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RFLP mapping of the genes controlling hybrid breakdown in rice (*Oryza sativa* L.)

Abstract Complementary recessive genes *hwd1* and *hwd2* controlling hybrid breakdown (weakness of F_2 and later generations) were mapped in rice using RFLP markers. These genes produce a plant that is shorter and has fewer tillers than normal plants when the two loci have only one or no dominant allele at both loci. A cultivar with two dominant alleles at the *hwd1* locus and a cultivar with two dominant alleles at the *hwd2* locus were crossed with a double recessive tester line. Linkage analysis was carried out for each gene independently in two F_2 populations derived from these crosses. *hwd1* was mapped on the distal region of rice genetic linkage map for chromosome 10, flanked by RFLP markers C701 and R2309 at a distance of 0.9 centiMorgans (cM) and 0.6 cM, respectively. *hwd2* was mapped in the central region of rice genetic linkage map for chromosome 7, tightly linked with 4 RFLP markers without detectable recombination. The usefulness of RFLP mapping and map information for the genes controlling reproductive barriers are discussed in the context of breeding using diverse rice germplasm, especially gene introduction by marker-aided selection.

Key words Reproductive barrier · Rice (*Oryza sativa* L.) · Gene mapping · Restriction fragment length polymorphism (RFLP)

Introduction

While reproductive barriers are essential to maintain the identity of species, subspecies and ecotypes, these barriers inhibit breeding efforts to introduce genes in wide crosses. Within Asian cultivated rice (*Oryza sativa* L.), a genetically diverse species consisting of two subspecies and many ecotypes (Chang 1976), 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 1987; Ise et al. 1992).

Reproductive barriers have been classified into several types (Stebbins 1950). Hybrid inviability (weakness) and hybrid sterility are genetic disorders observed in F_1 hybrids. The third type of hybrid breakdown includes both sterility and weakness observed in the F_2 or later generations. There have not been many reports of hybrid breakdown in rice. The segregation of weak or chlorotic plants in the F_2 generation was observed in crosses between cultivars belonging to different subspecies or to the same subspecies (Oka 1957b; Sato et al. 1984; Okuno 1985; Ise et al. 1992). One of these phenomena is controlled by complementary recessive genes, and about 30% of the F_2 have reduced plant height and number of tillers (Okuno 1985). As this abnormality is not apparent in the seedling stage, the number of plants available for selection in breeding will be significantly reduceed. This hybrid breakdown was found to be controlled by a pair of complementary genes or duplicate genes.

The Location on genetic linkage maps of the genes controlling hybrid breakdown has not yet been determined, perhaps due to their complicated mode of inheritance. Recently, the chromosomal locations

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of interacting non-allelic genes affecting with hybrid sterility found in F_2 generation have been reported (Wu et al. 1995). Mapped RFLP markers provide accurate and efficient means of determining the position of genes on a linkage map.

We report here the location of the genes controlling hybrid breakdown found in a cross between two cultivars, 'Sasanishiki' and 'Col. No.15', using RFLP markers.

Materials and methods

Plant material

A pair of complementary recessive genes have been found which result in hybrid breakdown (Okuno 1985). Plants with one or no dominant allele showed a weak plant type with a shorter stature and fewer tillers than the normal type. Two cultivars, each having a different dominant allele, were each used as one of the parents to construct the mapping populations. The 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*. A cross between these cultivars and the tester line (W26), which is recessive homozygote for both loci, was used to generate the F_2 mapping population. F_1 plants from both cross combinations have only one dominant allele and subsequently show a weak plant type. In the F_2 generation one of the loci is recessive, and segregation of plant type is determined by the genotype for the other locus. We analyzed 225 F_2 plants from the cross between W26 and 'Siborunauli 1', and 184 plants from the cross between 'Col. No. 15' and W26. In these cross combinations, differences in plant type were only apparent after the three- or four-leaf stage. Plant type was judged at the heading stage by tiller number: the weak type has fewer than 10 tillers (4 on average) and the normal type has more than 15.

RFLP analysis

After the plant type was determined, leaves were collected, and total DNA was extracted using standard procedures. DNA samples were digested with restriction enzymes and blotted onto a positively charged nylon membrane after electrophoresis. Southern hybridization and signal detection was conducted using an ECL direct nucleic acid labeling and detection kit (Amersham). RFLP markers mapped on the rice genetic linkage map (Kurata et al. 1994) were used for mapping. Restriction enzymes which revealed polymorphism in parental DNAs were selected from eight restriction enzymes (*Apa*I, *Kpn*I, *Dra*I, *Hin*dIII, *Bam*HI, *Bgl*II, *Eco*RI and *Eco*RV) for F_2 analysis. Segregation patterns in the F_2 populations were detected with selected probe-enzyme combinations and scored.

Data analysis

Segregation patterns of DNA markers and plant type in F_2 populations were analyzed with MAPMAKER/EXP 3.0 (Lander et al. 1987). The map position of two loci controlling hybrid breakdown was determined by multipoint linkage analysis.

Results

Segregation of plant type in F_2 populations

Out of 225 F_2 plants from the cross between W26 and 'Siborunauli 1', 52 showed a normal plant type and 173 showed a weak plant type. This fits the expected ratio of 1:3 (normal:weak) for a single recessive gene as expected at the 5% level. In the F_2 population of the cross between 'Col. No. 15' and W26, 56 showed a normal plant type and 128 showed a weak plant type. This also fits the expected ratio of 1:3, (normal:weak) for a single recessive gene at the 5% level.

Mapping of the genes controlling hybrid breakdown

From the cross between W26 and 'Siborunauli 1' linkage analysis revealed that one of the loci, *hwd1*, is located in the proximal region of the rice genetic linkage map for chromosome 10. Nine RFLP markers located on this region were used for detailed analysis. The gene was flanked by C701 and R2309 at a distance of 0.9 centiMorgans (cM) and 0.6 cM, respectively (Fig. 1). Since two restriction enzymes (*Dra*I and *Eco*RV) were found to reveal different genotypes with probe C288 in 1 F_2 plant, this probe produced

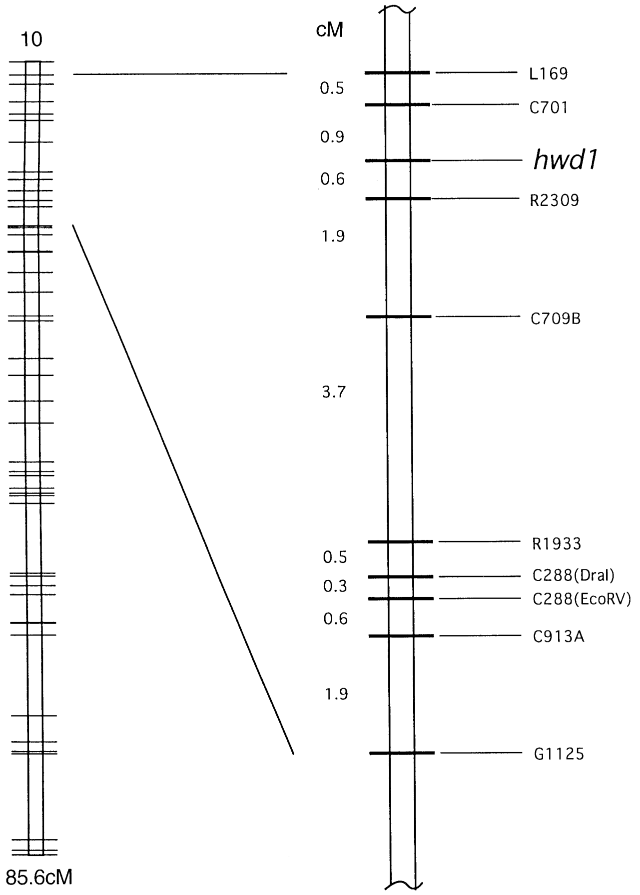

Fig. 1 A linkage map showing the position of *hwd1* constructed with 225 F_2 individuals from the cross between W26 and Siborunauli 1 (*right*). The frame of the map of chromosome 10 (Kurata et al. 1994) is shown on *left*

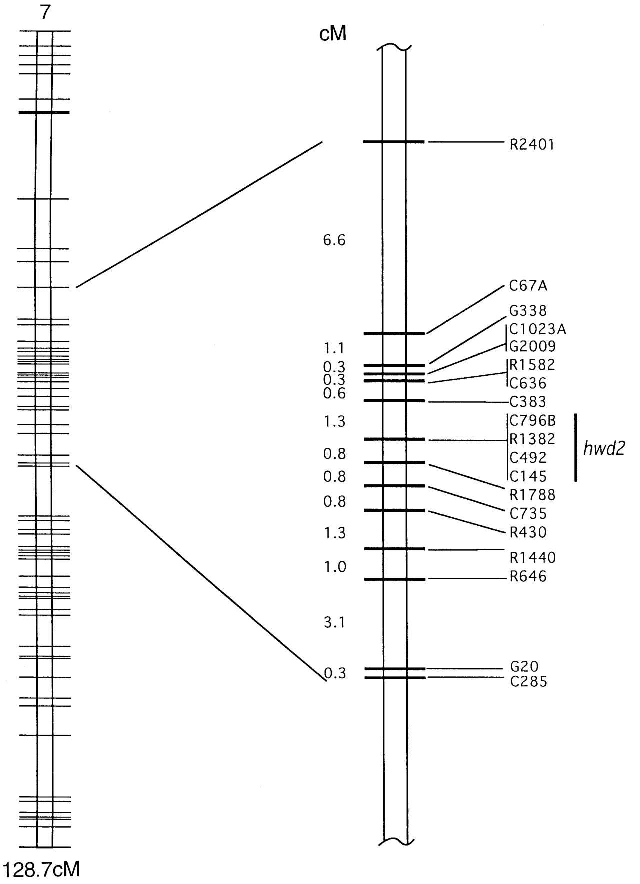

Fig. 2 A linkage map showing the position of *hwd2* constructed with 184 F_2 individuals from the cross between Col. No.15 and W26 (*right*). The frame of the map of chromosome 7 (Kurata et al. 1994) is shown on *left*

2 markers 0.3 cM apart. The distance between RFLP marker L169 and G1125 was 10.9 cM. Order of the markers on this region was almost the same as that on the map constructed in the cross between 'Nipponbare' and 'Kasalath', except for the position of markers C288 and C913A (Kurata et al. 1994).

In the F_2 population from the cross between 'Col. No. 15' and W26, 19 RFLP markers in the central region of rice genetic linkage map on chromosome 7 were used to determine the map position of *hwd2*. Four RFLP markers (C796B, R1382, C145 and C492) cosegregated with plant type (Fig. 2). The distance between the RFLP markers R2401 and C285 was 18.3 cM.

Discussion

Mapping was carried out for complementary genes controlling the hybrid breakdown (designated as *hwd1* and *hwd2*) found in 'Sasanishiki' and 'Col. No. 15'. The genes were mapped on chromosomes 7 and 10, and no other genes seemed to control segregation of plant type in the cross combinations used. Marker orders on the neighboring region of *hwd1* and *hwd2* were almost the same as those reported by Kurata et al. (1994), with the exception of 2 markers on chromosome 10. The reason for this difference is unknown and could be due to differences in chromosomal structure among cultivars or to experimental errors. Cryptic structural rearrangements have been implicated in the region causing spikelet sterility during differentiation of cultivated rice (Li et al. 1997). To clarify this point detailed analysis should be carried out on this region using a larger population and different cross combinations. Complementary recessive genes which produce a plant type similar to that produced by the genes used in this study were reported by Oka (1957b). An allelism test or mapping of the genes reported by Oka should be carried out to clarify whether they are identical to *hwd1* and *hwd2* or not.

Map information of the gene controlling reproductive barriers is useful in marker-aided selection. In the transfer of quantitative trait loci (QTLs) located in neighboring regions of *hwd1* and *hwd2* to a desirable genetic background, the breeding efficiency will depend on the genotype of both the donor and recipient parent for *hwd1* and *hwd2*. When the combination of these genes results in undesirable plants the number of plants available for selection will greatly reduced. To obtain recombinants between QTL and *hwd1* or *hwd2*, selection needs to be carried out in a large population. Knowledge of the map position of the genes controlling reproductive barriers is helpful in the design of experiments in marker-aided selection.

Rapid progress in QTL analysis enables the identification of QTLs associated with agronomic traits of importance. Several QTLs have been reported on chromosomes 7 and 10, where *hwd1* and *hwd2* are located (Redona and Mackill 1996; Xiao et al. 1996a; Lin et al. 1996; Li et al. 1997). Since QTL analysis can reveal cryptic but useful characteristics, both rice cultivars and their wild relatives have the potential to improve rice (Xiao et al. 1996b). Since hybrids from wide crosses are likely to have reproductive barriers, research into reproductive barriers can be helpful to overcome this problem.

Some genes controlling reproductive barriers are reported to be located in a cluster. In *Drosophila* the genes causing hybrid sterility lie in a cluster that was determined by fine genetic linkage analysis (Palopoli and Wu 1994; Cobot et al. 1994; Davis and Wu 1996). In these studies, phenotypic variations in fertility seemed to be caused by different combinations of genes in one region. Similar observations have been obtained in rice. The gamete eliminator and its regulating elements seem to be located on the region between the *waxy* (*wx*) and *photosensitivity-1* (*Se_1*) loci on chromosome 6 (Sano 1990).

The Discrimination of tightly linked alleles is possible by analysis of large populations using fine genetic linkage maps. Such detailed analysis provides a better understanding of the genetic system of the genes controlling reproductive barriers. The map information obtained in this study may be useful for such a detailed analysis.

Determination of genotypes for the genes controlling hybrid breakdown in rice cultivars is helpful for the selection of parents in a crossing program. The geographical distribution of *hwd1* and *hwd2* was analyzed in 100 Asian rice cultivars (Okuno 1986). In that study, a geographical cline was observed in the frequency of the genotypes. Cultivars with two dominant alleles were found mainly in Japan, the Philippines and Indonesia. The frequency of cultivars with four dominant alleles increased in south China, Vietnam, Laos, Myanmar and Thailand. Since 70% of the cultivars had two dominant alleles (similar to 'Siborunauri 1' and 'Col. No. 15'), hybrid breakdown was revealed in the progenies from some cross combinations. Determination of the genotypes for both loci is now in progress using test crosses.

This study revealed the detailed map position of two genes controlling hybrid breakdown in cultivated rice. RFLP mapping and the determination of genotypes for the genes controlling reproductive barriers make it possible to understand the genetic basis of reproductive barriers in cultivated rice. Such analyses broaden the choice of breeding materials and are useful for efficient rice improvement by marker-aided selection.

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References

Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. Euphytica 25:425–441

Chu YE, Oka HI (1972) The distribution and effect of genes causing F_1 weakness in *Oryza breviligulata* and *O. glaberrima*. Genetics 70:163–173

Cobot FL, Davis AW, Johnson NA, Wu CI (1994) Genetics of reproductive isolation in the *Drosophila simulans* Clade: complex epistasis underlying hybrid male sterility. Genetics 137:175–189

Davis AW, Wu CI (1996) The broom of the sorcerer's apprentice: the fine structure of a chromosomal region causing reproductive isolation between two sibling species of *Drosophila*. Genetics 143:1287–1298

Ise K, Sekizawa K, Sato H (1992) Inheritance of hybrid weakness in indica/japonica rice crosses. Int Rice Res Notes 17–4:5

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamot Y, Kirihata T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Sasaki T, Minobe Y (1994) A 330 kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

Lander ES, Green P, Abrahanson J (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li Z, Pinson SRM, Paterson AH, Park WD, Stancel JW (1997) Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. Genetics 145: 1139–1148

Lin HX, Qian HR, Zhuang JY, Lu J, Min SK, Xiong ZM, Huang N, Zheng KL (1996) RFLP mapping of QTLs for yield and related characters in rice (*Oryza sativa* L.). Theor Appl Genet 92: 920–927

Oka HI (1957a) Genic analysis for the sterility of hybrids between distantly related varieties of cultivated rice. J Genet 55:397–409

Oka HI (1957b) Phylogenetic differentiation of cultivated rice. XV. Complementary lethal genes in rice. Jpn J Genet 32:83–87

Oka HI (1974) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetics 77:521–534

Okuno K (1985) Complementary recessive genes controlling hybrid breakdown found in a varietal cross of rice. Rice Genet Newsl 2:52–54

Okuno K (1986) Geographical distribution of complementary recessive genes controlling hybrid breakdown of rice. Rice Genet Newsl 3:44–45

Palopoli MF, Wu CI (1994) Genetics of hybrid male sterility between *Drosophila* sibling species: a complex web of epistasis is revealed in interspecific studies. Genetics 138:329–341

Redona ED, Mackill DJ (1996) Mapping quantitative trait loci for seedling vigor in rice using RFLPs. Theor Appl Genet 92: 395–402

Sano Y (1990) The genic nature of gamete eliminator in rice. Genetics 125:183–191

Sano Y, Chu YE, Oka HI (1979) Genetic studies of speciation in cultivated rice, 1. Genic analysis for F_1 sterility between *Oryza sativa* L. and *O. glaberrima* Steud. Jpn J Genet 54:121–132

Sato YI, Morishima H (1987) Studies on the distribution of complementary gene causing F_1 weakness in common rice, its wild species, 2. Distribution of two complementary genes, *Hwc-1* and *Hwc-2* genes, in native cultivars and its wild relatives of tropical Asia. Euphytica 36:425–431

Sato YI, Matsuura S, Hayashi K (1984) The genetic basis of hybrid chlorosis found in a cross between Japanese native cultivars. Rice Genet Newsl 1:106–107

Stebbins GL (1950) Isolation and the origin of species. In: Stebins GL Jr (ed) Variation and evolution in plants. Columbia University Press, New York, pp 189–250

Wu P, Zhang G, Huang N, Ladha JK (1995) Non-allelic interaction conditioning spikelet sterility in an F_2 population of indica/japonica cross in rice. Theor Appl Genet 91:825–829

Xiao J, Li J, Yuan L, Tanksley SD (1996a) Identification of QTLs affecting traits of agronomic importance in a recombinant inbreed population derived from a subspecific rice cross. Theor Appl Genet 92:230–244

Xiao J, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li J, Yuan L (1996b) Genes from wild rice improve yield (Scientific correspondence). Nature 384:223–224