Genetic characteristics of Korean weedy rice (*Oryza sativa* L.) by RFLP analysis

Young-Chan Cho[1,3], Tae-Young Chung[1] & Hak-Soo Suh[2]
[1] *Department of Biology Resources, National Agricultural Science and Technology Institute, RDA, Suweon 441–707, Rep. of Korea;* [2] *College of Natural Resources, Yeungnam University, Kyongsan 712–749, Rep. of Korea;* [3] *Present address: Rice Breeding Division, National Crops Experiment Station, RDA, Suweon 441–100, Rep. of Korea*

Summary

The genetic characteristics and classification of 24 strains of Korean weedy rice, two strains of foreign red rice, three *Japonica* cultivars, one *Tongil* cultivar and one *Indica* cultivar (*Oryza sativa* L.) were investigated at the DNA level using the restriction fragment length polymorphism (RFLP) method. Eighty-three random combinations between six restriction enzymes and forty genomic DNA probes (RG# and KR#) were assayed. Thirty-seven (92.5%) out of the forty probes used showed polymorphisms among the 31 accessions assayed. A high level of polymorphism was found between short and long grain type Korean weedy rices, whereas fewer polymorphisms were presented among strains within each grain type. A dendrogram summarizing genetic similarity coefficients among thirty-one accessions was constructed based on their DNA polymorphisms. The Korean weedy rice strains were classified into two groups identical to the short and long grain types classified by morphological and physiological characters. From the RFLP analysis, it was deduced that the short grain strains of Korean weedy rice belonged to *Japonica*, while the long grain strains were closer to *Indica* than to *Japonica*, and were differentiated into a local ecotype surviving in the growth conditions in the southern part of the Korean peninsula.

Introduction

Rice is the staple food in Korea, and although no wild relatives occur, Korean weedy rice (*Oryza sativa* L.; locally called 'Red rice', 'Aengmi' and 'Sharei') survive in farmers' fields. Due to their weedy characteristics such as long seed dormancy, easy grain shattering and drought tolerance, weedy rice persists as weeds in rice fields since time immemorial (Hara, 1942; Oka, 1988). The seed coat color of Korean weedy rice varies from dark purple, red and brown to white or colorless (Heu et al., 1990; Suh et al., 1992a). Korean weedy rice strains can be classified into short and long grain types (Suh et al., 1992a). Short grain Korean weedy rices were distributed all over the country, while long grain types were presented only in the Nakdong River and Seomjin River basins of the southern part of the Korean peninsula (Hara, 1942; Suh et al., 1992a). Korean

weedy rice is considered a useful germplasm because it successfully acclimatised and adapted to the natural growing conditions (Heu, 1990; Suh et al., 1992a). It also has many useful genes for blast resistance (Cho & Suh, in preparation), seedling cold tolerance (Suh & Morishima, in preparation), and grain quality and germination characteristics (Suh & Ha, 1993a, 1993b).

Morphological and physiological characters, crossing-affinities, and biochemical composition of proteins and isozymes have been used to classify and identify the subspecies of *Oryza sativa* L. (Eun et al., 1989; Glaszmann, 1987; Heu et al., 1988, 1990; Oka, 1988; Suh et al., 1992b). Recently, the potential value of restriction fragment length polymorphism (RFLP) technology has been used for assessing genetic variation and phylogenetic relationships among populations and species of rice (Nakano et al., 1992; Wang et al., 1989, 1992). The aim of the present study was the eval-

uation of the genetic characteristics of Korean weedy rice and the identification of the genetic relationships among Korean weedy rice, *Japonica* and *Tongil* (*Indica/Japonica*) cultivars growing in Korea, and *Indica* cultivar and two foreign red rice accessions based on RFLP analysis.

Materials and methods

Plant materials

The genetic materials used in this study were 31 accessions including 24 strains of Korean weedy rice, one Chinese and one Indian red rice, *Japonica* cultivars Gihobyeo, Geumobyeo and Jodongji, and *Tongil* (*Indica/Japonica*) cultivar Milyang23, and *Indica* cultivar IR26. Korean weedy rices were collected from farmers' fields all over the country, and each strain was then maintained at Yeungnam University, Korea (Suh et al., 1992a).

Morphological and physiological characters

Grain length/width ratio. Samples of ten seeds from each accessions were taken and the length and width of the seeds was measured using vernier calipers. The ratio of mean grain length to width of seed of each sample provided an index of grain shape (Suh et al., 1992a).

Glume hair length. Samples of five seeds from each accessions were taken and the longest three glume hairs of each seed were measured using a microscope at 50 × magnification. Glume hair length is reported as the mean of each sample.

Phenol reaction. The dehulled rices and hulls of five seeds from each accessions were soaked in 2 ml of 2% phenol solution in a 23 × 180 mm tube, and kept at 30 °C for 3 days. The phenol reaction was evaluated from the color of the pericarp of dehulled rices and of the hulls (Suh et al., 1992a).

$KClO_3$ resistance. The seeds were placed on moistened filter papers in plastic tubes (45 × 85 mm) at 30 °C. Two groups of ten seedlings of 2 cm height were collected from each accessions. The roots of the first group were kept in 2 ml of 0.1% $KClO_3$ solution in 23 × 180 mm tubes, while these of the other group were kept in 2 ml distilled water. All tubes were kept

at 28 °C in darkness. After 5 days, the shoot length of the seedlings was measured. The ratio of the mean shoot length of $KClO_3$-treated plants to that of controls provided an index of $KClO_3$ resistance (Ueno et al., 1990).

DNA extraction and RFLP analysis

Leaves were harvested from five individuals of each strain grown in the field under normal agricultural conditions. Total DNA was extracted using the phenol-urea method (McCouch et al., 1988) from mixed leaves. DNA samples were assayed with 83 random combinations of six restriction endonuclease enzymes (*Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Sca* I, *Xba* I; KOSCO) and 40 genomic DNA probes. RG# probes of single copy clones (Chromosome number is in Table 2.) were from IR36 genomic DNA provided by Dr S.D. Tanksley, Cornell University, while KR# probes were from genomic DNA of Yeonkiaengmi 11, a Korean weedy rice, of short grain type. The probes were nonradioactively labelled with digoxigenin-dUTP using a DIG-labeling Kit (Boehringer Mannheim Biochemica) following the random hexamer priming method. Restriction endonuclease digestion, electrophoresis and Southern blotting were carried out as described by McCouch et al. (1988). Detection of chemiluminescent reactions was conducted as described by Ishii et al. (1990).

Data analysis

Each polymorphic fragment detected by Southern analysis was treated as a unit character: the presence or absence of a fragment was assigned a score of 1 or 0, respectively. All hybridizing major fragments detected in a single accession, regardless of frequency, were used to make comparisons among accessions. The genetic similarity coefficients among accessions assayed were quantified based on Nei's (1987) formula using NTSYS-pc software (Rohlf, 1992). The cluster analysis was performed using the unweighted pair-group method with arithmetic mean (UPGMA, Sokal & Michener, 1958).

Table 1. The list of Korean weedy rice and cultivated rice used for RFLPanalysis, and their grain length/width ratio, glume hair length, phenol reaction and KClO₃ resistance

No.	Weedy rice accessions	Group[a]	Grain length/width ratio	Glume hair length (mm)	Phenol reaction[b] Hull	Pericarp	KClO₃ resistance
1	Gihobyeo	Japonica	2.22	1.30	−	−	65.7
2	Geumobyeo	Japonica	2.24	1.34	−	−	75.0
3	Jodongji	Japonica	1.90	1.01	−	−	60.3
4	Kyongsanaengmi 14	Weedy (S)	2.09	1.12	+++	+	63.3
5	Geochangaengmi 12	Weedy (S)	2.28	0.88	−	−	61.5
6	Geumleungaengmi 22	Weedy (S)	2.30	1.13	++	+	88.8
7	Geumleungaengmi 33	Weedy (S)	2.18	0.78	+	++	75.6
8	Yeonkiaengmi 11	Weedy (S)	2.09	1.31	++	+	81.0
9	Kyongjuaengmi 10	Weedy (S)	1.83	0.98	+++	−	78.8
10	Suweonaengmi 7	Weedy (S)	2.14	0.94	−	++	84.1
11	Pyongtaegaengmi 4	Weedy (S)	2.28	1.01	−	+	74.4
12	Kanghwaaengmi 11	Weedy (S)	2.37	0.99	+	+	89.1
13	Danyangaengmi 1	Weedy (S)	2.32	1.18	++	−	77.4
14	Ssalsharei	Weedy (S)	2.24	1.02	++	−	84.3
15	Gwangyangaengmi 14	Weedy (S)	2.47	1.12	−	+	57.3
16	Seonsanaengmi 2	Weedy (S)	2.31	1.12	−	−	63.4
17	Milyang 23	Tongil	2.78	0.69	++	+	25.4
18	IR 26	Indica	3.87	0.59	+++	+++	32.0
19	Kyongsanaengmi 2	Weedy (L)	3.17	0.63	−	−	24.0
20	Milyangaengmi 1	Weedy (L)	2.95	0.72	++	+	24.7
21	Donggokaengmi 1	Weedy (L)	3.12	0.71	−	−	31.9
22	Changnyeongaengmi 1	Weedy (L)	3.15	0.52	−	−	22.4
23	Changnyeongaengmi 5	Weedy (L)	3.06	0.66	+++	++	21.2
24	Hamyangaengmi 2	Weedy (L)	2.78	0.70	−	−	21.4
25	Chilgokaengmi 1	Weedy (L)	3.22	0.71	+++	+	24.4
26	Masanaengmi 1	Weedy (L)	3.05	0.67	++	++	27.0
27	Guryeaengmi 9	Weedy (L)	2.70	0.54	++	−	30.1
28	Cheongdoaengmi 1	Weedy (L)	3.16	0.71	−	−	31.0
29	Jangseongaengmi 1	Weedy (L)	2.85	0.69	−	−	19.4
30	Chinese 85–17	Foreign	3.33	0.59	−	+	37.0
31	ARC 10239	Foreign	2.93	0.61	+++	+	29.1

[a] Japonica: *Japonica* cultivar; Indica: *Indica* cultivar; Tongil: *Tongil* (*Indica/Japonica*) type cultivar; Foreign: long grain foreign red rice; Weedy: Korean weedy rice; (S): short grain type; (L): long grain type.

[b] Phenol reaction: +, positive; −, negative.

[c] Percentage of the relative shoot length treated with 0.1% KClO₃ and distilled water for 5 days at 28 °C.

Results

Morphological and physiological characters

Data for morphological and physiological characters are reported in Table 1. These characters have been used as criteria to classify *Oryza sativa* L. into *Japonica* and *Indica* (Oka, 1988; Ueno et al., 1990). Based on the data obtained, the Korean weedy rice collected have been also classified into two groups. The first group was a short grain type with a grain length/width ratio of 1.83–2.47, glume hair length of 0.78–1.31mm and KClO₃ resistance of 57.3–89.1%. The second group was a long grain type with a grain length/width ratio of 2.78–3.22, glume hair length of 0.52–0.71mm and KClO₃ resistance of 19.4–37%. Eleven out of 13 short grain Korean weedy rices showed a positive reaction to phenol in the hull and/or pericarp, and only five out of eleven long grain Korean weedy rices showed a positive reaction to phenol. Generally, *Japonica* rice
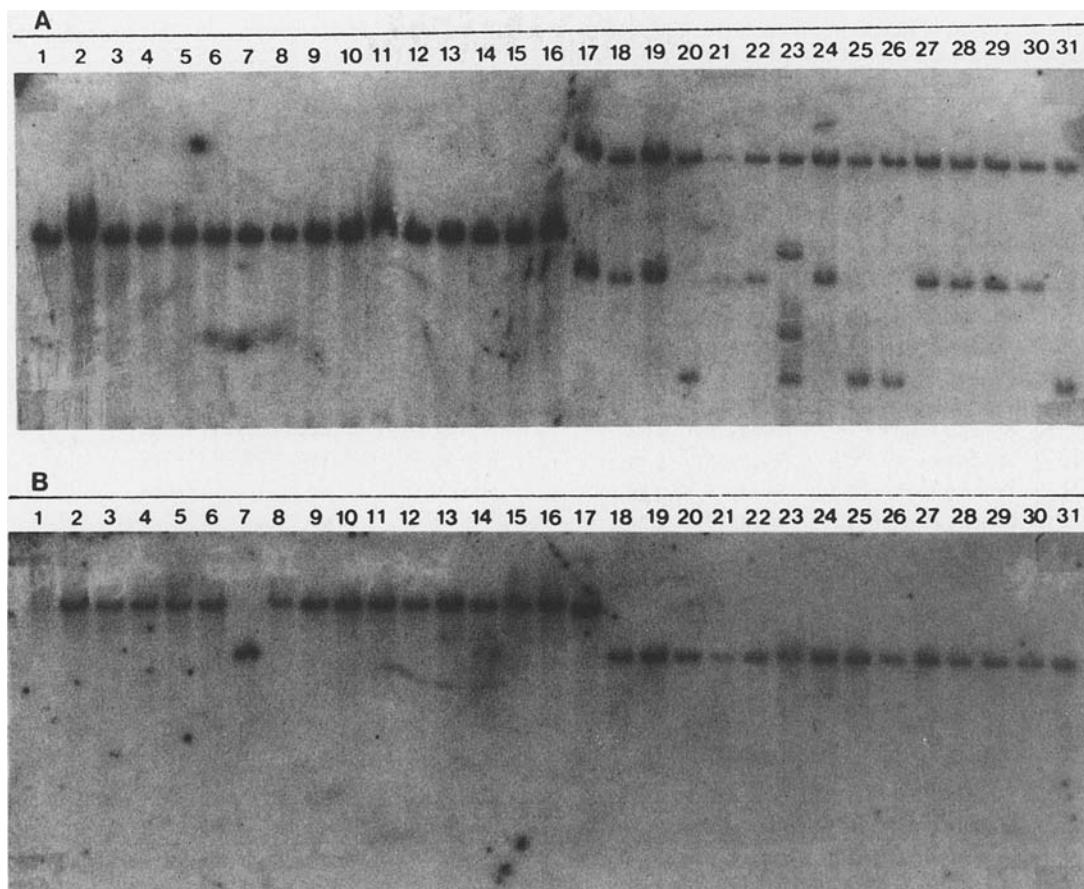
106

Fig. 1. Southern blot patterns observed with probe RG109 (A: the DNA was digested with *Eco* RV) and probe RG346 (B: the DNA was digested with *Eco* RI) using DIG-labeled probes on genomic DNA from 24 strains of Korean weedy rice (no. from 4 to 16 and from 19 to 29), three *Japonica* cultivars (nos 1, 2, 3), one *Tongil* (*Indica/Japonica*) cultivar (no. 17), one *Indica* cultivar (no. 18), and two strains of foreign red rice (nos 30, 31).

shows a negative reaction and *Indica* rice shows a positive reaction to phenol, respectively (Oka, 1988; Suh et al., 1992). Based on grain shape, glume hair length and KClO₃ resistance, short grain Korean weedy rices could be classified into *Japonica* and long grain Korean weedy rices into *Indica* rice, however, phenol reaction was not consistent with the classification of *Indica* and *Japonica*.

RFLP variation and genetic characteristics

Based on RFLP data of 31 genotypes assayed, 37 out of 40 probes (92.5%) showed polymorphisms with at least one enzyme. The probes RG29, RG131 and RG169 did not reveal any polymorphism with the six restriction enzymes tested. For most probe/enzyme combinations, the band patterns observed in Southern blots showed

a typical single copy variation. The band patterns of probes RG146 and RG241 could not be explained by single locus variation; individuals were variable in three or four bands (data not shown). Probe RG109 showed polymorphisms not only between short and long grain Korean weedy rices, but also within long grain weedy rices (Fig. 1A). Probe RG346 had a similar behavior with the exception of the accession Geumleungaengmi 33 which, in spite of having short grain type, showed the typical band of long grain weedy rices (Fig. 1B). In total, 118 polymorphic bands were detected by 37 probes which revealed on the average 3.2 unique fragments per probe.

The genetic characteristics of the Korean weedy rice compared to the *Japonica* cultivar, Gihobyeo, and the *Indica* cultivar, IR26, are shown in Table 2. Probes RG77, RG252 and RG214 revealed the com-

Table 2. Comparison of RFLP characteristics of Korean weedy rice with those of the *Japonica* cultivar Gihobyeo and *Indica* cultivar IR26

No.	Red rice accessions	#1[a] RG109	#1 RG77	#1 RG350	#2 RG690	#2 RG236	#2 RG780	#3 RG462	#3 RG83	#3 RG256	#4 RG120	#4 RG252	#4 RG227	#5 RG393	#5 RG143	#5 RG214	#6 RG346	#6 RG470	#6 RG13	#7 RG64	#7 RG213	#7 RG146	#8 RG528	#8 RG711	#8 RG165	#8 RG477	#9 RG28	#9 RG885	#9 RG598	#10 RG553	#10 RG134	#10 RG241	#11 RG211	#12 RG396	#12 RG341	#KR KR9	#KR KR97	#KR KR181	G[b]	P	U	R
1	Gihobyeo	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A[c]	0.84	0	0.16	0
2	Geumobyeo	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.74	0.05	0.18	0.03
3	Jodongji	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.74	0	0.18	0.08
4	Kyongsananengmi 14	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.81	0.03	0.16	0
5	Geochangaengmi 2	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.72	0.05	0.18	0.05
6	Geumleungaengmi 22	A	U	A	A	A	A	A	C	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.63	0.14	0.16	0.05
7	Geumleungaengmi 33	A	U	A	A	A	A	A	U	A	A	U	A	A	A	B	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.55	0.16	0.16	0.13
8	Yeonkiaengmi 11	A	U	A	A	A	A	A	U	A	A	C	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.71	0.08	0.16	0.05
9	Kyongjuaengmi 10	A	C	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.68	0.08	0.18	0.05
10	Suweonaengmi 7	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	G	A	A	A	A	A	A	0.68	0.11	0.18	0.03
11	Pyongtaegaengmi 4	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	0.72	0.05	0.18	0.05
12	Kanghwaaengmi 11	A	U	A	A	A	A	C	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.68	0.08	0.16	0.08
13	Danyangaengmi 1	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	A	A	A	0.65	0.08	0.16	0.11
14	Ssalsharei	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	0.68	0.08	0.16	0.08
15	Gwangyangaengmi 14	A	U	A	A	A	A	A	U	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.69	0.05	0.18	0.08
16	Seonsananengmi 2	A	C	A	B	A	A	A	C	A	A	U	A	A	A	A	A	U	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	0.69	0.08	0.18	0.05
17	Milyang 23	B	B	C	B	B	A	C	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	E	B	B	B	B	B	B	0.03	0.33	0.03	0.61
19	Kyongsananengmi 2	B	U	A	B	A	A	A	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	C	A	A	B	0.11	0.30	0.08	0.51
20	Milyangaengmi 1	C	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	C	A	B	B	0.08	0.30	0.08	0.54
21	Donggokaengmi 1	B	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	C	A	A	B	0.11	0.27	0.08	0.54
22	Changnyeongaengmi 1	B	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	F	B	C	A	A	A	B	B	E	A	A	A	A	B	A	A	B	0.11	0.27	0.08	0.54
23	Changnyeongaengmi 5	C	U	A	D	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	C	A	A	B	0.05	0.37	0.08	0.50
24	Hamyangaengmi 2	B	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	B	A	A	B	0.11	0.24	0.08	0.57
25	Chilgokaengmi 1	C	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	C	A	A	B	0.05	0.33	0.08	0.54
26	Masananengmi 1	C	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	A	B	C	A	A	A	B	B	D	A	A	A	A	C	A	A	B	0.05	0.33	0.08	0.54
27	Guryeaengmi 9	B	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	H	B	C	A	A	A	B	B	D	A	A	A	A	C	A	A	B	0.08	0.27	0.08	0.57
28	Cheongdoaengmi 1	B	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	F	B	C	A	A	A	B	B	E	A	A	A	A	C	A	A	B	0.08	0.35	0.08	0.49
29	Jangseongaengmi 1	B	U	A	B	A	A	B	C	U	A	U	U	A	U	A	A	U	A	A	A	F	B	C	A	A	A	B	B	E	A	A	A	A	C	A	A	B	0.08	0.27	0.08	0.57
30	Chinese 85-27	B	A	B	D	A	A	C	B	U	D	B	B	B	B	B	C	B	B	B	B	C	B	C	B	B	B	B	C	D	D	I	C	B	B	C	B	B	0.05	0.27	0.08	0.59
31	ARC 10239	C	A	B	B	A	A	C	U	D	B	B	B	B	C	B	C	B	B	C	B	B	B	B	B	B	B	B	C	E	E	E	E	B	B	A	B	A	0.11	0.37	0.18	0.44
18	IR*26	B	U	B	B	A	A	C	B	U	D	B	B	B	C	B	C	B	B	C	C	C	B	C	C	B	B	B	B	D	E	F	B	B	B	B	B	B	0	0.18	0.18	0.82

[a] #: The number of chromosome by Kinoshita (1990); KR#, genomic probes of Korean weedy rice.

[b] G: ratio of Gihobyeo band pattern; R: ratio of IR 26 band pattern; P: ratio of specific band pattern of weedy rice; U: ratio of common band pattern between Gihobyeo (*Japonica*) and IR 26 (*Indica*).

[c] A: Gihobyeo band pattern; B: IR 26 band pattern; C, D, E, F, G, H, and I: specific band patterns of weedy rice; U: common band pattern both Gihobyeo (*Japonica*) and IR 26 (*Indica*).
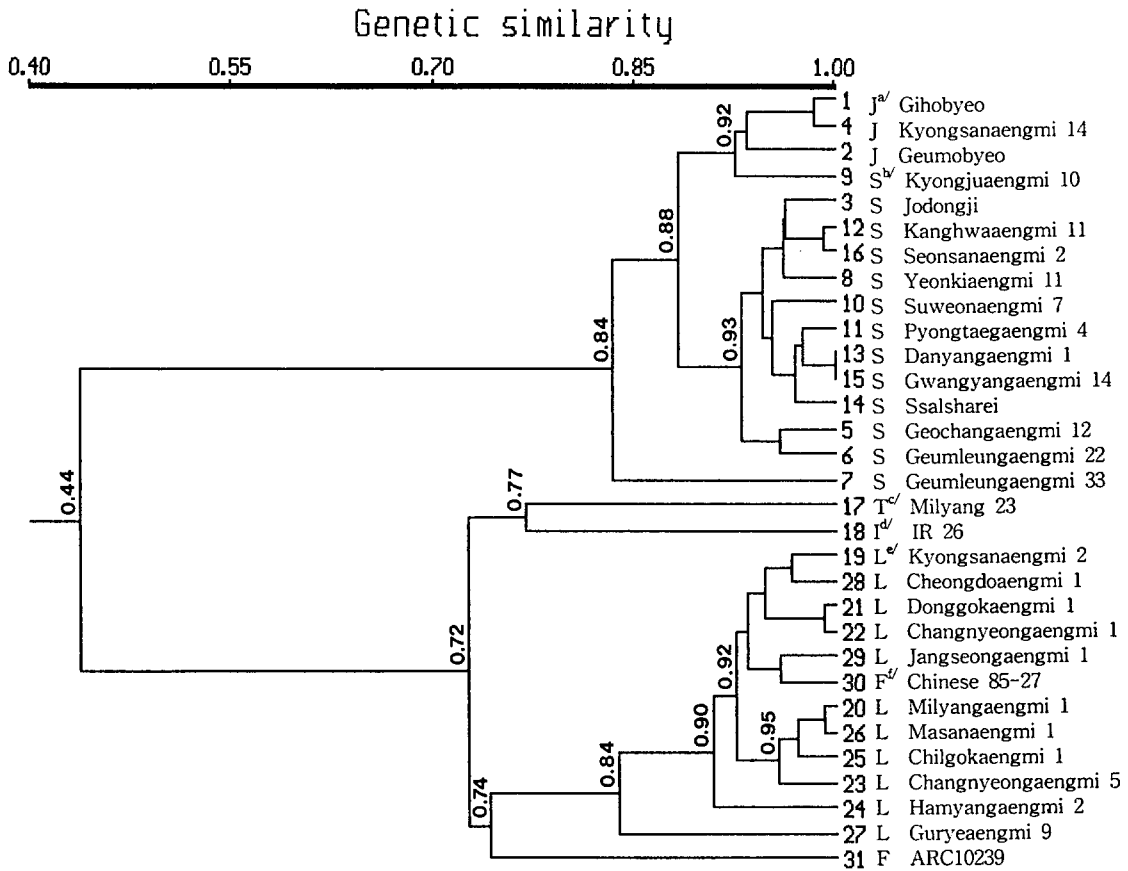
Genetic similarity

0.40   0.55   0.70   0.85   1.00

1 J[a/] Gihobyeo
4 J Kyongsanaengmi 14
2 J Geumobyeo
9 S[b/] Kyongjuaengmi 10
3 S Jodongji
12 S Kanghwaaengmi 11
16 S Seonsanaengmi 2
8 S Yeonkiaengmi 11
10 S Suweonaengmi 7
11 S Pyongtaegaengmi 4
13 S Danyangaengmi 1
15 S Gwangyangaengmi 14
14 S Ssalsharei
5 S Geochangaengmi 12
6 S Geumleungaengmi 22
7 S Geumleungaengmi 33
17 T[c/] Milyang 23
18 I[d/] IR 26
19 L[e/] Kyongsanaengmi 2
28 L Cheongdoaengmi 1
21 L Donggokaengmi 1
22 L Changnyeongaengmi 1
29 L Jangseongaengmi 1
30 F[f/] Chinese 85-27
20 L Milyangaengmi 1
26 L Masanaengmi 1
25 L Chilgokaengmi 1
23 L Changnyeongaengmi 5
24 L Hamyangaengmi 2
27 L Guryeaengmi 9
31 F ARC10239

0.92 0.88 0.84 0.93 0.77 0.72 0.74 0.84 0.90 0.95 0.92 0.44

Fig. 2. Dendrogram showing the genetic relationships among 24 strains of Korean weedy rice, three *Japonica* tester cultivars, one *Tongil* (*Indica/Japonica*) cultivar, one *Indica* tester cultivar, and two strains of the foreign red rice of *Oryza sativa* based on cluster analysis of RFLPs. The numbers above dendrogram are the genetic similarity coefficients scales according to Nei(1987). [a] J: *Japonica* cultivars; [b] S: short grain Korean weedy rices; [c] T: *Tongil* (*Indica/Japonica*) type cultivar; [d] I: *Indica* cultivar; [e] L: long grain Korean weedy rices; [f] F: long grain foreign red rices.

mon DNA fragments in most short and long grain Korean weedy rices and Gihobyeo and IR26. Probes RG83, RG528, RG165 and RG553 revealed the common DNA fragments only in most short grain Korean weedy rices with Gihobyeo and IR26, however, did specific polymorphisms in long grain weedy rices. Probes RG213 and RG134 revealed variable polymorphic DNA fragments in short and long grain Korean weedy rices compared to Gihobyeo and IR26. Probes RG146, RG477 and RG241 showed the common DNA fragments with Gihobyeo in most short grain weedy rices, while the long grain weedy rices showed specific fragments different from IR26. For probes RG350 and RG780, the long grain weedy rices showed the band pattern of Gihobyeo different from IR26. In contrast, probes RG711, RG28 and RG341 revealed the band of IR26 in most short grain weedy rices. Overall, short

grain Korean weedy rices had 65–81% genetic similarity with *Japonica* Gihobyeo except Geumleungaengmi 33, and 5–13% with *Indica* IR26. Long grain Korean weedy rices had 49–57% genetic similarity with IR26 and 5–11% with Gihobyeo. Short grain Korean weedy rices had 13–18% common genetic compositions with Gihobyeo and IR26, and all long grain weedy rices had 8% common genetic compositions. Of short grain Korean weedy rices, Geumleungaengmi 22, Geumleungaengmi 33, Kyongjuaengmi 10 and Ssalsharei had 14%, 16%, 11% and 11% specific genetic compositions of weedy rice, respectively, and the rest had 3–8%. However, all long grain Korean weedy rices had 24–37% specific genetic compositions of weedy rice. Chinese red rice 85–27 and ARC10239 had genetic compositions similar to those of long grain Korean weedy rices.

Cluster analysis by RFLP data

A dendrogram showing the genetic relationships among the 31 genotypes assayed was constructed based on RFLP data (Fig. 2). The short and long grain type strains of Korean weedy rice were classified into two main clusters. This was consistent with the classification according to the morphological and physiological characters of grain shape, glume hair length and $KClO_3$ resistance (Table 1). The genetic similarity coefficient calculated by Nei's method between the two main clusters was 0.44. Short grain weedy rices were classified into three sub-clusters within a 0.88 Nei's genetic similarity coefficient and were close to *Japonica* cultivars Gihobyeo, Geumobyeo and Jodongji. The long grain weedy rices were classified into four sub-clusters within a 0.92 Nei's genetic similarity coefficient. The long grain types Milyangaengmi 1, Masanaengmi 1, Chilgokaengmi 1 and Changnyeongaengmi 5 which showed positive phenol reaction, were classified into a sub-cluster within a 0.95 Nei's genetic similarity coefficient. The Nei's genetic similarity coefficient between long grain Korean weedy rices and *Indica* and *Tongil* cultivars was 0.72. This was due to the fact that long grain Korean weedy rices had 24–37% specific genetic backgrounds of weedy rice when compared with *Indica* IR26 (Table 2). Chinese red rice 85–27 from Anhui Province was closely related to the long grain type strains of Korean weedy rice.

Discussion

Cultivated rice was introduced into Korea from China about 4,000 years ago (Heu et al. 1991). Later, *Japonica* type rice had been cultivated for long time, and recently, *Tongil* (*Indica/Japonica*) type varieties were cultivated. Now, only *Japonica* varieties are cultivated as commercial rice in Korea. No wild rice occurs in Korea. However, Korean weedy rice, locally called 'Red rice', 'Aengmi' and 'Sharei', is distributed as a weedy form in farmers' fields (Hara, 1942; Suh et al., 1992a). The origin of weedy rices in Korea is still unknown. The Korean weedy rices were classified into two groups using the following morphological and physiological characteristics: grain shape, glume hair length and $KClO_3$ resistance. One group comprised the short grain type including *Japonica* cultivars, and the other the long grain type including *Indica* and *Tongil* cultivars. Generally, *Japonica* rice shows a negative reaction and *Indica* rice shows a positive reaction to

phenol (Oka, 1988; Suh et al., 1992). However, most of the Korean weedy rices showed the opposite reactions to phenol (Table 1). These results indicate that the genetic backgrounds of Korean weedy rice are complex. Based on RFLP analysis, Korean weedy rices could be classified into two main clusters of short and long grain types. This result was consistent with the classification based on morphological and physiological characteristics. Based on their genetic relationships by RFLP analysis, short grain Korean weedy rices were very close to *Japonica*, and long grain Korean weedy rices were closer to *Indica* and *Tongil* (*Indica/Japonica*) than to *Japonica* cultivar in spite of having different genetic backgrounds from the *Indica* and *Tongil* cultivars surveyed. It was evident that long grain Korean weedy rices had much more specific genetic backgrounds of weedy rice than do short grain Korean weedy rices when compared to representatives of locally grown varieties (Table 2). We have not enough information whether the specific genetic background of the Korean weedy rice found in this study will be consistent with other *Japonica* or *Indica* cultivars, however, we suppose that Korean weedy rice, especially long grain type, evolved differently from typical *Indica*. Suh et al. (1992b) reported that short grain Korean weedy rices showed 35.2–45.8% cross–sterility with *Japonica* cultivars and 58.2–67.6% with *Indica* cultivars, while long grain Korean weedy rices had 67.7–78.6% cross-sterility with *Japonica* cultivars and 49.3–53.8% with *Indica* cultivars in agreement with results from Hara (1942) and Heu et al.(1990). Therefore, we concluded that the specific genetic compositions of Korean weedy rice might be related to the cross-sterility. Hara (1942) reported that among the Korean weedy rice collected from farmers' fields, the short grain types were *Japonica* and the long grain types belonged to the class *Indica*. Heu et al. (1991) reported that some Korean native varieties belonged to *Indica* and that some Korean weedy rice strains had characteristics of *Indica* rice, and that a rice sample which was enshrined in the Buddha's image in 1302 was deduced to be a mixture of *Indica* and *Japonica* from its grain shape and amylose content. Therefore, they concluded that the *Indica* rice might have been introduced and grown in Korea before 1300. Some tropical weedy rices are known to originate from cross between wild and cultivated rice or between *Japonica* and *Indica* cultivated rice (Suh & Morishima, 1995). Chinese red rice 85–27 collected from the Anhui Province in China was closely related to the long grain strains of Korean weedy rice, and our result is consistent with previous results based

on crossing-affinities (Suh et al., 1992b) and isozyme analyzes (Cho et al., 1993). Oka (1988) also reported that the Korean weedy rice of *Japonica* type was morphologically similar to Chinese red rice 'Lutao'. Suh et al. (1992b) argued that long grain Korean weedy rices growing only in the Nakdong River and Seomjin River basins of the southern part of Korea were originally imported from the Yangtze basins (Anhui and Jiangsu Provinces) of China. We hypothesize that long grain Korean weedy rice might have been introduced with *Indica* rice from China, or originated from natural cross between *Indica* and *Japonica* rice in the same period, because majority of RFLP characteristics are common with *Indica* and some of them are common with *Japonica*.

Based on our results, we suggest that the short grain type strains of Korean weedy rice can be classified as *Japonica*. However, long grain Korean weedy rices are closer to *Indica* than to *Japonica*, and are differentiated into a local ecotype surviving under the cultural conditions of *Japonica* cultivars in the southern part of the Korean peninsula.

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