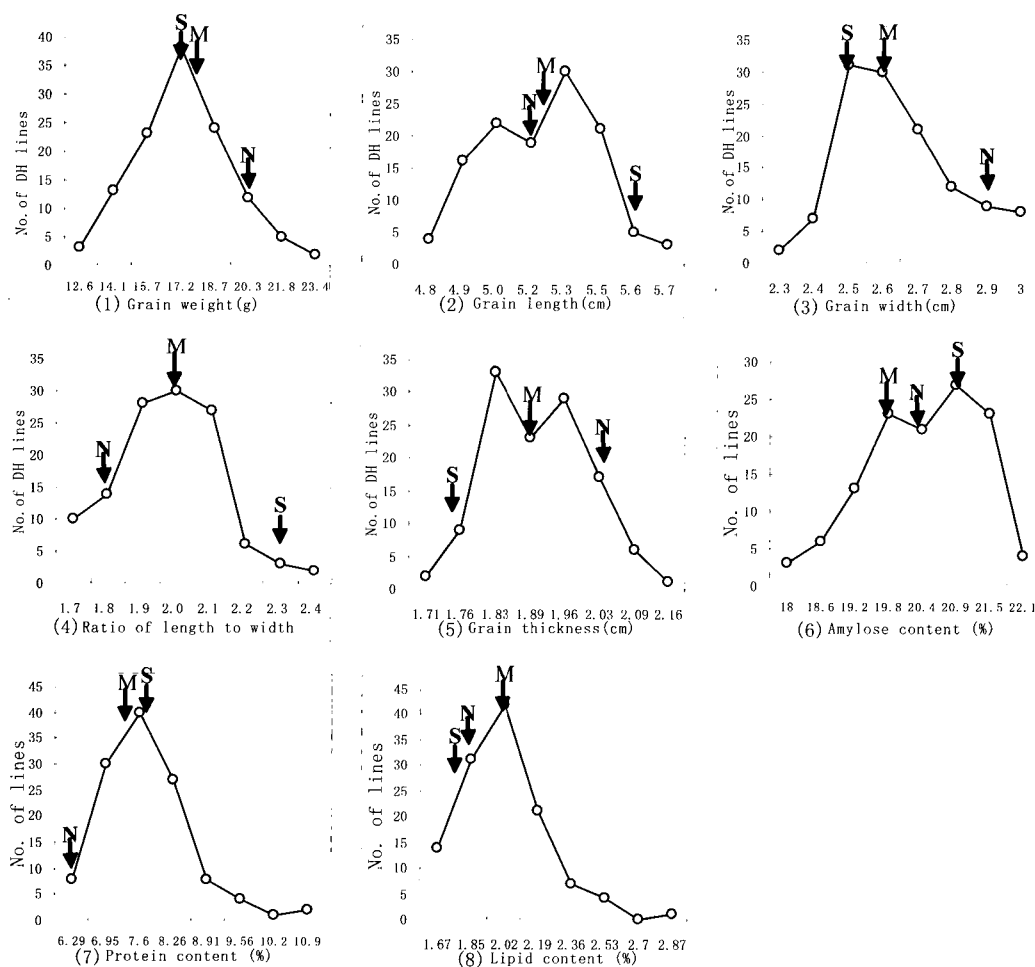
The analyses of variance suggested that there was a significant difference on amylose content of two parents ( $p < 0.01$ ), but not on protein and lipid content ( $p > 0.05$ ). As well as appearance characters, all three physicochemical properties of 120 DH lines had highly significant differences ( $p < 0.0001$ ). The means of two parents for protein and lipid content of brown rice exhibited less than those of DH

population, but the opposite result was observed on amylose content (Table 2). The mean amylose content of DH lines, 20.69%, was 4% and 2% lower than that of ‘Samgangbyeo’ and ‘Nagdongbyeo’, respectively, manifesting that more than 50% of DH lines were transgressive segregations with a range from 18.03% to 22.77% (Fig. 2 (6)). The mean of protein content in DH population (Fig. 2 (7)) was 17% higher than that of ‘Nagdongbyeo’, but similar to the value of ‘Samgangbyeo’ and ‘Nagdongbyeo’, potentially implying that protein content was controlled by at least two non-allelic QTLs. The distribution of lipid content in 120 DH lines ranged from 1.67% to 3.06% with a mean value of 2.10% which was higher than that of both two parents, indicating that more than 75% of DH lines were trans-

gressive segregations (Fig. 2 (8)). Further study integrating to the marker-based mapping to detect QTLs related to amylose, protein and lipid content will be unfolded.

### Correlation coefficients

That is easy to understand significantly positive or negative correlations ( $P < 0.01$ ) among appearance characters were shown in Table 3. Highly significant correlations were observed between grain width and grain weight ( $r = 0.85$ ), or the ratio of grain length to width ( $r = -0.81$ ), as we expect. The grain thickness significantly correlated to the grain weight ( $r = 0.60$ ) and width with a positive value, but to the ratio of grain length to width ( $r = -0.60$ ) with a negative value. Significant negative correlations were



**Fig. 2.** Distributions of grain appearance characters and chemical constituents in DH population from a cross ‘Samgangbyeo’ (S) and ‘Nagdongbyeo’ (N). The means of traits for the DH lines (M) and both parents were indicated by arrows.

**Table 3.** Correlation coefficients among eight grain characteristics of 120 DH lines from a cross ‘Samgangbyeo’ and ‘Nagdongbyeo’

Grain properties	X1	X2	X3	X4	X5	X6	X7	X8
Grain weight (X1)	1.00							
Grain length (X2)	0.42**	1.00						
Grain width (X3)	0.85**	0.10 <sup>ns</sup>	1.00					
Ratio of length to width (X4)	-0.49**	0.50**	-0.81**	1.00				
Grain thickness (X5)	0.60**	-0.10 <sup>ns</sup>	0.63**	-0.60**	1.00			
Protein content (X6)	-0.40**	-0.09 <sup>ns</sup>	-0.37**	0.26**	-0.43**	1.00		
Lipid content (X7)	-0.37**	-0.16 <sup>ns</sup>	-0.27**	0.14 <sup>ns</sup>	-0.32**	-0.02 <sup>ns</sup>	1.00	
Amylose content (X8)	0.27**	0.11 <sup>ns</sup>	0.18 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.33**	-0.57**	-0.10 <sup>ns</sup>	1.00

\*\*means significant at the 1% level; <sup>ns</sup>means not significant.

observed between grain weight and ratio of length to width ( $r = -0.49$ ), or protein content ( $r = -0.40$ ), or lipid content ( $r = -0.37$ ). There were significant negative correlations between grain width and protein content ( $r = -0.37$ ), and between grain width and lipid content ( $r = -0.27$ ), with same trends observed between grain thickness and protein or lipid content ( $r = -0.43$ ,  $r = -0.32$ ). A highly significant negative correlation between protein content and amylose content ( $r = -0.57$ ) was shown. Grain thickness significantly correlated to amylose content ( $r = 0.33$ ). Thus it can be seen that the nutrition qualities including amylose, protein and lipid content have great influences on the grain appearance of brown rice, as Hu *et al.* reported (2004).

## DISCUSSION

Through displaying of frequency distribution of all traits in this study, grain weight, length, width and protein content were considered that controlled by at least two non-allelic QTLs.

According to studying for amylose content in recent years, a prevailing view considered that amylose content was controlled by a major gene and some minor modifying genes. A previous study showed that the amylose content is controlled by the *Wx* locus or a genomic region tightly linked to this locus on chromosome 6 (Tan *et al.*, 1999). Different inherited population were used for amylose content showed varied frequency distributions of phenotype no more than fitted to the ratio of 3:1 and 1:1, such as F<sub>2</sub> and

RIL with that of 3:1 and 1:1 ratios (Tan *et al.*, 1999) and DH with that of 1:1 ratio (He *et al.*, 1999), all of them merely supporting the same result. In the present study, the phenotypic distribution for amylose content could not offer much more genetic information to support similar viewpoints but have to recur to QTL detection.

A highly negative correlation between protein content and lipid content had been reported to indicate a linkage and two epistatic loci of protein and lipid content possibly account for the phenotypic correlation (Hu *et al.*, 2004). There was not a significant correlation between them in this research with a potential reason accounted for extra-genetic effects. In the future study, DNA markers will be used for detecting QTLs of appearance and physicochemical characters. A trend for fine-scale mapping and characterization of detected QTLs including positional cloning of genes at QTLs to improve rice grain quality will be achieved.

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