

GENETIC HETEROGENEITY FOUND IN CROPS PLANTED IN SMALL ISLANDS

Katsuyuki ICHITANI and Yoshimichi FUKUTA

Abstract

Some native crop cultivars planted in small islands differ genetically from those planted in other regions in Japan. Among such genes conferring genetic heterogeneity in small islands, *Hwc2* gene in rice was examined. Frequency of *Hwc2* gene has showed a latitudinal cline of Japonica types of the insular region. In this study, first, linkage analysis of *Hwc2* locus was performed. *Hwc2* gene cosegregated with *ph* gene on rice chromosome 4. This result was consistent with our previous study. Then, the significance of the chromosomal region of the *Hwc2* locus and the genetic heterogeneity of crops in small islands are discussed.

Key words: Genetic heterogeneity, *Hwc2*, Linkage analysis, Rice

Introduction

Some native crop cultivars planted in small islands differ genetically from those planted in other regions in Japan. For example, KAWASE and SAKAMOTO (1982) examined phenol color reaction (see Materials and Methods) of 376 strains of foxtail millet, *Setaria italica* (L.) P. Beauv., collected from different areas throughout Eurasia including Japan. Strains that showed the positive phenotype of phenol color reaction were found in Tsushima, the Nansei islands, Taiwan, Philippines, and India with high frequency. However, these cultivars were rarely found in other regions. Another example is red rice. It was expelled from most of Japan, but it is still planted for religious purposes in Homan Shrine in the Tanegashima island, and Takuzutama Shrine in the Tsushima island (WATABE, 1993). Such genetic heterogeneity is thought to be closely related to routes of the crops and geographical heterogeneity of these small islands.

Weak growth occurring in hybrids derived from crosses between two normal strains is called hybrid weakness. F₁ weakness of intraspecific hybridization has been reported in some cereal crops such as wheat *Triticum aestivum* L. (CALDWELL and COMPTON, 1943; TSUNEWAKI, 1970) and barley *Hordeum vulgare* L. (WIEBE, 1934; TAKAHASHI and HAYASHI, 1972). There are some hybrid weakness phenomena in rice, *Oryza sativa* L., (SATO, 1997). Among them, F₁ weakness found in crosses of a Peruvian rice cultivar Jamaica and Japanese lowland cultivars is controlled by a set of complementary genes, *Hwc1* and *Hwc2* (AMEMIYA and AKEMINE, 1963). *Hwc1* and *Hwc2* are carried by Jamaica and Japanese lowland cultivars, respectively. Genotypes of *Hwc1* and *Hwc2* loci are *Hwc1Hwc1hwc2hwc2* for Jamaica, and *hwc1hwc1Hwc2Hwc2* for Japanese cultivars.

Cultivated Asian rice is classified into two groups, Indica and Japonica. Japonica cultivars comprise two varietal groups, temperate and tropical types, the latter including cultivars classified in earlier studies as Javanica types (GLASZMANN, 1987; MACKILL,

1995). Distribution of *Hwc2* gene-carrier was surveyed in Asian native cultivars (SATO and HAYASHI, 1983); the *Hwc2* gene was prevalent among temperate Japonica, but not among tropical Japonica or Indica. Most Japanese native cultivars carry the *Hwc2* gene while those from the Nansei islands and Taiwan carry *hwc2* with relatively high frequency. These Japanese cultivars carrying *hwc2* have many traits that are specific to tropical Japonica while most Japanese cultivars are temperate Japonica. Therefore, *hwc2* gene in rice is among the genes conferring genetic heterogeneity in small islands.

We identified the chromosomal location of the *Hwc2* locus by using 127 recombinant inbred (RI) lines segregating at the *Hwc2* locus (ICHITANI et al., 2001). These RI lines are produced by continually selfing or sib-mating progeny of individual members of an F_2 population until homozygosity is achieved (BURR and BURR, 1991). Each RI line is fixed for a different combination of linked blocks of parental alleles; consequently, an RI family constitutes a permanent population in which segregation is fixed. Suppose that a hybrid weakness gene segregates in a set of RI lines. Crosses between RI lines and a line carrying the complementary hybrid weakness gene produce normal plants and weak ones showing hybrid weakness as in F_2 and backcross population. Plants showing weakness are difficult to analyze genetically. Therefore, F_2 or backcross is not an appropriate population for mapping *Hwc2*. The RI lines are appropriate for mapping *Hwc2* because RI lines in themselves, not the hybrids expressing weakness, can be analyzed for further genetic study. Information about presence or absence of hybrid weakness phenomena can easily be combined to accumulate a genetic data set of each RI line. This confers great advantages over usual segregating populations such as F_2 and backcross.

The present study examines 27 more RI lines; the more accurate location is determined. Then, significance of the chromosomal region of the *Hwc2* locus and genetic heterogeneity of crops planted in small islands are discussed.

Materials and Methods

A total of 191 RI lines have been developed by FUKUTA et al. (1999) from the cross between a temperate Japonica cultivar Akihikari and an Indica cultivar Milyang 23. Preliminary experiments indicated that Akihikari carries the *Hwc2* gene while Milyang 23 carries neither *Hwc1* nor *Hwc2* (Fig. 1). Genotypes of 183 restriction fragment length polymorphism (RFLP) loci which almost cover the entire rice genome have been identified for F_5 individuals, from which each F_6 line was produced by the single seed descent method. Phenol reaction, one criterion to classify cultivars into Indica and Japonica (SATO, 1991), is controlled by a gene *Ph* on chromosome 4 (NAGAO and TAKAHASHI, 1963). Milyang 23, like most Indica cultivars, carries the dominant allele *Ph*, changing hull color into black after soaking in phenol solution. On the other hand, Akihikari, like most Japonica cultivars, carries the recessive allele *Ph*, leaving hull color in a phenol solution unchanged. The genotype of each RI line at the *Ph* locus has already been determined by ICHITANI et al. (2001). The 27 RI lines which had not been examined by ICHITANI et al. (2001) were crossed to the *Hwc1* carrier Jamaica as a pollen donor. Then, they were sown on petri dishes containing 5-mm-deep tap water. They were left in the dark at 28 °C for five days. Next, their roots were observed to find the genotype of RI lines at the *Hwc2* locus. At least six hybrid seedlings were observed in each cross combination. Estimation of the genotype of each RI line and linkage analysis for RFLP, *Ph* and *Hwc2* followed ICHITANI et al. (2001).

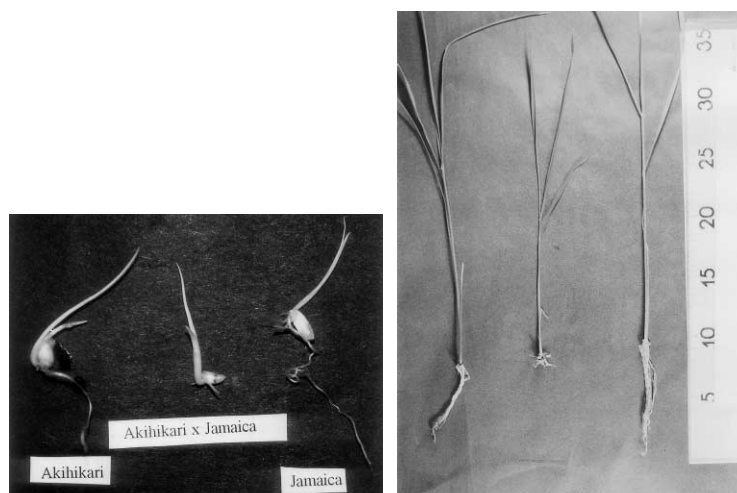


Fig.1. Growth of 'Akihikari' (left), 'Jamaica' (right), and their hybrid (center) seedlings. (a) Five days after sowing date. (b) One month after sowing date.

Results

The 27 RI lines were classified into three types: 10 lines fixed for *Hwc2* allele, 12 lines fixed for the *hwc2* allele, and 5 lines which had not reached fixation. The *Hwc2* gene cosegregated with *ph* gene on rice chromosome 4. Addition of this result to ICHITANI et al. (2001) led to the segregation ratio $58Hwc2Hwc2 : 81hwc2hwc2 : 15Hwc2hwc2$; this ratio deviated somewhat from 15: 15: 2, the expected ratio of gene constitution of F_6 lines bred through single seed descent method ($\chi^2 = 6.87331$, d. f. =2, $0.05 > P > 0.01$). The result of linkage analysis indicated that *Hwc2* and *Ph* were located together between the two RFLP loci, *XNpb264* and *XNpb197*, on the long arm of the chromosome 4. The genetic distance between *Hwc2* and *Ph* was modified by 0.11 cM to 0.49 cM from the 0.6 cM of ICHITANI et al. (2001). On the other hand, the genetic distance between *XNpb234* and *Hwc2* was longer than that in ICHITANI et al. (2001) by 0.15cM; the genetic distance between *Ph* and *XNpb197* was almost unchanged. The four loci, *Hwc2*, *Ph*, *XNpb264*, and *XNpb197*, were rearranged in the order of *XNpb264* - *Hwc2* - *Ph* - *XNpb197*.

Discussion

From a phylogenetic point of view, it is interesting that the two loci *Hwc2* and *Ph*, both of which have been associated with varietal differentiation, are closely linked to each other. Many loci controlling morphological and physiological traits related to adaptability are located near the *Hwc2* and *Ph* loci (ICHITANI et al., 2001). Moreover, recent advances in molecular marker-assisted analyses have shown that the gall midge resistance gene *Gm2* locus (CHAUDHARY et al., 1986; CAUSSE et al., 1994), quantitative trait loci (QTLs) for cool tolerance at the booting stage (SAITO et al., 1995), drought resistance (CHAMPOUX et al., 1995), and the number of large vascular bundles in the peduncle (SASAHARA et al., 1999) were all located near the two loci, even though the linkage between the two

loci and QTLs was indirectly obtained from that between these QTLs and molecular markers linked to *Ph*. These facts suggest that these adaptability-gene combinations, rather than *Hwc2* or *Ph*, might cause or promote varietal differentiation. Genes on the *Hwc2* and *Ph* loci might be mutated and be dragged by surrounding gene combinations.

One indication that the *Ph* - *Hwc2* region may be involved in adaptation is seen in Japanese cultivars. AMEMIYA and AKEMINE (1963) reported that, unlike Japanese lowland cultivars, Japanese upland ones examined up to now carried *hwc2*. On the other hand, ISHIKAWA et al. (1992) reported that many Japanese upland cultivars carry *Ph* gene, though they carry the Japonica type allele at most isozyme loci and are classified as temperate Japonica. There is no information about correspondence between the two studies, but there is some possibility that Japanese upland cultivars conserve the *Ph* - *hwc2* linkage block. Rice is easily infected with blast fungus under the upland condition. To cope with blast, upland cultivars carry blast resistance genes. Linkage between *Ph* and a blast resistance gene(s) was detected in some Japanese upland cultivars (MARUYAMA et al., 1983; HIGASHI and SAITO, 1985; GOTO, 1988). The linkage block containing *Ph* and a blast resistance gene(s) is thought to have derived from exotic cultivars, probably Indica, and have survived introgression with temperate Japonica genetic background. Tight linkage between *Ph* and *hwc2* suggests that the *hwc2* gene may have been dragged by *Ph* and a blast resistance gene(s). A similar linkage block containing a blast resistance gene(s) and an upland-cultivar-specific isozyme gene on chromosome 11 was suggested by ISHIKAWA et al. (1997).

Hybrid weakness genes may be thought to be useless for practical breeding; however, a breeding scheme utilizing such genes is proposed for preventing the spread of transgene and genetic contamination of cultivars (SATO, 1997; SATO and INAMURA, 1989; YONEZAWA et al., 1990; MORISHIMA, 2001). There is a high probability that such potentially useful genes be identified in local cultivars planted in small islands. Moreover, genes can be tools with which origins and routes of crops can be traced. Local cultivars can be useful for ethnology and folklore, too. Skewed geographic distribution of the *Hwc2* gene in rice presented a good opportunity to reevaluate the idea that rice and rice cropping had proceeded to Japan northwards along the Nansei islands or the path on the sea (*kaijo no michi*) proposed by YAGAGITA (1961). WATABE (1993) reported that a traditional method of using cattle to plow paddy fields in the Nansei islands is also seen in insular regions of Southeast Asia. WATABE (1993) also reported that a few native cultivars in the Yaeyama islands looked like Bulu, a group of tropical Japonica native cultivars grown in Indonesia. These findings shed new light on rice diffusion and rice cropping. However, most local cultivars planted in small islands tend to be replaced by improved cultivars and are in danger of extinction. Exploitation and conservation of genetic resources is required for the future.

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