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Distribution of the genes causing F_2 chlorosis in rice cultivars of the Indica and Japonica types

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Summary. Chlorotic plants were segregated in F_2 populations in varietal crosses of common rice. The genetic basis and distribution of the genes causing F_2 chlorosis in native cultivars were studied to examine the role of the F_2 chlorosis in varietal differentiation of rice. It was proven that this F_2 chlorosis was controlled by a set of duplicate genes, *hca-1* and *hca-2*. The *hca-2* gene was widely distributed in native cultivars of the Japonica type, while many Indica types carried its dominant allele *hca-2*$^+$. Japanese cultivar J-147 carried *hca-2*. The *hca-1* gene was frequently distributed in cultivars containing the *Hwc-2* gene for F_1 weakness. We concluded that F_2 chlorosis does not cause or promote varietal differentiation in rice.

Key words: F_2 chlorosis – Reproductive barrier – *Oryza sativa* – Gene distribution – Indica-Japonica differentiation

Introduction

Various reproductive barriers have been found in crosses between distantly related cultivars of common rice (*Oryza sativa* L.). Partial F_1 sterility is commonly observed in various crosses (Oka 1958; Jennings 1966). F_1 weakness (Oka 1957; Amemiya and Akemine 1963), F_2 weakness (Oka 1957; Okuno 1986) and F_2 sterility (Oka 1978; Oka and Doida 1962; Yokoo 1984) have also been reported in various crosses between distantly related cultivars. Three reproductive barriers have been recognized that restrict gene exchange between cultivars and develop particular gene and character associations that characterize varietal groups. The distribution pattern of genes responsible for those reproductive barriers also reflects the phylogenetic relationships of the varietal groups, as reported for F_1 necrosis and chlorosis in wheat (Hermsen 1963; Tsunewaki 1970; Zeven 1980), F_1 lethality in cotton (Stephens 1950), F_1 lethal in *Mimulus gutatus* (Christie and MacNair 1987), F_1 chlorosis (Takahasi et al. 1970) and F_1 weakness (Konishi and Sato 1982) in barley.

A case of F_2 chlorosis in rice was discovered in a cross between two Japanese native cultivars (Sato et al. 1984). This paper attempts to analyze the genetic basis of F_2 chlorosis and survey the distribution of relevant genes in Inica and Japonica type cultivars. Gene distribution was also examined in relation to the distribution of the *Hwc-2* gene responsible for F_1 weakness (Amemiya and Akemine 1963; Sato and Hayashi 1983; Sato and Morishima 1987).

Materials and methods

Genetic basis of F_2 chlorosis

Five F_2 populations derived from crosses of five cultivars of different origins with a tester strain J-147 (a native cultivar from the central region of Japan) were examined. Also observed were F_3 lines derived from 144 randomly taken normal F_2 plants of J-321 × J-147, each consisting of 200 or more plants. Numbers of normal and chlorotic plants in F_2 populations and F_3 lines were recorded 3 weeks after germination.

Distribution of the gene causing F_2 chlorosis

One hundred seventy-one cultivars collected from various localities in Asia were crossed with the test-strain J-147. The F_1 plants were grown in a paddy field. Several panicles of F_1s were bagged at their flowering time to prevent outcrossing. More than 200 selfed seeds of each F_1 plant were sown on a nursery bed and normal and chlorotic seedlings were classified and counted. A portion of those cultivars (111 in total number) were crossed with another tester, Jamaica, having *Hwc-1* (a native cultivar from Peru) to examine whether or not they carried the *Hwc-2* gene causing F_1 weakness.

Classification of cultivars into the Indica and Japonica types

To classify the tested cultivars into Indica and Japonica types three discriminant characters were recorded: phenol reaction (Ph), susceptibility to pottasium chlorate ($KClO_3$ susceptibility, K) and apiculus hair length (Hr). Classification was made by using a discriminant function: Z = Ph + 1.313K −0.82 Hr. For details, the reader may refer to Oka (1958) and Sato et al. (1986).

Results

Symptom of F_2 chlorosis

The first symptom of chlorosis was discoloration of the tips of the second or third leaf. The yellowish part gradually expanded and the whole plant died within 20 days. No chlorotic plants survived to maturity. Therefore, they could not transmit the chlorosis genes to the progeny. No such chlorosis was observed in F_1 plants.

Table 1. Segregation for normal and chlorotic plants in five F_2 populations. Chi-square for heterogeneity = 1.64, df = 4, not significant (ns)

Cross[a]	No. of plants			% of chlorotic plants	Chi-square (15:1)
	Normal	Chlor.	Total		
J-321 × J-147	849	63	912	6.9	0.67 ns
J-164 × J-147	312	20	332	6.0	0.01 ns
Th-19 × J-147	175	11	186	5.9	0.04 ns
La-3 × J-147	289	15	304	4.9	0.90 ns
P-77 × J-147	336	28	394	7.1	0.49 ns

[a] Varietal names were given by accession number in our lab. The symbols J, Th, La and P indicate their collection site as Japan, Thailand, Latin America and the Philippines, respectively

Table 2. Segregation of the chlorotic plants in randomly selected F_3 lines derived from F_2 of J-321 × J-147

	No. of F_3 lines with normal:chlorotic plants				Chi-square (4:4:7)
	15:1	3:1	1:0	Total	
Observed	37	35	72	144	0.69 (ns)
Expected	38.4	38.4	67.2	144.0	

Genetic basis of F_2 chlorosis

Numbers of normal and chlorotic plants observed in five F_2 populations are given in Table 1. The relative frequency of chlorotic segregants varied between 0.047 and 0.071, which fit the expected ratio of 0.0625 (1/16) under the assumption of a duplicate gene system. The segregation pattern of chlorotic plants observed in the F_3 lines of J-321 × J-147 is given in Table 2. F_3 lines showing 15 : 1, 3 : 1 and 1 : 0 ratios were 37, 35 and 72, respectively. A duplicate gene system in which double recessive homozygotes express chlorosis can be adopted to these segregation patterns. When the parental genotypes are assumed to be *AAbb* and *aaBB*, the F_3 lines showing 15 : 1 (*AaBb*), 3 : 1 (*Aabb* and *aaBB*) and 1 : 0 (*A.BB*, *AABb*, *AAbb* and *aaBB*) ratios are expected to be 4 : 4 : 7. The observed frequencies fit this expected ratio (Table 2). Thus, it was asserted that this F_2 chlorosis was controlled by a couple of recessive duplicate genes. According to the rule of gene symbolization given by the Rice Genetics Cooperative, these two recessive genes are symbolized *hca-1* (carried by J-147) and *hca-2* (carried by J-321), respectively (*hc:* hybrid chlorosis, *a*: the first set of duplicate genes, see Sato 1988).

Distribution of hca-1 and hca-2 genes in the Indica and Japonica cultivars

A total of 171 cultivars were tested for *hca-2* or *hca-2*$^+$ carriers according to the presence or absence of chlorotic segregants in respective F_2 populations. Of them, 112 were *hca-2* carriers. The distributions of *hca-2* and *hca-2*$^+$ genes in the Indica and Japonica cultivars as classified by their Z values are given in Table 3. The Z values showed a bimodal feature in the cultivars tested, indicating that the cultivars were divisible into two

Table 3. Frequency distribution of Z value in *hca-2* and *hca-2*$^+$ carriers. Chi-square for the assumption for random combination = 66.5, $P < 0.001$

	Z value										Total
	−1.0	−0.5	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	
	Japonica						Indica				
	1	4	4	1	2	2	8	15	11	8	5	
hca-2$^+$ carrier	12 (36.6)						47 (22.4)				59
	5	24	33	18	10	4	5	4	4	2	3	
hca-2 carrier	94 (69.4)						18 (42.6)				112
Total	106						65				171

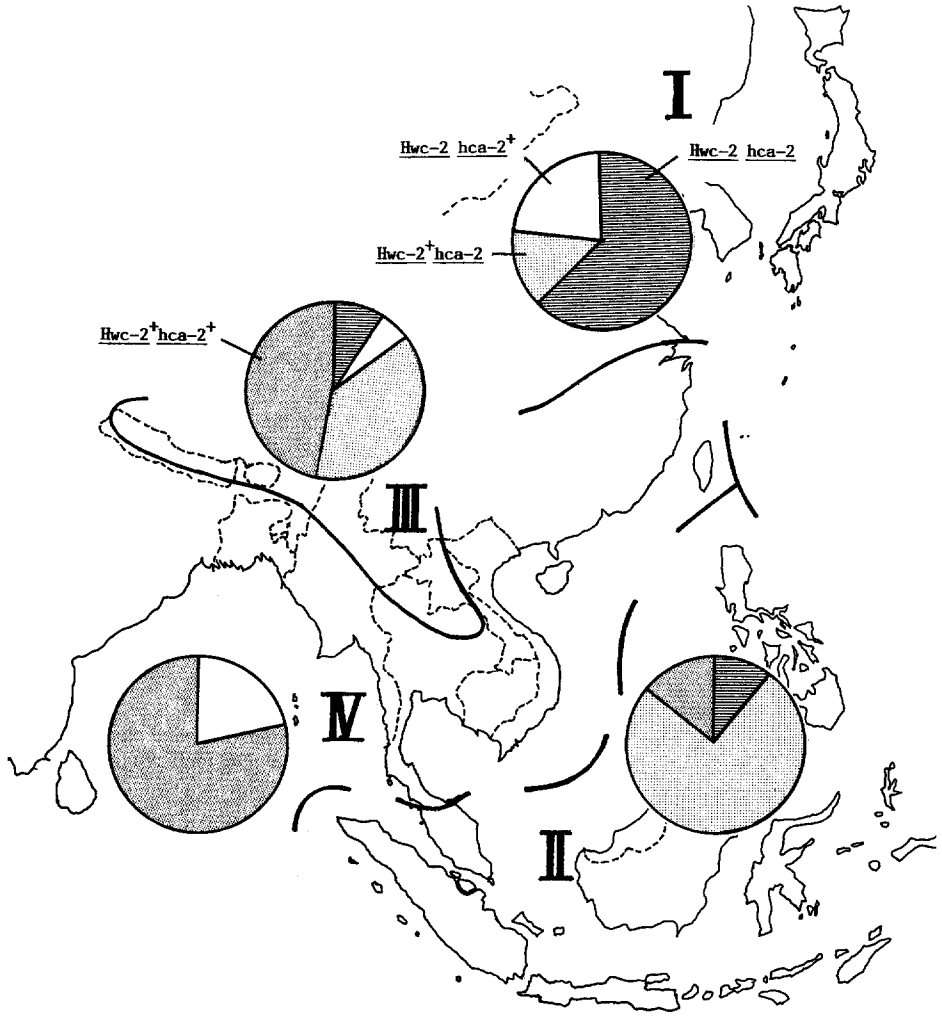

Fig. 1. Geographical distribution of cultivars with four genotypes defined by *Hwc-2* and *hca-2* genes

Table 4. Distribution of *hca-2* gene in the cultivars with *Hwc-2* and *hwc-2* carriers. $\chi^2 = 10.92, p < 0.01$

	Indica cvs with			Japonica cvs with			Pooled		
	Hwc-2	*hwc-2*	Total	*Hwc-2*	*hwc-2*	Total	*Hwc-2*	*hwc-2*	Total
hca-2$^+$	1	31	32	6	1	7	7 (15.1)	32 (23.9)	39
hca-2		3	3	36	33	69	36 (27.9)	36 (44.1)	72
Total	1	34	35	42	34	76	43	68	111

types. We defined the Indica and Japonica types with their Z values greater and not greater than 1.25, respectively. Both the temperate and tropical Japonica types fell in the same range of Z value. Frequency of *hca-2* was much higher in Japonica types (94 out of 106 cultivars) than in Indica types (18 out of 65). The dominant allele *hca-2*$^+$ was predominant in Indica types (47 out of 65) and rare in Japonica types (12 out of 106). Chi-square test proved that the difference in frequency was highly significant ($\chi^2 = 66.5$, $P < 0.001$).

One of the complementary genes causing F_1 weakness, *Hwc-2*, is also frequent in the Japonica and rare in the Indica type (Sato and Morishima 1987). It is known whether most cultivars tested for *hca-2* have *Hwc-2*. Accordingly, cultivars tested were divided into four types: (1) *Hwc-2 hca-2*$^+$, (2) *Hwc-2*$^+$ (*= hwc-2) hca-2*$^+$, (3) *Hwc-2 hca-2* and (4) *Hwc-2*$^+$ *hca-2*. The frequencies of the four genotypes in the Indica and Japonica types are given in Table 4. A majority of the Indica cultivars (32 out of 35) had *Hwc-2*$^+$ *hca-2*$^+$, causing neither F_1

weakness nor F_2 chlorosis in test crosses. The rest consisted of three *Hwc-2*$^+$ *hca-2* carriers and one *Hwc-2 hca-2*$^+$ carrier. Among 76 Japonica cultivars, 36 were *Hwc-2 hca-2* carriers causing both F_1 weakness and F_2 chlorosis that were not found in the Indica type. The rarest, *Hwc-2*$^+$ *hca-2*$^+$, was most frequent in the Indica type. In Table 4, the frequencies of the four genotypes expected from random combination of alleles at the two loci are shown in parentheses. Two types, *Hwc-2*$^+$ *hca-2*$^+$ and *Hwc-2 hca-2*, which are predominant in the Indica and Japonica types, respectively, were more frequent than expected values, while *Hwc-2 hca-2*$^+$ and *Hwc-2*$^+$ *hca-2* were less than expected. Chi-square tests showed that the difference between observed and expected frequencies was significant. This indicates that *hca-2* tended to associate with *Hwc-2* in the Japonica type.

A tester line Taichung 65 (T65), which has been frequently used for various test crosses, was found to be an *hca-2* carrier. The occurrence of F_2 chlorosis has not been reported in crosses with T65 except for a cross with J-147. This suggests that *hca-1* found in J-147 is a rare allele, although no particular crosses with a *hca-2* carrier were made.

Geographical distribution of the four genotypes based on Hwc-2 and hca-2 loci

Geographical distribution of the four genotypes, *Hwc-2 hca-2*, *Hwc-2*$^+$ *hca-2*, *Hwc-2 hca-2*$^+$ and *Hwc-2*$^+$ *hca-2*$^+$, is shown in Fig. 1. In this figure Asia is divided into four regions: (1) Japan, Korea and northern China, (2) the Philippines, Malaysia and Indonesia, (3) Northern Laos and Thailand, upper Burma, Assam and Sikkin of India, Bhutan and Nepal, and (4) the area from India proper to southern China and Taiwan.

In regions 1 and 2 *hca-2* carriers were predominant. Carriers of *Hwc-2* were predominant in region 1. Cultivars with *hwc-2 hca-2*$^+$ were most frequent in region 4. Region 3 showed a high diversity of all four genotypes.

Discussion

Post-mating reproductive barriers reported so far in common rice are due to either interlocus interaction or interallelic interaction, except for cytoplasmic-genic sterility. The genes responsible for the barriers, especially for F_1 sterility, are considered to be linked with marker genes whose frequencies differ between the Indica and Japonica types, e.g., glutinous endosperm (*Wx/wx*), red pericarp (*Rc/rc*), and antocyanin pigmentation (*A/a*) (Oka 1974). The roles of these barriers in varietal differentiation have attracted the interest of rice scientists.

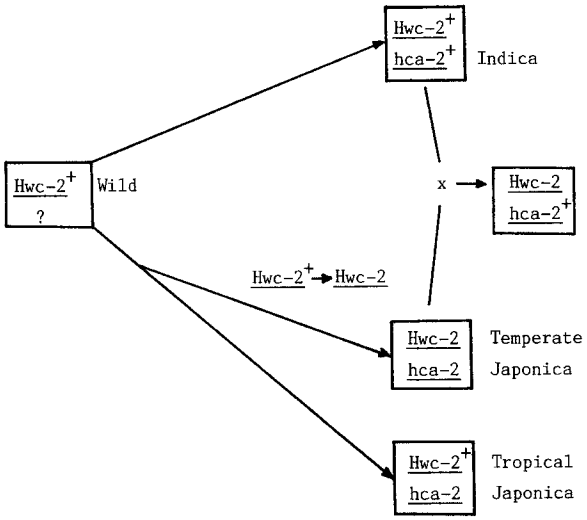

Fig. 2. Phylogenesis of the Indica and Japonica types based on *Hwc-1* and *hca-2* loci

If a reproductive barrier plays an effective role or promotes Indica-Japonica differentiation by restricting gene exchange, the two complementary or duplicate genes causing a barrier must be separately distributed in the two varietal groups. In the present F_2 chlorosis, *hca-1* is a rare allele while *hca-2* was frequent in the Japonica type. Such a low frequency of one complementary or duplicate gene set also occurs in F_1 weakness in rice (Oka 1957; Amemiya and Akemine 1963), in F_1 chlorosis (Takahashi and Hayashi 1972) and in F_1 weakness in barley (Konishi and Sato 1982). One of the genes for F_2 weakness (Oka 1957; Okuno 1986) also seems to be infrequent. These rare alleles are recently born by mutation. The reproductive barriers mentioned above would not cause or promote Indica-Japonica differentiation. The distribution pattern of the genes causing these reproductive barriers may suggest phylogenetic relationships of varietal groups developed by other evolutionary forces. Preferential distribution of *hca-2* in the Japonica type suggests that this gene was born in the primitive Japonica type no later than the time it evolved from its ancestors. The distribtuion of *hca-2* in wild strains must be surveyed to learn more about this relationship.

In the case of F_1 weakness, the distribution pattern of the *Hwc-2* gene in wild and cultivated strains suggested its origin to be in an early stage of differentiation of the temperate Japonica type (Sato and Morishima 1987). Combining this and the present data, we can assume differentiation of the Indica and Japonica types as illustrated in Fig. 2. The primitive Japonica types were differentiated from wild ancestors

accompanying an F_2 chlorosis gene *hca-2*. Then, *Hwc-2* gene was born in the primitive Japonica types. It is reasonable to consider that the *Hwc-2 hca-2*$^+$ type was derived from hybridization between the Indica type (*Hwc-2*$^+$ *hca-2*$^+$) and *Hwc-2 hca-2* types, although the time and place have not been specified.

Okuno (1986) reported a set of duplicate genes causing F_2 weakness and their distribution in Asian cultivars. However, their distribution in the Indica and Japonica types was not sufficiently surveyed. There remains some other reproductive barriers (including hybrid sterility) to be analyzed regarding genetic basis and the distribution of genes. Allelism tests and gene surveys will enable us to further understanding differentiation of rice cultivars by use of reproductive barriers as reflectors.

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