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Distribution and RFLP Mapping of Complementary Genes Causing Hybrid Breakdown in Asian Cultivated Rice, *Oryza sativa* L.

Kazutoshi OKUNO* and Shuichi FUKUOKA

Department of Genetic Resources I, National Institute of Agrobiological Resources (NIAR) (Tsukuba, Ibaraki 305-8602 Japan)

Abstract

Weak plants were found in the BC_1F_1 generation in a backcrossing program aimed at introducing the wx gene from a Thai cultivar, Col. No. 15, into a Japanese cultivar, Sasanishiki, in Asian cultivated rice. These weak plants were characterized by poor growth and discoloration at the tillering stage, but they were fertile. Hybrid breakdown, which is defined as hybrid weakness and sterility detected in the F2 and later inbred generations from varietal crosses, is controlled by a pair of complementary recessive genes, *hwd1* and *hwd2*, at unlinked loci. Two dominant genes at either the same or different loci, *Hwd1/Hwd1 hwd2/hwd2, hwd1/hwd1 Hwd2/Hwd2* or *Hwd1/hwd1 Hwd2/hwd2* are needed for normal growth. Using tester lines homozygous for a pair of complementary recessive genes selected in the BC1F3, the genotypes for hybrid breakdown of 100 Asian rice cultivars were tested based on the phenotype of F1 plants. Clinal variation for hybrid breakdown was observed. Cultivars with 2 dominant alleles at either hwd1 or hwd2 locus, were mainly found in insular Asia (Japan, Philippines and Indonesia), while the frequency of cultivars with 4 dominant alleles was more common in cultivars from continental Asia. Linkage analysis using RFLP markers mapped over 12 rice chromosomes indicated that hwd1 from Col.No.15 was located between RFLP markers, C701 and R2309, on chromosome 10, and *hwd2* of Sasanishiki was tightly linked to 4 RFLP markers on chromosome 7. Role of hybrid breakdown in genetic differentiation of Asian cultivated rice is discussed.

Discipline: Plant breeding/Genetic resources
Additional key words: reproductive barrier, hybrid weakness, geographical differentiation, RFLP markers

Introduction

Isolating mechanisms prevent or restrict the exchange of genes between and within species and may be either external or internal in type[18]. External barriers include eco-geographical isolation of interspecific and intraspecific variation and result in unique genotypes adapted to distinct environments. Internal barriers restrict gene flow between plants growing sympatrically and play an important role in plant speciation and genetic differentiation.

In addition to partial cross-incompatibility[14], different types of reproductive barriers have been found in varietal crosses of Asian cultivated rice, *Oryza sativa* L. These post-hybridization reproductive barriers include, hybrid weakness or F_1 lethality[1,8,16], hybrid sterility[9,10], hybrid breakdown[3,6,8,11-13,19], hybrid chlorosis[17] and distorted segregation[7]. Among these post-hybridization reproductive barriers, hybrid breakdown which is defined as weakness and sporophytic sterility found in the F_2 and later inbred generations, is distinct from F_1 weakness or lethality. F_1 weakness and hybrid breakdown have been detected in remote crosses of Asian cultivated rice. Whereas a pair of complementary dominant genes causes weakness and lethality in F_1 plants[1,8,16], a pair of complementary or duplicate recessive genes is responsible for weakness in the F_2 and later generations[3,8,13]. These characters are free from direct artificial selection and are useful for research into evolution and differen-

Present address:
* Department of Research Planning and Coordination, NIAR (Tsukuba, Ibaraki, 305-8602 Japan)

tiation in plants. The geographical distribution of genes conferring reproductive barriers reflects the phylogenetic relationship of the varietal groups of rice.

In a breeding program aimed at producing isogenic lines for the *wx* genes, 30 glutinous cultivars from different Asian countries were used for crosses to introduce the *wx* gene into the Japanese cultivar, Sasanishiki. When a Thai glutinous cultivar of upland rice, Col. No. 15 was used for the cross, weak plants appeared among BC_1F_1 plants backcrossed to Sasanishiki.

In this report, the genetic basis of hybrid breakdown found in the above cross, the geographical distribution of the genes responsible for hybrid breakdown, mapping of genes using RFLP markers and the role of hybrid breakdown in genetic differentiation of Asian cultivated rice will be discussed.

Inheritance of hybrid breakdown[13,15]

To analyze the mode of inheritance of weakness, various populations were produced. Reciprocal crosses were made between Sasanishiki and Col. No. 15. F_1 plants were backcrossed to both cultivars and were also self-pollinated. Weak BC_1F_1 and BC_2F_1 segregants were crossed to Sasanishiki. Seeds from self-pollinated spikelets of weak BC_1F_1 plants were bulked to detect segregation in the BC_1F_2 generation. Each BC_1F_2 plant was separately harvested to analyze segregation in the BC_1F_3 generation. All the materials were transplanted in the field at the same time and observed for their growth and morphology. BC_1F_2 bulked populations originating from weak BC_1F_1 segregants were planted in the field and investigated for heading time, culm length and the number of panicles per plant.

The segregation for hybrid weakness in different generations is shown in Table 1. F_1 plants of reciprocal crosses between Sasanishiki and Col. No. 15 showed vigorous growth and were fertile. BC_1F_1 plants backcrossed to both cultivars segregated into 3 normal : 1 weak types. Weakness did not appear at the seedling stage. The weak BC_1F_1 segregants became yellow at the tillering stage and stunted. Weak plants produced one or a few panicles with fertile seeds. When weak BC_1F_1 and BC_2F_1 segregants were backcrossed to Sasanishiki, BC_2F_1 and BC_3F_1 plants segregated into 1 normal : 1 weak plants. BC_1F_2 bulked populations derived from weak BC_1F_1 plants segregated into 1 normal : 3 weak plants. BC_1F_3 lines derived from a random sample of BC_1F_2 plants segregated into 1 normal : 2 heterozygous : 1 weak lines. F_2 plants from reciprocal crosses between Sasanishiki and Col. No. 15 segregated into 11 normal : 5 weak plants. Also, F_2 plants of reciprocal crosses between Sasanishiki and weak BC_1F_3 segregants showed a good fit to the segregation ratio 1 normal : 3 weak plants.

The frequency distribution for heading time, culm length and number of panicles per plant in the BC_1F_2 bulked population is shown in Fig. 1. Heading time occurred from August 5 to August 30 (99 to 124 days after sowing) and no relation between heading time and weakness was detected. Three-fourths of BC_1F_2 plants had a very short culm and few panicles and the others showed normal growth, reflecting segregation for hybrid weakness.

The genetic basis of hybrid breakdown found in the progeny of the cross between Sasanishiki and

Table 1. Segregation ratios for hybrid weakness observed in each generation

Generation	Cross-combination[a]	Number of plants			Ratio expected	χ^2
		Normal	Seg.[b]	Weak		
F_1	Sas/Col. 15	45		0	1 : 0	0.000
	Col. 15/Sas	105		0	1 : 0	0.000
F_2	Sas/Col. 15	474		226	11 : 5	0.350
	Col. 15/Sas	326		165	11 : 5	1.267
BC_1F_1	Sas/Col. 15//Sas	30		12	3 : 1	0.286
	Sas/Col. 15//Col. 15	23		8	3 : 1	0.011
BC_2F_1	Col. 15/Sas//2*Sas	15		13	1 : 1	0.143
BC_3F_1	Sas/Col. 15//3*Sas	22		15	1 : 1	1.324
BC_1F_2	Sas/Col. 15//Sas	46		142	1 : 3	0.028
BC_1F_3	Sas/Col. 15//Sas	46	83	54	1 : 2 : 1	2.279
F_2	Sas/weak BC_1F_3 segregant	85		255	1 : 3	0.000
	Weak BC_1F_3 segregant/Sas	130		473	1 : 3	3.808

a): Sas; Sasanishiki, Col. 15; Col. No. 15. b): Lines segregating into weak and normal plants.

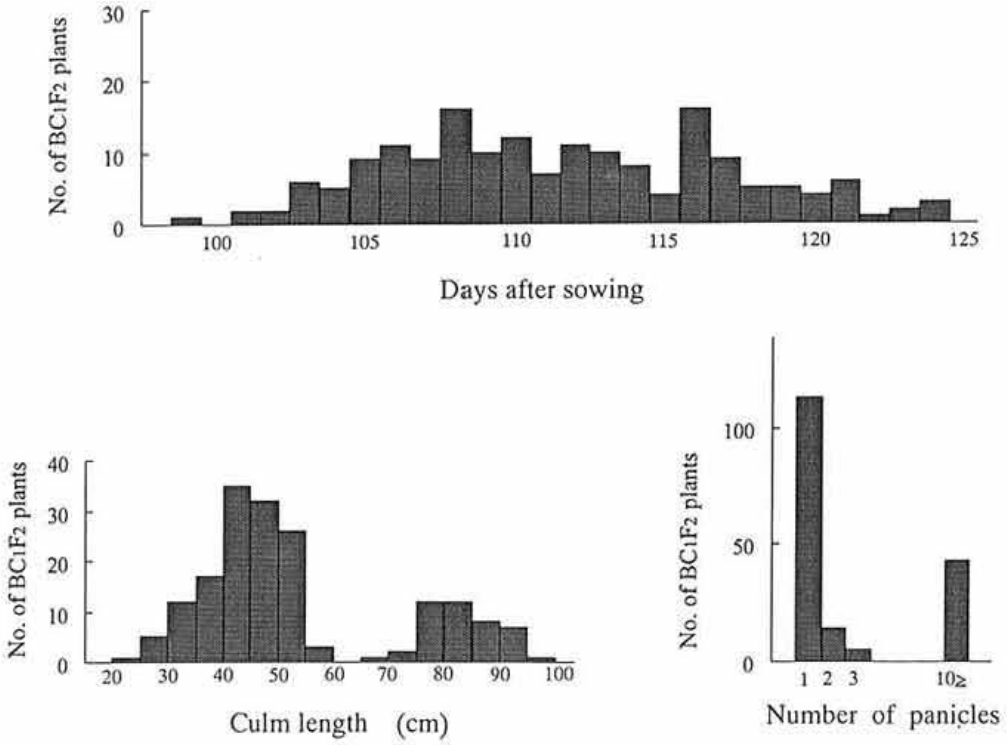

Fig. 1. Frequency distribution for heading time, culm length and number of panicles per plant in BC_1F_2 population from weak BC_1F_1 plants of the cross, Sasanishiki/Col. No. 15//Sasanishiki

Col. No. 15 could be elucidated by assuming the presence of a pair of complementary loci in the same manner as postulated by Oka[8]. The segregation ratio for weak and normal plants in all the generations analyzed fitted well to the expected ratios in each population for this mode (Table 1). Based on this model, if Sasanishiki and Col. No. 15 have the genotypes, AAbb and aaBB, segregants with only one or no dominant gene, Aabb, aaBb and aabb, are likely to express the weakness. At least 2 dominant genes at either the same or different loci, AAbb, aaBB and AaBb, are necessary for normal growth.

Based on the results obtained in this study, a pair of complementary loci causing hybrid breakdown in Asian cultivated rice was symbolized as *hwd1* and *hwd2*. Col. No. 15 and Sasanishiki carry recessive alleles at the *hwd1* and *hwd2* loci, respectively.

Distribution of a pair of complementary genes for hybrid breakdown[15]

Early maturing and fertile BC_1F_3 lines in which double recessive homozygotes express weakness, were selected as tester lines. Using BC_1F_3 lines lacking dominant genes, *hwd1/hwd1 hwd2/hwd2*, for hybrid breakdown, a total of 100 Asian rice cultivars were tested for complementary genes at a pair of unlinked loci. Five to 10 F_1 plants from each of the crosses were grown in the field and analyzed for weakness based on their growth pattern and morphology. F_2 plants from reciprocal crosses between Sasanishiki and one of the tester lines were also investigated.

When the F_1 plants between a tester line and a given cultivar show vigorous growth, the cultivar is likely to carry 2 pairs of dominant genes, *Hwd1/Hwd1 Hwd2/Hwd2*. When the F_1 plants show weakness, the cultivar is likely to carry one pair of dominant genes, *Hwd1/Hwd1 hwd2/hwd2* or *hwd1/hwd1 Hwd2/Hwd2*. When the F_1 plants were intermediate between normal and weak plants, their F_2 progeny was further tested to determine the genotype for hybrid breakdown of cultivars.

Geographical distribution of a pair of complementary genes causing hybrid breakdown in Asian cultivated rice is presented in Fig. 2. Clinal variation was observed in the frequency of genotypes for hybrid breakdown among Asian cultivars. A majority of cultivars with 2 dominant genes, *Hwd1/Hwd1 hwd2/*

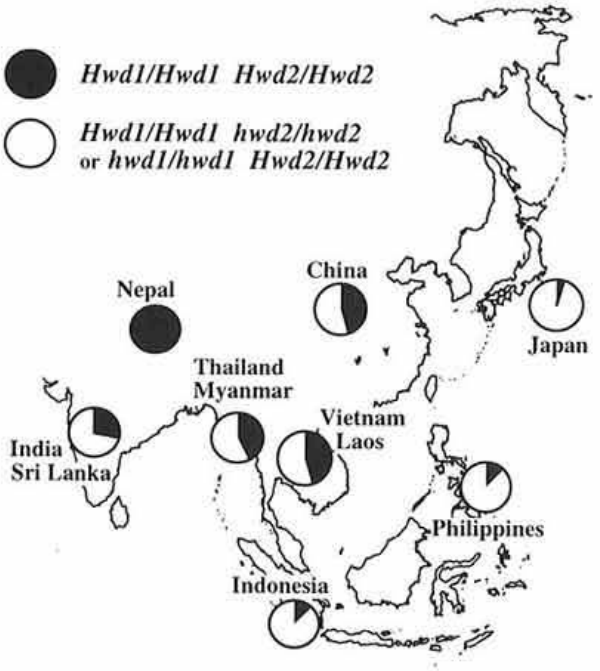

Fig. 2. Geographical distribution of a pair of complementary genes responsible for hybrid breakdown in Asian cultivated rice

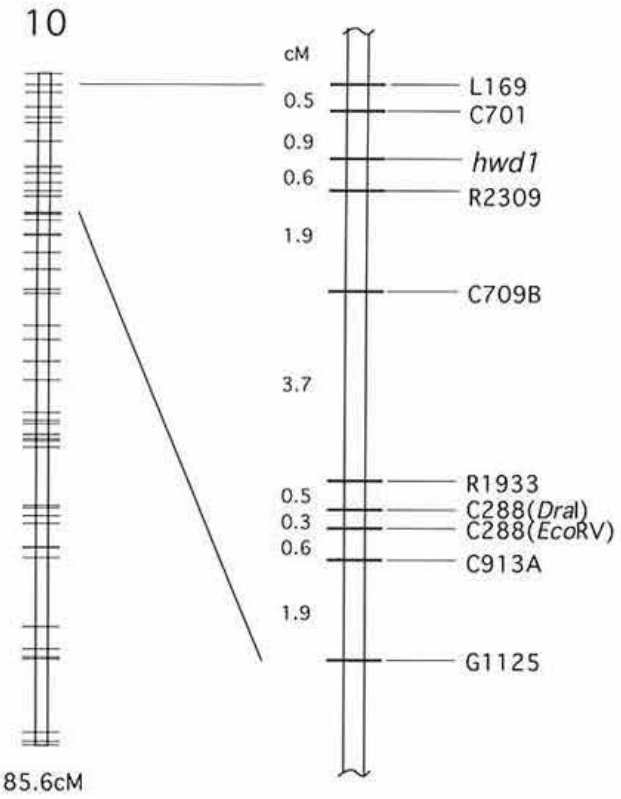

Fig. 3. Linkage map showing the position of *hwd1* responsible for hybrid breakdown in rice
Loci are shown on the right side map and the distance (cM) among RFLP markers is shown on the left side map of chromosome 10.

hwd2 or *hwd1/hwd1 Hwd2/Hwd2*, was found in insular Asia, Japan, Philippines and Indonesia. On the other hand, the frequency of cultivars with 4 dominant genes, *Hwd1/Hwd1 Hwd2/Hwd2* was more common in continental Asia, China, Vietnam, Laos, Thailand and Myanmar. All the Nepalese cultivars analyzed in this study carried 4 dominant genes.

Mapping of complementary genes using RFLP markers[2)]

The location of the complementary genes causing hybrid breakdown was analyzed using RFLP markers. Two cultivars with 2 dominant genes at either locus were used for one of the parents to produce mapping populations. Nepalese cultivar Siborunauli 1 is dominant for *hwd1* and recessive for *hwd2*. Thai cultivar Col. No. 15 is dominant for *hwd2* and recessive for *hwd1*. Crosses between these cultivars and one of the tester lines (W26) which is recessive homozygote for these 2 loci were made to produce F_2 mapping populations. F_1 plants from both cross-combinations had only one dominant allele and showed weakness. Two hundred and twenty-five F_2 plants from the cross between W26 and Siborunauli 1 and 184 F_2 plants from the cross between Col. No. 15 and W26 were analyzed for segregation into weak and normal plant types.

Total DNA was isolated from the leaves of each F_2 plant using standard procedures and was digested with restriction enzymes. Southern hybridization and signal detection were conducted using ECL direct nucleic acid labeling and a detection kit (Amersham). RFLP markers mapped on the rice linkage map[4)] were used for mapping. Segregation patterns of RFLP markers and plant types in F_2 populations were analyzed with MAPMAKER/EXP 3.0[5)]. The map position of 2 loci controlling hybrid breakdown was determined by multipoint linkage analysis.

F_2 plants from the cross between W26 and Siborunauli 1 segregated into 52 normal : 173 weak types. F_2 plants from the cross between Col. No. 15 and W26 segregated into 56 normal : 128 weak types. These data fitted to the expected ratio of 1 : 3. Linkage analysis using F_2 population from the cross between W26 and Siborunauli 1 revealed that the gene *hwd1* was located in the proximal region on chromosome 10. The gene, *hwd1*, was linked between RFLP

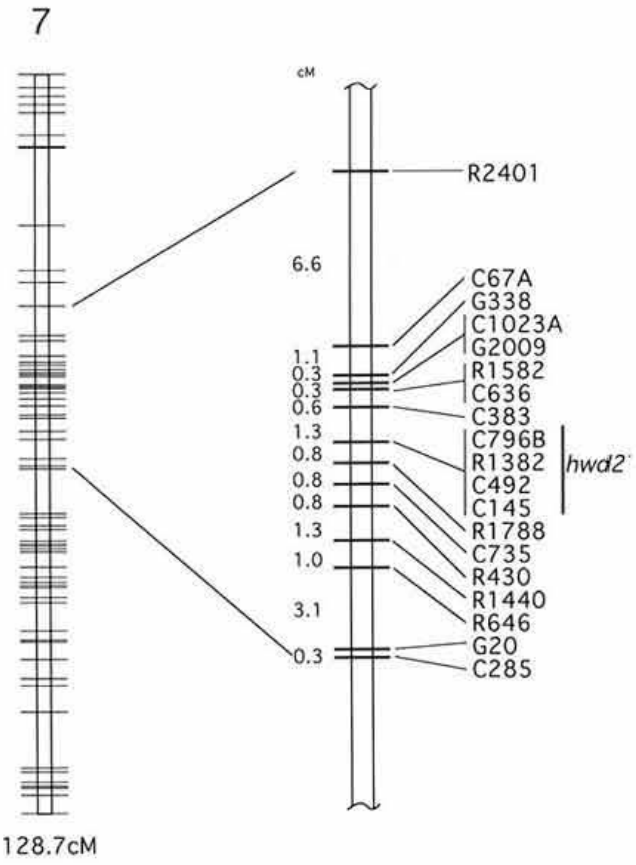

Fig. 4. Linkage map showing the position of *hwd2* responsible for hybrid breakdown in rice
Loci are shown on the right side map and the distance (cM) among RFLP markers is shown on the left side map of chromosome 7.

markers C701 and R2309 at a distance of 0.9 centi-Morgans (cM) and 0.6 cM, respectively (Fig. 3). In the F_2 population from the cross between Col. No. 15 and W26, 4 RFLP markers (C796B, R1382, C145, C492) co-segregated with plant types, indicating that *hwd2* was located in the central region of chromosome 7 (Fig. 4).

Role of hybrid breakdown in varietal differentiation of Asian cultivated rice

Hybrid weakness in F_1 and hybrid breakdown including weakness in F_2 and later generations have been observed in particular crosses among Asian rice cultivars. The former occurs under a complementary dominant gene system and F_1 plants heterozygous for 2 complementary loci are not viable[1,8,16]. The latter occurs under a complementary or duplicate recessive gene system and F_2 progeny segregates into weak[8,3,13], partially sterile[6,11,12,19] or chlorotic[17] plants according to Mendelian segregation ratios.

Hybrid weakness analyzed in this study was detected in F_2 and later generations in the cross between a Japanese and a Thai cultivar. Therefore, this weakness is categorized into hybrid breakdown and is controlled by a pair of complementary recessive genes with a segregation ratio of 11 normal : 5 weak plants in the F_2. These results are the same as those reported in the F_2 of a cross between an Indian cultivar and a Japanese cultivar[8]. On the other hand, a duplicate recessive gene system with a segregation ratio of 15 normal : 1 weak or chlorotic plants in the F_2 has also been reported[3,17]. If hybrid breakdown contributes to promoting *indica-japonica* differentiation of Asian cultivated rice, these complementary or duplicate genes causing hybrid breakdown must be distributed independently in *indica* and *japonica* rices. In this study, one of the complementary genes for hybrid breakdown, *hwd1*, was carried by the *indica* cultivar, Col. No. 15, and the other gene, *hwd2*, the *japonica* cultivar, Sasanishiki. These findings suggest that the genes may have caused or accelerated *indica-japonica* differentiation. However, to gain a better understanding of the role of hybrid breakdown in varietal differentiation, the distribution pattern of *hwd1* and *hwd2* in rice cultivars with the genotypes, *Hwd1/Hwd1 hwd2/hwd2* and *hwd1/hwd1 Hwd2/Hwd2*, will be further analyzed.

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