Development of Near Isogenic Lines and Genetic Analysis for Alkali Digestibility of Rice Grain

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ABSTRACT : In order to develop near isogenic lines (NILs) the heterozygous rice plants for alkali digestibility value (ADV) were selected and tested in every generation from F_5 to F_9 of a cross, Seratus malam/Suwon 345. Finally several sets of NILs, which were six low ADV lines and four medium-high ADV lines, were selected among F_{10} lines. No differences of the plant growth characteristics, amylose content and protein content of rice grain were found between low and medium-high ADV lines. Rice flour of low ADV lines showed longer gel length in gel consistency test than medium-high ADV lines, and also showed different gelatinization characteristics measured by rapid visco analyzer compared with medium-high ADV lines. The result of genetic analysis indicated that ADV-NILs developed were differed in a pair of major gene controlling ADV, and low ADV was dominant over medium-high ADV.

Keywords : rice, alkali digestibility value (ADV), near isogenic lines (NILs), genetic analysis

Isogenic lines are the set of plant materials differing genotypically by one or a few number of alleles. Two isogenic lines for alkali digestibility in rice would have the exactly same genetic composition with the exception of allele(s) governing alkali digestibility value (ADV) of rice grain. Near isogenic lines (NILs) can be developed by successive selfing of the heterozygous plant for target character or by backcrossing to the recurrent parent several times.

NILs have been used to determine the effect of particular allele(s) on the performance and to clarify the expression pathway of the target gene. Recently NILs are widely used to identify DNA markers linked to the economically valuable genes of the crop plant (Young *et al.* 1988). DNA markers are useful for cultivar identification, genetic mapping, germplasm evaluation, and indirect selection in breeding procedure.

ADV is the indirect value for the gelatinization temperature of starch granules in rice grain, and has been considered as an important trait for the selection of good cooking and eating quality (Juliano 1985). ADV of rice grain is determined by both plant genotype and growing environment. Air temperature during ripening period among environmental factors largely affected on ADV (Heu *et al.* 1979), and its variation due to growing season or cultivating year was also found (Heu *et al.* 1976).

One or two major genes related with ADV were reported and low ADV was dominant over high ADV (Kahlon 1964, Heu and Park 1979, Park and Heu 1981). It was also reported that high ADV was dominant in other crosses (Choi and Choi 1980). But the results of genetic analysis reported were not clear because alkali digestibility is the character of endosperm which has triploid (3n) genome. The large variation due to growing environments also made it difficult to find the ADV gene action on cooking and eating quality of rice grain (Kim *et al.* 1994).

The primary objectives of this study were to develop NILs for ADV of rice grain, and to get the precise information for the inheritance pattern of ADV and the pleiotrophic effect of gene(s) controlling ADV by using the NILs.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study were derived from a cross, Seratus malam/Suwon 345. Seratus malam was an Indonesian tropical japonica and was not homozygous in ADV. Suwon 345 was a typical Korean japonica showing medium-high ADV. Successive selfing of the heterozygous plants for ADV was adopted to develop NILs. The individual plants differed in ADV in the segregated lines were selected in each generation for progeny test from F_5 to F_9.

Twenty two F_{10} lines were grown to fix NILs under the normal cultural condition at paddy field in 1998. Twenty eight plants were planted in a row in each line and NILs tested were planted with two replicated randomized block design. Twenty plants of each pedigree line were harvested individually and tested for alkali digestibility of milled rice grain of every plant.

Two sets of NILs in F_9, of which one showed low ADV and another was medium-high, were crossed, and their F_1

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and F_2 plants were grown in 1998 growing season. F_2 seeds harvested from F_1 plants were milled and tested for alkali digestibility of individual grain, and ADV of F_3 seeds harvested from the individual F_2 plants were also tested to know the segregation mode of ADV.

ADV test

Milled rice grains were soaked in 9 ml of 1.4% KOH solution at 30°C for 23 hours and the degree of degradation for each grain was scored with the 1 to 7 grading system. Grade 1 was for the grain not degraded, 2 for the grain swollen, 3 for the grain swollen with incomplete and narrow collar, 4 for the grain swollen with complete and wide collar, 5 for the grain split and segmented with complete and wide collar, 6 for the grain dispersed and merging with collar, and 7 was for the grain completely dispersed and cleared (Juliano 1985). Number of grains tested were 120 grains for the each pedigree line, 36 to 42 grains for the each F_1 and F_2 plant.

Plant growth and grain quality test

Heading date, culm length, panicle length, number of panicle per hill, percentage of matured grain and 1000-grain weight of the each F_{10} line were measured for 10 plants for each line per replication. Bulked grains of the ten F_{10} lines were milled and measured for their amylose content with iodine colorimetric method (Juliano 1971), protein content with micro-Kjeldahl method, gel consistency with IRRI method (Juliano 1985), chroma values of brown rice using chroma meter (Minolta CR-310), and gelatinization properties of rice flour 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

Selection of NILs for ADV

Twenty two F_{10} lines planted were developed from the two F_6 lines (Fig. 1); 10 awnless lines derived from KR90045-812-2-8-3 and 12 awned lines from KR90045-812-2-8-5 (Table 2). There were no segregations in ADV and the agronomic characteristics in each F_{10} line tested (Table 1). This indicated that the plant materials tested were genetically fixed.

Six lines from KR90045-812-2-8-3 group and four lines from KR90045-812-2-8-5 group were selected based on their ADV (Table 1) and the data of seven agronomic characteristics measured (Table 2). Four low and two medium-high ADV lines from the former group, and two low and two medium-high ADV lines were selected from the latter

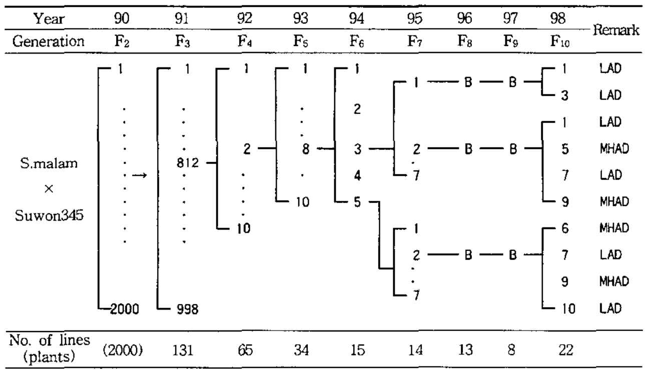

Fig. 1. Selection process of near isogenic lines (NILs) for alkali digestibility value (ADV).

Table 1. Alkali digestibility value of the selected F_{10} lines in Seratus malam/Suwon 345.

Parent or pedigree line	ADV							No. of plants (grains) tested	Mean ADV	Remark[†]
	1	2	3	4	5	6	7			
Seratus malam		119	13	3	116	151	6	34(408)	4.5	
Suwon 345				198	42			20(240)	4.3	
KR90045-812-2-8-3-1-B-B-3	1	239						20(240)	2.0	LAD
KR90045-812-2-8-3-1-B-B-6	3	237						20(240)	2.0	LAD
KR90045-812-2-8-3-2-B-B-1	12	222						20(234)	1.9	LAD
KR90045-812-2-8-3-2-B-B-7	18	222						20(240)	1.9	LAD
KR90045-812-2-8-3-2-B-B-5				208	32			20(240)	4.1	MHAD
KR90045-812-2-8-3-2-B-B-9				203	37			20(240)	4.2	MHAD
KR90045-812-2-8-5-2-B-B-7	1	239						20(240)	2.0	LAD
KR90045-812-2-8-5-2-B-B-10	1	239						20(240)	2.0	LAD
KR90045-812-2-8-5-2-B-B-6			3	213	24			20(240)	4.1	MHAD
KR90045-812-2-8-5-2-B-B-9				222	18			20(240)	4.1	MHAD

[†]MHAD and LAD mean medium-high ADV line and low ADV line, respectively.

Table 2. Agronomic characteristics of the selected F_{10} lines in Seratus malam/Suwon 345.

Parent or pedigree line	Heading date	Culm length (cm)	Panicle length (cm)	No. of panicle	Ripening ratio (%)	1000 gr. wt. (g)	Awn[†]	ADV
Seratus malam	Aug.20	98.0	22.0	8.3	72.6	20.50		
Suwon 345	Aug.18	78.9	20.7	12.1	80.7	24.92		
KR90045-812-2-8-3-1-B-B-3	Aug.10	82.1	22.6	9.1	73.1	24.46	-	LAD
KR90045-812-2-8-3-1-B-B-6	Aug.10	82.6	22.4	9.8	73.4	24.23	-	LAD
KR90045-812-2-8-3-2-B-B-1	Aug. 9	83.2	24.2	9.1	73.3	25.36	-	LAD
KR90045-812-2-8-3-2-B-B-7	Aug.10	81.5	22.9	9.3	73.9	25.68	-	LAD
KR90045-812-2-8-3-2-B-B-5	Aug.10	82.4	23.4	8.8	77.1	24.97	-	MHAD
KR90045-812-2-8-3-2-B-B-9	Aug.11	81.6	23.2	8.9	75.4	25.08	-	MHAD
LSD(0.05)	1.49	3.34	1.19	1.30	9.48	0.96		
KR90045-812-2-8-5-2-B-B-7	Aug. 9	83.3	25.4	8.6	81.7	23.76	+	LAD
KR90045-812-2-8-5-2-B-B-10	Aug. 9	82.3	24.0	8.9	75.1	24.87	+	LAD
KR90045-812-2-8-5-2-B-B-6	Aug.10	84.9	25.1	8.3	74.4	26.48	+	MHAD
KR90045-812-2-8-5-2-B-B-9	Aug. 9	84.9	25.2	7.9	80.9	25.90	+	MHAD
LSD(0.05)	1.41	3.31	0.74	1.17	6.58	1.04		

[†]– : Awnless, + : Awned.

Table 3. Quality related characteristics of the selected F_{11} lines in Seratus malam/Suwon 345.

Parent or NIL	ADV	Amylose (%)	Protein (%)	Gel length (mm)	Chroma value		
					L	a	b
Seratus malam	2.0	23.1	9.2	63.0	32.1	5.27	6.7
Suwon 345	4.4	17.3	7.2	65.1	42.0	1.38	10.6
KR90045-812-2-8-3-1-B-B-3-B	2.0	17.9	7.6	90.3	46.0	1.06	10.2
KR90045-812-2-8-3-1-B-B-6-B	1.9	17.9	7.8	79.7	45.0	1.29	10.1
KR90045-812-2-8-3-2-B-B-1-B	2.0	17.3	8.1	86.1	47.1	1.09	10.4
KR90045-812-2-8-3-2-B-B-7-B	2.0	16.9	7.3	81.7	47.0	1.23	10.7
KR90045-812-2-8-3-2-B-B-5-B	4.3	18.4	7.9	66.3	45.0	1.29	10.1
KR90045-812-2-8-3-2-B-B-9-B	4.7	17.6	7.7	66.1	45.4	1.22	10.3
KR90045-812-2-8-5-2-B-B-7-B	2.0	16.6	7.1	97.0	44.3	1.33	10.2
KR90045-812-2-8-5-2-B-B-10-B	2.0	17.3	7.4	82.5	45.8	1.15	10.2
KR90045-812-2-8-5-2-B-B-6-B	4.6	18.2	7.1	73.6	45.2	1.18	10.1
KR90045-812-2-8-5-2-B-B-9-B	4.3	18.7	7.2	67.7	44.8	1.21	10.1

group. Mean ADVs of them were 2.0, 4.2, 2.0 and 4.1, respectively. No difference in agronomic characteristics was found among the selected lines within the same group except 1000-grain weight in the latter group. Consequently it was estimated that ADV-NILs in F_{10} were genetically fixed. They were four low and two medium-high ADV lines which were awnless, and two low and two medium-high ADV lines which were awned.

Quality related characteristics of NILs

Amylose content of rice grain, protein content of brown rice, gel consistency of rice flour and chroma value of brown rice grain of ADV-NILs selected were compared in Table 3. No difference in amylose content, protein content and chroma value was found among NILs within the same group. However, low ADV lines showed longer gel length in gel consistency test than medium-high ADV lines. This result showed the same tendency with the previous report (Kim *et al.* 1992) in which the gel length was longer in lower ADV lines.

Gelatinization characteristics of rice flour of NILs measured by rapid visco analyzer were presented in Table 4. Low ADV lines showed higher initial pasting temperature, maximum viscosity and breakdown viscosity compared with medium-high ADV lines. However, setback viscosity,

Table 4. Visco-amylogram characteristics of the selected F_{11} lines in Seratus malam/Suwon 345.

Parent or NIL	Initial pasting temp. (°C)	Viscosity, RVU						B/A	C/B	C/A
		Max.(A)	Min.(B)	Final(C)	A-B	C-B	C-A			
Seratus malam	74.3	249	130	253	119	123	4	0.52	1.95	1.02
Suwon 345	68.9	368	147	271	221	124	-97	0.40	1.84	0.74
KR90045-812-2-8-3-1-B-B-3-B	76.7	309	121	220	188	99	-89	0.39	1.82	0.71
KR90045-812-2-8-3-1-B-B-6-B	76.8	295	115	214	180	99	-81	0.39	1.86	0.73
KR90045-812-2-8-3-2-B-B-1-B	76.0	290	110	201	180	91	-89	0.38	1.83	0.69
KR90045-812-2-8-3-2-B-B-7-B	76.9	303	114	204	189	90	-99	0.38	1.79	0.67
KR90045-812-2-8-3-2-B-B-5-B	69.6	264	125	226	139	101	-38	0.47	1.81	0.86
KR90045-812-2-8-3-2-B-B-9-B	66.5	262	136	236	126	100	-26	0.52	1.74	0.90
KR90045-812-2-8-5-2-B-B-7-B	78.6	313	121	216	192	95	-97	0.39	1.79	0.69
KR90045-812-2-8-5-2-B-B-10-B	78.5	315	126	224	189	98	-91	0.40	1.78	0.71
KR90045-812-2-8-5-2-B-B-6-B	70.8	274	135	247	139	112	-27	0.49	1.83	0.90
KR90045-812-2-8-5-2-B-B-9-B	70.5	267	136	248	131	112	-19	0.51	1.82	0.93

Note : A-B, C-B and C-A indicate breakdown, consistency and setback viscosity, respectively.
B/A, C/B and C/A indicate breakdown, consistency and setback ratio, respectively.

Table 5. Frequency distribution of ADV of the F_2 seeds in a cross between medium-high ADV line and low ADV line.

Cross combination	ADV							No. of grains	χ^2 (3 : 1)	P
	1	2	3	4	5	6	7			
KR90045-812-2-8-3-2-B-B (P_1)				43	17			60		
KR90045-812-2-8-3-4-B-B(P_2)	1	83						84		
KR90045-812-2-8-5-1-B-B(P_3)				48	12			60		
KR90045-812-2-8-5-4-B-B(P_4)		84						84		
F_2 seed (P_1/P_2))	1	304	11	87	59	6		468	6.46	0.05~0.01
F_2 seed (P_3/P_4)		340	2	85	64	1		492	7.48	0.01~0.001

breakdown and setback ratios of low ADV lines were lower than those of medium-high ADV lines. This result is partly supported by the previous report (Kim *et al.* 1998) in which ADV showed negative correlationship with breakdown viscosity among gelatinization characteristics in japonica rice.

Genetic analysis of ADV

F_2 seeds of the two crosses between low and medium-high ADV-NILs selected from F_9 pedigree field were tested for their ADV. The frequency distribution of ADV for the F_2 grains showed that more low ADV seeds were segregated than medium-high ADV seeds. But chi-square test indicated that the segregation ratio of low and medium-high ADV did not fit to 3 : 1 in both crosses (Table 5). This result didn't agree to the previous reports in which the segregation of low and high ADV was fit to 3 : 1 ratio (Heu & Park 1979a), or the segregation of low and medium ADV to 3 : 1 ratio in F_2 seeds (Park & Heu 1981).

The biased segregation ratio from the expected 3 : 1 ratio in Table 5 might be caused by the following reasons. Because ADV of each rice grain is the endosperm character determined by triploidy(3n) genotype of each grain, the dosage effect of ADV gene in seed endosperm might modify the segregation mode of ADV for F_2 grains. Another reason might be the modifier gene action to the major gene(s) for ADV. Heu and Park (1979a) reported that a pair of major gene and a few modifier genes controlled ADV of rice grains in the crosses between low and high ADV varieties.

F_2 plants of a cross between low and medium-high ADV-NILs were classified into three types. They were F_2 plants bearing homozygous low ADV F_3 seed grains, F_2 plants bearing F_3 seed grains segregating in ADV, and F_2 plants producing homozygous medium-high ADV F_3 grains. Number of F_2 plants showing homozygous low, segregating and homozygous medium-high in ADV were 26, 55 and 25, respectively (Table 6). This was fit to the segregation ratio of 1 homozygous low : 2 segregating : 1 homozygous medium-high. This result indicated that ADV of rice grain followed the single gene inheritance pattern.

Frequency distribution of F_2 plants in Table 6 was not affected by the dosage effect of ADV gene because it was

Table 6. Frequency distribution of F_2 plants showing homozygous low, heterozygous and homozygous medium-high in ADV of rice grains of a cross between medium-high ADV line and low ADV line.

Cross combination	ADV			No. of plants	χ^2 (1 : 2 : 1)	P
	homozygous low	heterozy-gous	homozygous mediumhigh			
KR90045-812-2-8-3-2-B-B(P1)			10	10		
KR90045-812-2-8-3-4-B-B(P2)	10				10	
$F_2(P_1/P_2)$	26	55	25	106	0.17	0.90~0.95

based on number of plants differed in genotypes for ADV. Therefore, it could be concluded that ADV-NILs developed were differed in a major gene controlling ADV. Low ADV lines showed the dominant gene action over medium-high ADV lines. The biased segregation ratio of ADV in F_2 grains might be caused by the dosage effect of ADV gene in seed endosperm.

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