Geographical variation of the genes controlling hybrid breakdown and genetic differentiation of the chromosomal regions harboring these genes in Asian cultivated rice, *Oryza sativa* L.

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The geographical variation of two genes, *hwd1* and *hwd2*, controlling hybrid breakdown (F_2 weakness) was surveyed in 239 Asian rice cultivars. The cultivars with the genotype *Hwd1/Hwd1 Hwd2/Hwd2* were predominant in South Asia and South-east Asia, while those with the genotype *Hwd1/Hwd1 hwd2/hwd2* were predominant in Japan, the Philippines, and Yunnan province, China. Only one cultivar from Laos had the genotype *hwd1/hwd1 Hwd2/Hwd2*. Preferential association was found between the presence or absence of *hwd2* and the two major cultivar groups (indica and japonica) based on diagnostic RFLP markers. Dominant homozygotes were frequent in indica rice cultivars, while recessive homozygotes were frequent in japonica rice cultivars. This association was not clear for cultivars from the center of genetic diversity of rice, ranging from Vietnam to Bangladesh. Polymorphisms were surveyed for RFLP markers on chromosomes 10 and 7, harboring *hwd1* and *hwd2* loci, respectively. The region around *hwd2* tended to be differentiated in allelic combination among cultivars into the indica and japonica rice groups. No such trend was found around the *hwd1* region. These facts suggest the hybrid breakdown controlled by this genic system is a rare phenomenon and consequently did not contribute to cultivar differentiation of rice.

INTRODUCTION

Reproductive barriers are essential for maintaining the identity of species. The mechanisms of this phenomena in interspecific hybrids and their evolutionary roles have been discussed by many researchers and were reviewed by Stebbins (1950). Reproductive barriers observed in hybrids and their progenies were classified into three types (Stebbins, 1950). They are hybrid inviability (or weakness) and hybrid sterility observed in F_1 hybrids, and hybrid breakdown observed in the F_2 or later generations. Hybrid breakdown is expressed as sterility or weakness or both.

In rice the above three types of reproductive barriers have been reported by many workers (Oka, 1957a,b; Chu and Oka, 1972; Oka, 1974; Sano et al., 1979; Sato et al., 1984; Okuno, 1985; Sato and Morishima, 1988; Ise et al., 1992). Hybrid inviability (or weakness) and hybrid sterility have been widely observed both in inter- and intraspecific crosses. Hybrid breakdown has been reported only in intraspecific

crosses and its evolutionary role remains unclear.

The significance of reproductive barriers in genetic differentiation depends on the frequency of its occurrence and its selective advantage. Although many genes for hybrid sterility have been reported in rice, the geographical variation of these genes have not been analyzed due to the difficulty in identifying the genes. On the other hand, the geographical variation of the genes causing F_1 hybrid weakness was analyzed in inter- and intraspecific crosses of rice (Sato and Hayashi, 1983; Sato and Morishima, 1987). Those studies revealed that F_1 hybrid weakness is a rare phenomenon and consequently that it can not be considered to be important in differentiation of rice. The distribution of the genes causing hybrid breakdown (F_2 weakness) have not yet been analyzed in detail.

The genetic differentiation of the chromosomal region harboring the gene responsible for reproductive barriers is an important factor to consider in relation to its distribution. Sato and Hayashi (1983) reported that the differential distribution of the alleles at the locus *Hwc2* (formerly designated *L-2-a*), one of the complementary genes caus-

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ing F_1 weakness, between indica rice (*hwc2*) and japonica rice (*Hwc2*). Genes which confer selective advantage have been suggested to have an influence on the distribution of the genes linked to them. The genetic differentiation of the chromosomal region harboring the genes responsible for hybrid breakdown should be analyzed to determine the evolutionary importance of the region.

Hybrid breakdown (F_2 weakness) observed in a cross between the Japanese cultivar Sasanishiki and Thai cultivar Col. No. 15 was first reported by Okuno (1985). The weak plants are significantly shorter in plant height and have fewer tillers than normal plants. Since the weak plants cannot be distinguished from normal plants before transplanting, this phenomenon seriously reduces the number of plants available for selection in cross breeding.

The genetic basis of this hybrid breakdown, in which about 30% of the F_2 population were weak, was explained by assuming a set of genes *hwd1* and *hwd2* (Okuno, 1985). We mapped the genes *hwd1* and *hwd2*, using RFLP markers on chromosomes 10 and 7, respectively (Fukuoka et al., 1998). Test crosses were carried out for 100 Asian cultivars using tester lines recessive at both loci (Okuno, 1986). In that study, although a geographical difference was observed in the frequency of the genotypes, the genotypes of 70% of the cultivars were not determined. Determining the geographical distribution of these genotypes is useful for understanding the significance of these genes in the intraspecific differentiation of Asian rice cultivars and for carrying out efficient cross breeding.

In this study, 239 Asian cultivars were investigated for their gene composition, regarding *hwd1* and *hwd2* by crossing with three testers, and the geographical variation of their genotypes was examined. Further, genetic diversity in the chromosomal regions harboring *hwd1* and *hwd2* was evaluated using RFLP markers linked to these genes.

MATERIALS AND METHODS

Cultivars used and genetic characterization. A total of 239 rice cultivars from 18 Asian countries and regions (Table 1) were used to determine the genotypes for the genes controlling hybrid breakdown, *hwd1* and *hwd2*. These cultivars were characterized by RFLP markers. RFLP markers are useful for the classification of rice and several individual RFLP markers can be used to classify rices into indica and japonica, ecogeographical races (Zheng et al., 1994; Qian et al., 1995; Li et al., 1998). The RFLP markers diagnostic for the cultivar groups were screened from the markers on the rice RFLP genetic linkage map (Kurata et al., 1994), using a core collection of 130 rice cultivars whose cultivar groups had been identified (data are not shown). Three RFLP markers, C601, G187, and C711, selected as diagnostic markers for distinguishing between indica and japonica rices, are located on chromosomes 2, 8, and 9, respectively. Cultivars with the alleles common to the typi-

Table 1. The number of cultivars from 18 Asian countries and areas used for this study and cultivar groups they belong to

Code No.	Country/Area	Cultivar group*			total
		I	II	III	
1	Sri Lanka	11	1	1	13
2	India	9	0	2	11
3	Bangladesh	12	0	2	14
4	India (Assam)	16	0	1	17
5	Nepal	10	2	0	12
6	Bhutan	4	9	0	13
7	Myanmar	12	1	0	13
8	Thailand	5	6	1	12
9	Cambodia	11	0	0	11
10	Laos	5	6	0	11
11	Vietnam (northwest)	2	9	0	11
12	Malaysia	6	6	0	12
13	Indonesia	5	7	2	14
14	Philippines	4	13	1	18
15	China (Yunnan)	5	8	0	13
16	China (south)	13	2	0	15
17	China (north)	7	8	0	15
18	Japan	0	14	0	14
	total	137	92	10	239

* Cultivar group was determined based on three diagnostic RFLP markers; C601, G187, and C711. Group I and II corresponds to indica and japonica, respectively. Group III is an intermediate type based on the three RFLP markers.

cal indica type at all three loci were classified as group I, and those with the alleles common to the typical japonica type at all three loci as group II. Group III consisted of intermediate cultivars which did not fall into group I nor group II.

Test crosses. To determine the genotypes of cultivars with regard to hybrid breakdown genes, they were crossed with three testers: a double recessive tester line (either of W26, W2, or W3; *hwd1/hwd1 hwd2/hwd2*), Sasanishiki (*Hwd1/Hwd1 hwd2/hwd2*) and Col. No. 15 (*hwd1/hwd1 Hwd2/Hwd2*) (Okuno, 1985). The system of genotype determination is summarized (Table 2). Plants with the genotypes *hwd1/hwd1 hwd2/Hwd2*, *hwd1/Hwd1 hwd2/hwd2*, and *hwd1/hwd1 hwd2/hwd2* showed weakness and plants of other genotypes showed normal plant growth (Okuno, 1985). When a cultivar to be tested has the genotype *Hwd1/Hwd1 Hwd2/Hwd2*, F_1 plants from a cross with a tester line (W26, W2, or W3) are expected to have normal height and normal number of tillers, while a cultivar which is *Hwd1/Hwd1 hwd2/hwd2* or *hwd1/hwd1 Hwd2/Hwd2* produces weak F_1 plants from crosses with the same tester line.

In the crosses with the tester cultivars, Sasanishiki and Col. No. 15, whether normal and weak plants segregate in a ratio of 11:5 in the F_2 generation depends on the genotype of the cultivar tested. When weak plants appear in the cross with either Sasanishiki or Col. No. 15, the cultivar tested is regarded as having a recessive allele at the locus harboring dominant alleles in the tester cultivar. Cultivars whose genotypes could not be determined with this system

Table 2. Summary of expected results of three test crosses for the three genotypes at the *hwd1* and *hwd2* loci in Asian rice cultivars

Genotypes of cultivars ^a	Testers genotype		
	W26,W3,W2 (<i>hwd1/hwd1</i> <i>hwd2/hwd2</i>)	Sasanishiki (<i>Hwd1/Hwd1</i> <i>hwd2/hwd2</i>)	Col. No. 15 (<i>hwd1/hwd1</i> <i>Hwd2/Hwd2</i>)
	Plant type of F ₁	Segregation in F ₂ ^b	Segregation in F ₂ ^b
<i>Hwd1/Hwd1 Hwd2/Hwd2</i>	Normal	No	No
<i>Hwd1/Hwd1 hwd2/hwd2</i>	Weak	No	Yes
<i>hwd1/hwd1 Hwd2/Hwd2</i>	Weak	Yes	No

^a Cultivars that did not show results similar to those indicated above were classified as unidentified.

^b Ratio of normal and weak plants in F₂ were expected to be 11:5 (normal: weak). Segregation which significantly deviated from this ratio were not used for further analysis in this study.

were classified as unidentified.

Evaluation of the genetic diversity of the chromosomes harboring *hwd1* and *hwd2*. The genes *hwd1* and *hwd2* are located on chromosomes 10 and 7, respectively (Fukuoka et al., 1998). To analyze the genetic diversity in the regions harboring *hwd1* and *hwd2*, RFLP markers located on the chromosomes 10 and 7 were surveyed in 160 cultivars, 93 belonging to group I and 67 belonging to group II. Group III and unclassified cultivars were not used in this analysis. Southern hybridization was conducted with 12 RFLP probes (12 loci) on chromosome 10 and 33 RFLP probes (34 loci) on chromosome 7. For alleles at each of the RFLP marker loci, the proportion of group I and group II

cultivars were calculated. Based on these proportions, each allele was determined as to whether it was specific to group I or group II. The agreement between the classification by the three diagnostic RFLP markers (C601, G187, and C711) and the classification by single RFLP marker was calculated for each locus. The percentage thus obtained was regarded as indicating the degree of association of each locus with the classification of cultivars into indica and japonica.

RESULTS

Genotype determination and geographical variation of *hwd1* and *hwd2*. The genotypes for *hwd1* and *hwd2* were determined by test crosses (Table 3). Fifty-three

Table 3. Genotype classification for *hwd1* and *hwd2* of cultivars

Genotype		<i>Hwd1/Hwd1</i> <i>Hwd2/Hwd2</i>			<i>Hwd1/Hwd1</i> <i>hwd2/hwd2</i>			<i>hwd1/hwd1</i> <i>Hwd2/Hwd2</i>			unidentified			total
Cultivar group*		I	II	III	I	II	III	I	II	III	I	II	III	
Code No.	Country/ Area													
1	Sri Lanka	9	0	1	1	1	0	0	0	0	1	0	0	13
2	India	7	0	2	1	0	0	0	0	0	1	0	0	11
3	Bangladesh	6	0	1	4	0	1	0	0	0	2	0	0	14
4	India (Assam)	5	0	1	5	0	0	0	0	0	6	0	0	17
5	Nepal	8	0	0	1	1	0	0	0	0	1	1	0	12
6	Bhutan	3	4	0	1	2	0	0	0	0	0	3	0	13
7	Myanmar	11	1	0	0	0	0	0	0	0	1	0	0	13
8	Thailand	5	3	1	0	2	0	0	0	0	0	1	0	12
9	Cambodia	10	0	0	0	0	0	0	0	0	1	0	0	11
10	Laos	4	4	0	0	1	0	1	0	0	0	1	0	11
11	Vietnam (northwest)	2	5	0	0	1	0	0	0	0	0	3	0	11
12	Malaysia	5	0	0	0	5	0	0	0	0	1	1	0	12
13	Indonesia	5	0	2	0	7	0	0	0	0	0	0	0	14
14	Philippines	2	1	0	0	10	0	0	0	0	2	2	1	18
15	China (Yunnan)	5	0	0	0	6	0	0	0	0	0	2	0	13
16	China (south)	7	1	0	0	1	0	0	0	0	6	0	0	15
17	China (north)	4	2	0	0	5	0	0	0	0	3	1	0	15
18	Japan	0	0	0	0	13	0	0	0	0	0	1	0	14
total		98	21	8	13	55	1	1	0	0	25	16	1	239
		127			69			1			42			

* Cultivar group was determined based on three diagnostic RFLP markers; C601, G187, and C711. Group I and II correspond to indica and japonica, respectively. Group III is an intermediate type based on the three RFLP markers.

percent of cultivars tested had the genotype *Hwd1/Hwd1 Hwd2/Hwd2* and 29% had *Hwd1/Hwd1 hwd2/hwd2*. The genotype *hwd1/hwd1 Hwd2/Hwd2* was rare and found in only one cultivar. The genotype of 18% of the cultivars could not be determined due to unexpected results of the three test crosses. The frequencies of the cultivars with the genotypes *Hwd1/Hwd1 Hwd2/Hwd2*, *Hwd1/Hwd1 hwd2/hwd2* and *hwd1/hwd1 Hwd2/Hwd2* were 64.5%, 35.0%, and 0.5%, respectively, when unidentified samples were excluded.

The distribution of genotypes revealed geographical variation in the frequency of *hwd1* and *hwd2* (Fig. 1). The frequency for the cultivar with the genotype *Hwd1/Hwd1 Hwd2/Hwd2* was highest in mainland Southeast Asian countries such as Cambodia, Myanmar, and Thailand. Those with the genotype *Hwd1/Hwd1 hwd2/hwd2* were mainly found in cultivars of Yunnan province of China, the

Philippines, and Japan. The cultivars with both genotypes were found in all countries and regions except Japan. The genotype *hwd1/hwd1 Hwd2/Hwd2*, which was first found in Col. No. 15, was only found in one other cultivar from Laos in this survey. Therefore, we can conclude that differentiation of the genetic system controlling this hybrid breakdown occurred in Asian cultivated rice mainly at the *hwd2* locus.

Relationship between cultivar groups classified by RFLP markers and genotypes for hybrid breakdown.

Cultivars for which respective hybrid breakdown genotypes were determined were further classified into groups I, II, and III based on RFLP (Table 3). Seventy-seven percent and 17% of the cultivars with the genotype *Hwd1/Hwd1 Hwd2/Hwd2* belonged to groups I and II, respectively. However, 19% and 80% of the cultivars with the genotype

Fig. 1. Geographical variation in the genotypes for *hwd1* and *hwd2* in Asian rice cultivars. The cultivars with unidentified genotypes have been excluded. Countries and areas are indicated with code numbers. The names of countries and areas are shown on the right.

Hwd1/Hwd1 hwd2/hwd2 belonged to groups I and II, respectively. This indicates preferential association between the cultivar groups defined by the allelic combination at the diagnostic RFLP markers and the *hwd2* locus. This association was not found in cultivars from Bangladesh, Bhutan, Laos and Vietnam (northwest). Only one cultivar Tamen from Laos with the *hwd1/hwd1 Hwd2/Hwd2* genotype was classified as group I. The cultivars unidentified

for the *hwd1* and *hwd2* loci were found most frequently in cultivars from south China and Assam, India.

Genetic differentiation of the chromosomes harboring hybrid breakdown genes. Genetic differentiation around the chromosomal regions harboring *hwd1* and *hwd2* were analyzed. Percentages of agreement between classification into cultivar groups I and II and the classification by each RFLP marker on chromosomes 7 and 10 were plotted along the respective linkage maps (Figs. 2 and 3). The percentages ranged from 58 to 100%. A low percentage at a given locus indicates that this region is not involved in intraspecific differentiation, while a high percentage indicates the region may possibly have a relationship with intraspecific differentiation. On chromosome 10, the percentage of agreement was low around the *hwd1* locus, though it was higher in other regions on this chromosome. However, most of the RFLP marker loci on chromosome 7 had high percentages of agreement with classification into cultivar groups. The percentages were higher than 80% for the 12 RFLP marker loci closest to *hwd2*.

DISCUSSION

In the present study, the geographical variation of *hwd1* and *hwd2* and genetic differentiation in the chromosomal regions harboring *hwd1* and *hwd2* were analyzed to clarify the significance of hybrid breakdown (F_2 weakness) in relation to the differentiation of Asian rice cultivars.

Geographical variation was observed in the distribution of *hwd2*. *Hwd2* was predominant in South Asia and

Fig. 2. Percentages of agreement of the RFLP markers distributed along a genetic linkage map of chromosome 10 with the cultivar-group classification. The frame of the genetic linkage map of chromosome 10 (Kurata et al., 1994) is shown below and position of *hwd1* is indicated with an arrow.

Fig. 3. Percentages of agreement of the RFLP markers distributed along a genetic linkage map of chromosome 7 with the cultivar-group classification. The frame of genetic linkage map of chromosome 7 (Kurata et al., 1994) is shown below and position of *hwd2* is indicated with an arrow.

Southeast Asia, while *hwd2* was predominant in Japan and the Philippines. Dicotony in the genotypes between two regions was reported for the *Hwc2* locus associated with F_1 weakness (Sato and Hayashi, 1983) and the *hca2* locus associated with F_2 chlorosis (Sato and Morishima, 1988). Using a double recessive tester line, Okuno (1986) previously reported that the frequency of cultivars with the genotype *Hwd1/Hwd1 Hwd2/Hwd2* was high in South China, Vietnam, Laos, Myanmar, and Thailand. Similar results in the present study were obtained using a different cultivar set. Sixty percent of the cultivars had this double dominant genotype in the present study, while 30% of the cultivars had this genotype in the previous study. The difference in frequency can be explained by the sample choice. The present materials included more samples from South and Southeast Asia, where the cultivars with *Hwd1/Hwd1 Hwd2/Hwd2* are commonly found.

The present study revealed that cultivar with the genotype *hwd1/hwd1 Hwd2/Hwd2* was rare. A level of low polymorphism at one of the loci for F_1 weakness and F_2 chlorosis has also been reported in rice and other crops (Sato and Hayashi, 1983; Takahashi and Hayashi, 1987; Konishi, 1987; Sato and Morishima, 1988). So far, the genotype *hwd1/hwd1 Hwd2/Hwd2* has been found in only one cultivar from Laos and Col. No. 15 from Thailand. Since they seem to have a close genetic relationship based on RFLP analyses, the allele *hwd1* found in these cultivars might have a common origin. These observations support the hypothesis that hybrid breakdown controlled by *hwd1* and *hwd2* did not cause or promote intraspecific differentiation.

Using three testers, we could not determine the genotypes of 18% of the cultivars for *hwd1* and *hwd2*. There are three possible explanations for this result. One is misjudgment of the F_1 phenotypes in the cross with the recessive tester line. Some F_1 plants had intermediate plant types and it was difficult to judge their phenotypes. A second possible explanation is the effect of unidentified genes causing similar F_1 weakness or hybrid breakdown. A third explanation is heterogeneity within a cultivar at the *hwd1* or *hwd2* locus. Since the heterogeneous cultivars in the chromosomal regions harboring *hwd1* and *hwd2*, which were detected by RFLP analysis, were excluded from this study, the third explanation is unlikely. One or more of these three possible explanations may be involved in the difficulty in determining the genotypes for hybrid breakdown in some cultivars.

A clear association was observed between the genotype at the *hwd2* locus and the cultivar group to which the cultivar belonged. Seventy-seven percent of cultivars with the *Hwd1/Hwd1 Hwd2/Hwd2* genotype belonged to group I (indica) and 80% of cultivars with the *Hwd1/Hwd1 hwd2/hwd2* genotype belonged to group II (japonica). A similar preferential association with indica and japonica groups was found in the analysis of the F_1 weakness gene

hwc2 (Sato and Hayashi, 1983). In the present study, the association was obscure in some areas such as northwest Vietnam and Laos. These areas are included in the center of the genetic diversity of rice revealed by esterase isozyme analyses (Nakagahra et al., 1975). The present study also suggests that the cultivars in these regions are more genetically diverse than those in the other regions (Table 3).

The present study confirms that RFLPs on chromosome 7, especially in the region around *hwd2*, could be used with a high percentage agreement with the classification into two cultivar groups, indica and japonica. This suggests that some genes responsible for indica/japonica differentiation are located in this region, although it is unclear what genes they are. QTLs concerned with agronomically important traits such as plant height and panicle length are located around the region of *hwd2* in an indica/japonica cross (Xiao et al., 1996; Li et al., 1998). Such observations suggest the involvement of this region in indica/japonica differentiation because the genes concerned with agronomic traits would be easily selected.

The present study revealed that hybrid breakdown (F_2 weakness) is a rare event and therefore, there would be little chance that this phenomenon would impede varietal improvement by cross breeding. Also the present study implies that the polymorphic locus *hwd2*, one of the hybrid breakdown loci, is located in a chromosomal region possibly associated with differentiation in Asian cultivated rice. However, it may be concluded that the genes for hybrid breakdown investigated in this study are not a factor causing genetic differentiation in rice.

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