Difference in Recombination Frequency between Chromogen (*C*) and Waxy (*wx*) Genes in Japonica and Indica Rice (*Oryza sativa* L.) by Using Japonica Lines with a Wide-compatibility Gene (*S-5ⁿ*)

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Recombination frequency between *C* (Chromogen) and *wx* (glutinous endosperm) has long been known to be around 23% in Japonica varieties. But the frequencies were 15.1~18.9% in the cross of Japonicas and Ketan Nangka (KN). KN is Javanica and donor of a wide-compatibility gene, a neutral allele for gamete abortion, *S-5ⁿ*, which was located between *C* and *wx*. The recombination frequency between *C* and *wx* was about 30% in the cross of KN and Indica variety, IR36. Japonica breeding lines, Nekken 1 (Norin PL 9) and Nekken 2 were developed by incorporating the *C-S-5ⁿ* segment from KN by backcrossing with Japonica cultivars two and three times, respectively. The variation of recombination frequencies was tested with these breeding lines instead of KN. In the cross of Miyukimochi (Japonica) and Nekken 1, the recombination frequency was $16.1 \pm 4.8\%$. Whereas, the frequency was $30.3 \pm 6.4\%$ in Nekken 2/IR36. Thus, the differential recombination frequencies found in KN/Japonica and KN/IR36 were also found in the crosses where KN is replaced by Japonica breeding lines into which the *C-S-5ⁿ* segment of KN was incorporated. In the evaluation of recombination frequencies, the effect of *S-5ⁿ* and *C* was confirmed. The fact that recombination frequency is affected by a genetic factor in the chromosomal segment of *C-S-5ⁿ* will be of interest in studies of recombination mechanism and in constructing molecular maps of rice.

KEY WORDS: *Oryza sativa*, recombination frequency, gamete abortion, wide corss.

Introduction

Hybrid sterility in wide crosses of rice had been a major barrier for breeders to utilize Indica-Japonica crosses. But a solution for this was proposed by IKEHASHI and ARAKI (1986). They reported a multiple allele locus in chromosome 6, at which an allelic interaction of two alleles out of three, i.e. *S-5ⁱ* from Indica, *S-5^j* from Japonica and *S-5ⁿ* from Javanica varieties, confers female gamete abortion which is revealed as semi-sterility of spikelets. Female gametes carrying *S-5^j* were aborted in the genotype of *S-5ⁱ/S-5^j*, while a neutral allele *S-5ⁿ* did not cause the abortion in the heterozygotes of *S-5ⁱ/S-5ⁿ* and *S-5^j/S-5ⁿ*. Since then, the *S-5ⁿ* allele has been used to overcome the hybrid sterility (ARAKI *et al.* 1989).

For incorporating the neutral allele into Indica or Japonica lines a marker gene *C* (Chromogen) is utilized, as the *S-5* is located between *C* and *wx* (glutinous endosperm) with a recombination frequency of 4~6% for *C* and *S-5* (IKEHASHI and ARAKI, 1988). But precise determination of the locus was not always easy, because the sterility shows a continuous variation due to environmental effects and some minor genic effects. It was also found that the recombination frequency between *C* and *wx* showed significant variation in wide crosses (IKE-

Received March 20, 1991.

Accepted November 19, 1991.

HASHI and ARAKI, 1986). This fact is interesting, because the recombination frequency for *C* and *wx* in Japonica crosses has long been known to take stable value with a mean of 23% (KINOSHITA, 1990). If a genetic or cytological factor which controls recombination frequency is found, it can be a tool for a further study on recombination mechanisms. And such a variation of recombination frequency in wide crosses should also be considered in molecular marker mapping of rice.

In this paper we report the variation of the recombination frequencies between various marker genes linked with *S-5* locus. In particular, we tested the effect of *S-5ⁿ* from a Japonica variety, Ketan Nangka (KN) on the recombination frequency. We found that a chromosomal segment of *C-S-5ⁿ* incorporated from KN into Japonicas was associated with significant variation of recombination frequencies.

Material and Methods

The varieties used and their marker genes linked with *S-5ⁿ* are listed in Table 1. The isozyme locus was determined by starch gel electrophoresis according to ISHIKAWA *et al.* (1989). The locus of *wx* was determined by SDS-PAGE according to SANO (1984) as the parent varieties are differentiated by *Wx^a* and *Wx^b* allele.

KN does not show hybrid sterility in its cross to Indica varieties such as IR26, IR36 and IR50 as well as Japonica varieties, such as Akihikari (AK), Nihonmasari (NM) and Miyukimochi (MM) which is a glutinous variety. This character of KN is due to the neutral allele, *S-5ⁿ* at *S-5* locus and so, varieties which has *S-5ⁿ* are called wide-compatibility varieties (WCV) (IKEHASHI and ARAKI, 1986). New breeding lines which possess *C* and *S-5ⁿ* from KN in the genetic background of Japonica were also tested instead of KN. One of the breeding lines Nekken 1 was selected from AK//KN/NM and the other Nekken 2 was from AK/3/AK//KN/NM.

Table 1. Varieties tested and their marker genes

Variety or line	Marker gene loci ²⁾				
	<i>wx</i>	<i>C</i>	<i>alk</i>	<i>Pgi-2</i>	<i>S-5</i>
Akihikari (AK)	<i>Wx^b</i>	<i>C⁺</i>	<i>alk</i>	<i>Pgi-2¹</i>	<i>S-5ⁱ</i>
Miyukimochi (MM)	<i>wx</i>	<i>C⁺</i>	<i>alk</i>	<i>Pgi-2¹</i>	<i>S-5ⁱ</i>
Nekken 1 ¹⁾	<i>Wx^b</i>	<i>C</i>	<i>alk</i>	<i>Pgi-2¹</i>	<i>S-5ⁿ</i>
Nekken 2 ¹⁾	<i>Wx^b</i>	<i>C</i>	<i>alk</i>	<i>Pgi-2¹</i>	<i>S-5ⁿ</i>
IR26	<i>Wx^a</i>	<i>C⁺</i>	<i>Alk</i>	<i>Pgi-2²</i>	<i>S-5ⁱ</i>
IR36	<i>Wx^a</i>	<i>C⁺</i>	<i>Alk</i>	<i>Pgi-2²</i>	<i>S-5ⁱ</i>

¹⁾ *C* and *S-5ⁿ* were incorporated from Ketan Nangka (KN)

²⁾ *wx*: waxy, *C*: chromogen, *alk*: alkali degeneration, *Pgi-2*:phosphoglucose isomerase, *S-5*: wide-compatibility.

The recombination frequencies between the marker genes were evaluated by a method of three way cross, while monitoring the effect of alleles at *S-5ⁿ* on spikelet fertility. According to IKEHASHI and ARAKI (1986), the effect of *S-5ⁿ* on spikelet fertility is visible by the three way crosses, namely Japonica/WCV//Indica or Indica/WCV//Japonica. While no spikelet sterility occurs in the first single cross, fertile plants and semi-sterile plants may segregate in the *F*₁ of the three way crosses. Then, those genotypes with *S-5ⁿ* would show a high frequency of fertile plants and those with an allele opposite to *S-5ⁿ* would show a low frequency of fertile ones. By the three way crosses it had been found that the recombination frequency between *C* and *wx* differed significantly between KN/IR36 and KN/Japonica.

In this experiment, we tested three way crosses of Nekken 1/MM/IR26 and Nekken 2/IR36//AK respectively, in 1989 and 1990. The two Indica varieties were known to show an identical behavior in terms of hybrid sterility. In the experiment about 200 plants were tested in each cross.

Results

Recombination frequency for *C*—*wx*

Recombination frequencies between *C* and *wx* measured in the crosses of KN and Japonica were 15.1 and 18.9% (Table 2). In this experiment the value was $16.1 \pm 4.8\%$ in MM/Nekken 1 (Table 2). The recombination frequencies between *C* and *wx* in the crosses of KN/IR36 ranged from 22.6 to 34.4% in the past experiments (Table 2). A similar value was obtained through male gamete formation in the cross of Kochihibiki//IR36/KN. In this experiment the frequency was $30.3 \pm 6.4\%$ in Nekken 2/IR36 (Table 2). Thus, the different recombination frequencies which had found between KN/IR36 and KN/Japonica were confirmed in 1989 and 1990. It was carried out by using Japonica lines into which the segment of *C*—*S-5ⁿ* was transferred from KN into Japonica.

Table 2. Recombination frequency of *C* and *wx*

Cross		Frequency (%)	No. of plants tested
Carriers of <i>S-5ⁿ</i> /Japonica			
Akihikari (AK)/Ketan Nangka (KN)//IR36	(1983) ³⁾	15.1 ± 9.6	53
KN/AK//Penuh Baru II ¹⁾	(1985) ⁴⁾	18.9 ± 7.3	111
Miyukimochi (MM)/Nekken 1//IR26	(1989)	16.1 ± 4.8	225
Carriers of <i>S-5ⁿ</i> /Indica			
IR36/KN/AK	(1983) ³⁾	32.1 ± 13.1	49
IR36/KN//Achar Bhog ²⁾	(1985)	27.5 ± 9.8	80
KN/IR36//Penuh Baru II	(1985) ⁴⁾	22.6 ± 7.6	115
Kochihibiki//IR36/KN	(1981) ³⁾	34.4 ± 11.4	67
Nekken 2/IR36//AK	(1990)	30.3 ± 6.4	198

¹⁾Penuh Baru II: Indonesia, ²⁾Achar Bhog: India, ³⁾IKEHASHI and ARAKI, 1986, ⁴⁾Ibid., 1988

Recombination frequencies between other marker genes

In the cross of Nekken 2/IR36//AK, recombination values among the four marker genes were evaluated (Fig. 1). It was indicated that the recombination frequencies were higher in this cross than those reported in ordinary Japonica crosses. The recombination frequency for *C* and *alk* was 21.6% while the frequency in ordinary crosses of Japonica varieties is about 11.0%.

The effect of *S-5ⁿ* on spikelet fertility

The progeny plants of the three way crosses were classified according to their marker genotypes, and the distribution of spikelet fertility was compared between the genotypes. The plants with those markers linked closely to *S-5ⁿ* showed higher fertility than those carrying opposite alleles (Table 3 and 4). The plants possessing *C* generally showed high fertility with some exceptions which seemed to be recombinants with *C* and the opposite allele of *S-5ⁿ*. The fact that the plants with *C* showed the highest spikelet fertility indicated the close linkage between *S-5ⁿ* and *C*. In the cross of MM/Nekken 1//IR26, the spikelet fertility was clearly differentiated by presence or absence of *C*. In the cross of Nekken 2/IR36//AK, the same difference was observed but not clear. In the latter cross, it was more difficult to determine the linkage between *C* and *S-5ⁿ* than it was in the former cross. The reason for this may be the higher recombination frequency between *C* and *S-5ⁿ* in the latter cross.

Discussion

SANO (1990) reported that an alien chromosomal segment containing a gamete-abortion gene *S-1* introduced from *Oryza glaberrima* into an Asian cultivated rice significantly depressed recombination values. He assumed that the chromosomal segment containing *S-1* caused the restriction of recombination around *S-1* itself and that the loci flanking to *S-1* tend to behave as a gene complex in the interspecific hybrid. KINOSHITA (1990) reviewed past data on recombination frequencies between *C* and *wx*, and indicated that there were remarkable variations in the values with an average of $22.9 \pm 0.51\%$. According to him there was a report by JODON who obtained the value of 32.2% in 1,129 F_2 plants.

In this experiment the recombination frequency was 16.1% in the cross between the carrier of *S-5ⁿ* and Japonica, while the frequency was 30.3% in the cross between the carrier of *S-5ⁿ* and Indica. It is of interest that the mean of 16.1% and 30.3% becomes 23.2%

Fig. 1. Linkage map for the markers located on chromosome 6 in the cross of Nekken 2/IR36//Akihikari

Table 3. Distribution of spikelet fertilities classified by marker genotypes in Miyukimochi/Nekken 1/IR26 (1989)

Genotype	Number of plants in spikelet fertility											Mean spikelet fertility(%)	
	-50	-55	-60	-65	-70	-75	-80	-85	-90	-100%	Total		
<i>C/C⁺</i>	5	2	3	2	6	14	19	18	16	15	100	77.9**	
<i>C⁺/C⁺</i>	16	15	24	29	21	15	3	1		1	125	60.4	
<i>wx/Wx^a</i>	17	13	19	25	19	11	7	4		2	117	61.2**	
<i>Wx^a/Wx^b</i>	4	4	8	6	8	18	15	15	16	14	108	75.7	
<i>C/C⁺ wx/Wx^a</i>	3	1	1				4	3		2	14	68.5	
<i>C/C⁺ Wx^a/Wx^b</i>	2	1	2	2	6	14	15	15	16	13	86	79.5	
<i>C⁺/C⁺ wx/Wx^a</i>	14	12	18	25	19	11	3	1			103	60.2	
<i>C⁺/C⁺ Wx^a/Wx^b</i>	2	3	6	4	2	4				1	22	61.1	

**Signification at 0.01.

Table 4. Distribution of spikelet fertilities classified by marker genotypes in Nekken 2/IR36//Akihikari (1990)

Genotype	Number of plants in spikelet fertility											Mean spikelet fertility(%)	
	-30	-40	-50	-60	-70	-80	-90	-100%	Total				
<i>Wx^b/Wx^b</i>	1	1	2	7	14	33	23	9	90	74.8**			
<i>Wx^a/Wx^b</i>	1	1	6	15	33	25	21	6	108	69.0			
<i>C/C⁺</i>	0	0	0	5	20	32	44	19	120	79.4**			
<i>C⁺/C⁺</i>	3	3	14	23	38	41	11	0	134	63.9			
<i>Pgi-2¹/Pgi-2¹</i>	0	0	0	4	13	18	29	14	78	79.5**			
<i>Pgi-2¹/Pgi-2²</i>	3	2	10	14	23	31	11	2	96	65.1			
<i>alk/alk</i>	0	1	1	6	19	30	32	16	105	77.5**			
<i>Alk/alk</i>	3	2	13	22	40	43	23	3	149	66.7			

**Significant at 0.01.

and very close to the average in Japonica crosses. The low and high values, respectively, in this experiment seem to deviate from the overall average by a definite length.

The reduction of the recombination frequency from the overall mean of 22.9 to 16.1% can be attributed to genetic function of the *C-S-5ⁿ* segment from KN by the reason proposed by SANO (1990). In fact, the recombination frequency between *C* and *S-5ⁿ* was close to 6% (IKEHASHI and ARAKI, 1988). On the other hand, the segment did not appear to lower from

the recombination frequency in the cross of Indica, where the value was close to the highest of the past records. But, whether the recombination frequency of 30.3% is lower than that in Indica/Indica crosses or not can not be precisely answered, because of the lack of *wx* allele in standard Indica varieties. And the recombination frequency in Indica/Japonica cross is also not available due to hybrid sterility. Though the reason for increase of recombination value in the crosses between WCV to Indica is not clear, at least it can be concluded that the alien chromosomal segment was associated with differential of recombination frequencies. Such a segment containing a gamete abortion locus may also possess some factor for recombination frequency.

Hybrid sterility in rice has long been conceived as a consequence of structural chromosomal differences among isolated subspecies leading to irregular pairing and unequal division (CHANDRARATNA, 1964). The variable recombination frequency found in this experiment suggests such an irregular chromosome pairing. But the assumption may not apply to this case, because in our crosses it was of female gamete rather than male gamete that was related to the markers in the *C-wx* segment. No segregation distortion of the markers for *S-5* was found via the male gamete.

Further study on a genetic factor for recombination frequency may provide us with a tool to study the recombination mechanism. Such variation of recombination frequency will also be interesting in constructing detailed gene maps of rice using restriction fragment length polymorphism (RFLP).

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日本型イネの雑種不稔緩和遺伝子 ($S-5^n$) 導入系統に日本型とインド型品種を交雑した場合のアントシアン色素原遺伝子 (C) ともち遺伝子 (wx) の組換え価の差異

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イネの染色体6におけるアントシアン色素原遺伝子 (C) ともち遺伝子 (wx) の間の組換え価は、日本型稲においてはおよそ23%であることが知られている。しかし、著者らの過去の実験では、ジャワ型イネの Ketan Nangka (KN) と日本型品種の雑種では、この組換え価が15.1-18.9%となり、KN とインド型イネの IR36との雑種ではこの組換え価がおよそ30%となった。KN はインド型イネと交配しても日本型イネと交配しても雑種不稔を生じない。KN のこの特性は、日印交配の F_1 で雑種不稔を生ずる遺伝子座 $S-5$ において、配偶子の排除を起こさない雑種不稔緩和遺伝子 $S-5^n$ を持つためであることが明らかにされている。すなわち、 C と wx の間にある $S-5$ 座では、インド型イネは対立遺伝子 $S-5^i$ 、日本型イネは対立遺伝子 $S-5^j$ を保有し、 $S-5^i/S-5^j$ のヘテロでは $S-5^j$ を持つ配偶子が排除されて、半不稔を示すが、 $S-5^i/S-5^n$ および $S-5^j/S-5^n$ では配偶子の排除が起こらないことが明らかにされている (IKEHASHI and ARAKI, 1986)。日印交配における雑種不稔を解決するため、KN から C を標識として $S-5^n$ を導入した日本型品種の熱研1号 (中間母本農9号) と同2号が育成された。前者は KN に日本型イネを2回、後者は3回交配して育成されたもので、KN からの C および $S-5^n$ を保有している。

本報告では、熱研1号および同2号が、KN と同様に日本型あるいはインド型品種と交配したときに異なった組換え価を示すかどうかを検討した。その結果、みゆきもち/熱研1号//IR26では $C-wx$ の組換え価として $16.1 \pm 4.8\%$ 、熱研2号/IR36//アキヒカリでは、それが $30.3 \pm 6.4\%$ であった (Table 2)。この結果から、KN の染色体6の $C-S-5^n$ の部分が、日本型品種に導入された後でも $C-wx$ の組換え価を変動させるものと推定された。なお、ここで得られた組換え価、 16.1% と 30.3% の平均は 23.2% で日本稲間の通常値に等しいことが注目された。

次に、三品種交配実験のみゆきもち/熱研1号//IR26と、熱研2号/IR36//アキヒカリの後代において、 $S-5^n$ と連鎖している標識遺伝子を持つ個体群と持たない個体群に分けて稔性の分布を調査した (Table 3, 4)。その結果、 $S-5^n$ と密接に連鎖している C を持つ個体、 C を持たない個体より概して稔性が高かった。 C を持っていない稔性が低いか、あるいは C を持たないのに稔性が高い個体は組換え体と判定された。 wx 座についても同様の結果が得られた。こうした組換え体と思われる個体は、みゆきもち/熱研1号//IR26では比較的良好に判別されたが、熱研2号/IR36//アキヒカリでは判別が困難であった。このことは後者の組合せで全般的に組換え頻度が高いことによると推察された。熱研2号/IR36の組合せでは、 $S-5$ 座の近傍の標準遺伝子間の組換え価も通常よりも高いと認められた (Fig. 1)。またこの組合せでは、 C 座と同様にホスホグルコースイソメラーゼ ($Pgi-2$) 遺伝子座も稔性と関係し、 $S-5$ 座の近傍にあることが示唆された。以上のように C および $S-5^n$ の効果は確認されたが、 $S-5$ 座と関連標識遺伝子の組換え価の推定は、稔性の分布が連続的であったので保留した。

結論として、KN に由来する $S-5$ 座を含む領域には組換え価を変動させる遺伝的、あるいは細胞学的要因が含まれているものと推定された。このように組換え価を変動させる要因は組換えの機構に関する研究においても、また現在進められている制限酵素断片長多型 (RFLP) マーカーによる染色体地図の作成においても考慮されるべきであろう。