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Physicochemical Properties of Non-glutinous, Dull, and Glutinous Rice Grain in Segregating Populations of Dull/Glutinous Crosses

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ABSTRACT

Dull grains segregated from F_3 and F_4 of the crosses between two dull mutants and a glutinous cultivar were compared with non-glutinous and glutinous segregants for their physicochemical properties. Amylose content of dull rice grain segregated from the dull/glutinous cross showed the intermediate value between glutinous and non-glutinous rice grain, whether it is controlled by the recessive or dominant gene. Alkali digestibility value (ADV) of dull rice grain was lower than that of glutinous or non-glutinous rice. A positive correlation was found between ADV and amylose content of homozygous non-glutinous or dull F_4 grains, but a negative relationship was observed in glutinous grains.

Protein content of dull grain was significantly higher than that of glutinous or non-glutinous grain segregated from the same cross, while those of glutinous and non-glutinous grains were not different. Among gelatinization characteristics, initial pasting temperature and peak viscosity of dull grains were higher than glutinous rice, and were not different with non-glutinous grain. Hot, cool and consistency viscosities of dull grain were intermediate between glutinous and non-glutinous rices. Dull grains showed the highest breakdown viscosity and the lowest setback viscosity among the three endosperm types.

Keywords : rice grain quality, dull endosperm, amylose, alkali digestibility value, protein, gelatinization characteristics.

Dull endosperm is a typical characteristic of the non-glutinous low amylose mutant in rice grain. The dull grain appears translucent and hazy white in endosperm color, showing an intermediate appearance between glutinous and non-glutinous rice grain.

Two dull endosperm rice mutants were newly induced by the treatment of N-methyl-N-nitroso-urea (MNU) on fertilized egg cell of a *japonica* variety, Hwacheongbyeo (Kim et al, 1991). Allelism test revealed that these were different from five dull genes reported in Japan (Omura & Satoh 1984, Yano et al. 1988).

One of the mutants was expressed by the two recessive genes, *du-6^a(t)* and *du-6^b(t)*, and another was controlled by a single dominant gene, *Du-7(t)* (Kim et al., 1992; Koh et al., 1997). It was also reported that *Du-7(t)* gene was only transmitted through female gamete and showed the pleiotrophic effect on pollen sterility(Koh et al., 1997). Consequently, *Du-7(t)* mutants were always heterozygote. The gene *du* controlling dull endosperm was different from *wx* locus and *wx* was epistatic to *du* (Okuno et al., 1983).

Amylose content of the dull mutants ranged from 7.1 to 13.6% while the original variety Hwacheongbyeo showed 17.5 to 18.2% amylose in the grain endosperm. Starch granule of dull mutants was smaller than the original variety, and multi-pores starch structure was found in dull endosperm (Kim et al., 1991). The dull endosperm mutants were smaller in grain size, lower in absolute density and hardness of grain, higher in water absorption rate of grain than Hwacheongbyeo, but alkali digestibility values (ADV) showed no difference. Amylogram characteristics of dull mutants were intermediate between glutinous and non-glutinous rice, and gel consistency was ranged from medium to soft (Koh et al., 1997).

Recently, utilization of dull rice grain for cooking and processing is widely studied because its starch has high viscosity and puffing characteristics after cooking. Rice variety having good eating quality was developed by using dull gene in Hokkaido, Japan (Kim et al., 1994). This study was conducted to evaluate the several physicochemical characters of non-glutinous, dull and glutinous rices segregated from the crosses between newly induced two dull mutants and a glutinous rice variety.

MATERIALS AND METHOD

Low amylose mutant lines, *du-6(t)* and *Du-7(t)*, were crossed to a japonica glutinous rice variety Jinbuchalbyeo in 1994. F_1 plants were grown in greenhouse and then F_2 populations were planted in

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the field in 1995. F_3 seeds borne on F_2 plants were harvested and dehulled. Glutinous, dull and non-glutinous rice grains were separated by the degree of translucency and color stained with iodine solution, and were planted as different F_3 bulk populations in the field in 1996. Homozygous glutinous, dull and non-glutinous F_3 plants were identified after harvesting, and bulked seeds of each endosperm type in F_4 population were planted in 1997.

Seeding and planting dates of F_3 were on May 4 and June 1, 1996, respectively. F_4 seeds were sown on May 3 and planted on June 5, 1997. Planting density was 30 × 15 cm with single plant per hill, and fertilizer levels of the field was 11-7-8 kg per 10a for N-P_2O_5-K_2O in both years.

Amylose content and alkali digestibity value (ADV) of parents and F_4 seeds harvested from each F_3 plants, which were separated into glutinous, dull and non-glutinous normal endosperm, were determined. Amylose content was analysed by the iodine colorimetric method using bulked milled rice grain of each plant. ADV of milled rice grain were scored at 23 hours after soaking in 1.4% KOH solution with a 1 to 7 grading system. Twelve grains of each F_3 plant were tested for ADV.

Protein content and gelatinization characteristics of parents and F_5 seeds of three endosperm types harvested from each F_4 plants were also observed. Total nitrogen(N) content of brown rice was measured by the micro-Kjeldahl method and protein content was calculated with N × 5.95. Gelatinization characteristics of milled rice flour were determined using rapid visco analyzer(RVA-3D Newport). Peak (P), hot(H) and cool(C) viscosity were measured by RVA, and viscosity of breakdown(P-H), setback (C-P) and consistency(C-H) were calculated.

RESULTS AND DISCUSSION

Amylose content

Amylose content of each plant was measured by using F_4 seeds harvested from homozygous glutinous, dull and non-glutinous F_3 plants. Number of plants observed were 160 glutinous, 47 dull and 65 non-glutinous in *du-6(t)*/Jinbuchalbyeo cross, and 142 glutinous and 109 non-glutinous in *Du-7(t)*/ Jinbuchalbyeo cross. Amylose contents of parents were 9.9% for *du-6(t)*, 10.4% for *Du-7(t)* and 7.0% for Jinbuchalbyeo. Frequency distributions for amylose content of milled rice endosperm types were quite different. Glutinous rice varied from 4.0 to 7.1 %, dull grain ranged from 8.7 to 12.2% and non-glutinous rice grains showed variation from 14.5 to 19.6% (Fig. 1).

Significant differences were recognized among mean amylose content of glutinous, dull and non-glutinous rice grain. Although amylose content of glutinous rice might be overestimated because of the same analysis procedure with non-glutinous rice, mean amylose content of glutinous, dull and non-glutinous F_3 plants segregated from the same cross was lowest, intermediate and highest among the three endosperm types, respectively.

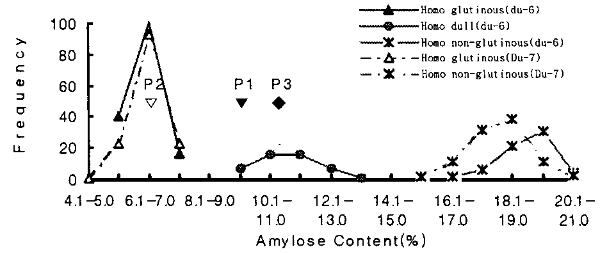


Fig. 1. Frequency distribution for amylose content of homozygous F_4 seeds of *du-6(t)* / Jinbuchalbyeo and *Du-7(t)* / Jinbuchalbyeo crosses.

The pattern of amylose content distribution in this study was almost the same as F_2 distribution (Okuno et al. 1983) in the cross between Kinmaze derived dull mutant and a Japanese glutinous rice. Homozygous dull plants were not segregated in *Du-7(t)* cross because of pleitrophic effect (Koh et al 1997) of *Du-7(t)* gene .

Three types of heterozygous F_3 plants for endosperm characteristics were identified in both crosses. First type was F_3 plant segregating dull and glutinous seeds, second type was non-glutinous and dull segregating plant and third type was the plant segregating glutinous, dull and non-glutinous endosperms. Mean amylose contents of dull and glutinous seeds segregated from each of 9 heterozygous F_3 plants in *du-6(t)*/Jinbuchalbyeo cross were 10.3% and 7.6%, respectively (Table 1). Amylose contents of non-glutinous and dull grains divided from each of 21 plants were averaged as 19.3% and 12.0%. Few F_3 plants segregating non-glutinous, dull and glutinous seeds were found in *du-6(t)* cross.

Endosperm characteristic of the heterozygous F_3 plants was mostly mixed with non-glutinous, dull and glutinous in *Du-7(t)* cross. Non-glutinous, dull and glutinous endosperms segregated from each of 143 heterozygous F_3 plants in *Du-7(t)*/Jinbuchalbyeo cross showed 15.8%, 11% and 6.2% of mean amylose content, respectively(Table 1). It is concluded that amylose content of dull rice grain segregated from the dull/glutinous cross showed an intermediate value between glutinous and non-glutinous rice grain, whether it is controlled by the recessive or dominant gene.

Table 1. Amylose content of non-glutinous, dull and glutinous F_4 seeds segregated from heterozygous plants of two rice crosses, *du-6(t)*/Jinbuchalbyeo and *Du-7(t)*/Jinbuchalbyeo

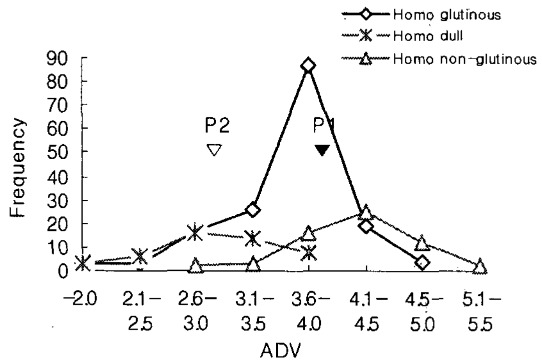
Cross	Dull · glut. segr.*		Non-glut. · dull segr.		Non-glut. · dull · glut. segr.		
	Dull	Glut.	Nonglut.	Dull	Nonglut.	Dull	Glut.
du-6(t)/ Jinbuchalbyeo	10.3a[b]	7.6b	19.3a	12.0b	-	-	-
Du-7(t)/ Jinbuchalbyeo	-	-	-	-	15.8a	11.0b	6.2c
No. of plant	9	9	33	21	146	143	144
C.V(%)	6.5	11.9	5.4	9.5	8.4	10.3	12.2

*F_3 plant segregating dull and glutinous F_4 seeds
[b]Mean values within each heterozygous type followed by same letter are not significantly different at 5% level using LSD

Alkali digestibility value (ADV)

ADV of F_4 grains harvested from each homozygous F_3 plant for endosperm type showed a different distribution pattern between glutinous, dull and non-glutinous seeds in *du-6(t)*/Jinbuchalbyeo cross (Fig. 2). ADV of glutinous rice varied from grade 2.0 to 4.7, dull grains ranged from grade 2.0 to 4.0 and non-glutinous endosperms were distributed from grade 3.0 to 5.2. Mean ADV was significantly different between three endosperm types segregated from the same cross. Non-glutinous rice showed the highest ADV and dull grain was lowest among them, while ADVs of parents were 3.6 for *du-6(t)* and 3.0 for Jinbuchalbyeo.

In *Du-7(t)* cross homozygous non-glutinous segregants showed a wider variation of ADV, which ranged from 2.5 to 4.8 than glutinous rice which varied from 3.0 to 4.2, although ADVs of their parents were 3.7 for *Du-7(t)* and 3.0 for Jinbuchalbyeo. However, mean ADVs of two endosperm types were similar.



Fri. 2. Frequency distribution for alkali digestion value of homozygous F_4 seeds of *du-6(t)*/Jinbuchalbyeo cross.

Dull and glutinous rice grains separated from each herozygous F_3 plant for endosperm type were not different in mean ADV, and non-glutinous endosperm showed higher ADV compared with that of dull grains divided from the heterozygous plant in *du-6(t)* cross (Table 2). Among three endosperm

Table 2. Alkali digestibility value of non-glutinous, dull and glutinous F_4 seeds segregated from heterozygous plants of two rice crosses, *du-6(t)*/Jinbuchalbyeo and *Du-7(t)*/Jinbuchalbyeo

Cross	Dull · glut. segr.		Non-glut. · dull segr.		Non-glut. · dull · glut. segr.*		
	Dull	Glut.	Nonglut.	Dull	Nonglut.	Dull	glut.
du-6(t)/ Jinbuchalbyeo	3.3a[b]	3.4a	4.3a	3.3b	-	-	-
Du-7(t)/ Jinbuchalbyeo	-	-	-	-	3.9a	3.4b	3.8a
No. of plant	10	10	33	33	143	140	140
C.V(%)	15.5	14.2	11.7	16.5	8.3	12.7	8.3

*F_3 plant segregating non-glutinous, dull and glutinous F_4 seeds
[b]Mean values within each heterozygous type followed by same letter are not significantly different at 5% level using LSD

Table 3. Correlation coefficients between alkali digestibility value and amylose content of F_4 seeds of two rice crosses, *du-6(t)*/Jinbuchalbyeo and *Du-7(t)*/Jinbuchalbyeo

Cross	*du-6(t)*/Jinbuchalbyeo			*Du-7(t)*/Jinbuchalbyeo	
	Non-glut.	Dull	Glut.	Non-glut.	Glut.
No. of plant	59	47	159	98	140
r	0.712**	0.405**	-0.665**	0.493**	-0.334**

** Correlation coefficients significant at 1% level

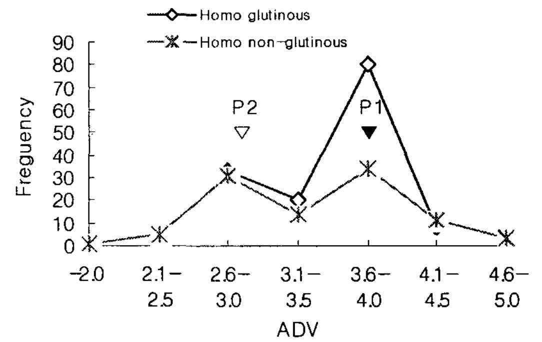


Fig. 3. Frequency distribution for alkali digestion value of homozygous F_4 seeds of *Du-7(t)* /Jinbuchalbyeo cross.

types separated from each heterozygous plant of a *Du-7(t)* cross non-glutinous and glutinous rice indicated similar ADV, and dull grains were lower in mean ADV. The results of Figure 2 and Table 2 indicate that ADV of dull grain is lower than that of glutinous and non-glutinous rice, when they are segregated from the same cross.

A positive correlationship was found between ADV and amylose content of homozygous non-glutinous or dull F_4 grains, but a negative relationship was observed in glutinous grains (Table 3). The reverse relationship between non-glutinous and glutinous rice is obtained because non-glutinous or dull endosperm starch has amylose and amylopectin but glutinous starch is composed of only amylopectin.

Protein content

Protein content of brown rice was measured by using F_5 seeds harvested from 82 homozygous glutinous F_4 plants, 76 dull plants and 70 non-glutinous plants in the cross of *du-6(t)*/Jinbuchalbyeo. Mean protein contents of parents were the same as 6.3% for *du-6(t)* and Jinbuchalbyeo. Homozygous glutinous F_5 grains varied from 4.3% to 8.0% of protein content and non-glutinous seeds ranged from 5.4% to 8.3%, but they were distributed with a similar pattern. But protein content of dull endosperm plants varied from 5.9% to 9.0% and showed a different distribution pattern (Fig. 3). Mean protein content of dull grains was significantly higher than that of glutinous or non-glutinous grains, while those of glutinous and non-glutinous grains were not different.

Gelatinization characteristics

F_5 rice grains harvested from each of 7 homozygous glutinous F_4 plants, 6 dull plants and 8 non-glutinous plants were tested for their gelatinization properties of milled rice flour. The plants used were selected randomly in *du-6(t)*/Jinbuchalbyeo cross.

Table 4. Gelatinization characteristics of non-glutinous, dull and glutinous F_5 rice seeds of *du-6(t)*/ Jinbuchalbyeo cross

Genotype	Initial pasting temp.(℃)	Viscosity(RVU)					
		Peak	Hot	Cool	Break-down	Setback	Consis-tency
P_1, *du-6(t)*	75.4	280.0	151.0	215.0	129.0	-65.0	64.0
P_2, Jinbuchalbyeo	65.7	84.0	42.0	56.0	42.0	-28.0	14.0
F_5 seed	69.3	273.4	124.8	224.5	148.6	-48.9	99.8
Non-glutinous Dull	71.3	270.0	83.2	123.2	186.8	-146.8	40.0
Glutinous	63.8	121.0	47.9	36.1	73.1	-57.9	15.3
Mean	68.0	221.6	87.2	141.8	134.4	-79.9	54.5
CV(%)	3.2	8.6	12.1	7.9	10.1	22.9	15.9
LSD(0.05)	2.4	21.4	11.9	12.6	15.2	20.5	9.7

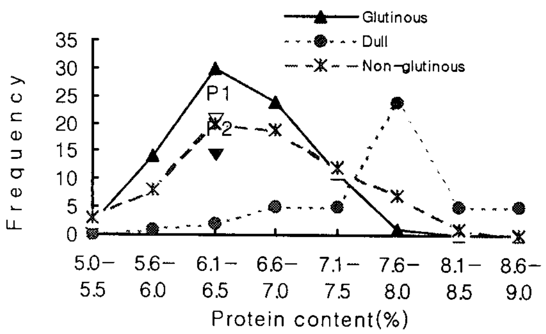


Fig. 4. Frequency distribution for protein content of non-glutinous, dull and glutinous F_5 seed of *du-6(t)*/Jinbuchalbyeo cross.

Glutinous parent Jinbuchalbyeo showed significantly lower values of initial pasting temperature, peak, hot, cool, breakdown and consistency viscosities compared with those of dull parent *du-6 (t)*. It is because glutinous rice starch is extremely softer than non-glutinous rice (Lim et al. 1995, Koh et al. 1997).

Highly significant differences of 7 gelatinization characteristics measured were found among three endosperm types segregating in F_4 population (Table 4). Mean initial pasting temperature and peak viscosity of dull grains were higher than those of glutinous rice and were not different from non-glutinous grain. Hot, cool and consistency viscosities of dull grains was intermediate between glutinous and non-glutinous rice grains. Dull grains showed the highest breakdown viscosity and lowest in set-back viscosity.

Taken together, dull grain segregated from the cross between dull mutant and glutinous variety differed in physicochemical characteristics related with cooking and processing quality. Futher studies are needed for specific utilization of dull rice grains.

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