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Linkage Studies in Rice (*Oryza sativa* L.) On Some Mutants for Physiological Leaf Spots

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Nine of the physiological leaf spot mutants were described and investigated linkage relation with marker genes. All of them were governed by single recessive genes respectively and the causal genes were designated as a series of *spl* (spotted leaves) as *spl*₁, *spl*₂, ..., *spl*₉.

With eight of them except for *spl*₉, the linkage groups were determined as follows: *spl*₁—a linkage group corresponding to A type of trisomics which never corresponded to any of twelve linkage groups published by Nagao and Takahashi, @&-linkage group X, *spl*₃—XI, *spl*₄—I, *spl*₅—IV, *spl*₆—III, *spl*₇ and *spl*₈—IX.

INTRODUCTION

Several mutants for physiological leaf spots, which is characterized by reddish or blackish brown spots and discolorations of leaves, stems and sometimes glumes were reported in rice and causal genes of them have been designated as a series of *bl* (Jones, 1952; Nagao and Takahashi, 1963; Nagao et al., 1964; Takahashi et al., 1968).

On the other hand, the authors reported some mutants showing physiological leaf spots and designated the causal genes as a series of *spl* (spotted leaves) in order to avoid the confusions of them with the *bl* mutants reported previously and to include any mutants for physiological leaf spots into a series of gene symbol regardless of the color of leaf spots. Namely, they are *spl*₁ (Iwata and Omura, 1975), *spl*₂ (Omura and Iwata, 1972) and *spl*₃, *spl*₄ and *spl*₅ (Iwata and Omura, 1977).

This paper deals with nine mutants of the physiological leaf spots including these.

MATERIALS AND METHODS

The mutants used are shown in Table 1. All of them are governed by single recessive genes, respectively.

Two mutants, *spl*₁ and *spl*₂, are originated from spontaneous mutation. Four, *spl*₃, *spl*₄, *spl*₅ and *spl*₇, were induced in the gamma-field of Institute of Radiation Breeding, National Institute of Agricultural Science, and *spl*₉ is also a induced mutant by irradiation and was introduced from Division of Genetics, National Institute of Agricultural Science. Two, *spl*₆ and *spl*₈, were

Table 1. List of physiological leaf spot mutants used and their causal genes.

Strain number	Original variety	Source	Gene symbol
HO 698	Banshinriki-byogata ¹⁾	Spontaneous	<i>spl</i> ₁
HO 696	Katsumonbyo ¹⁾	"	<i>spl</i> ₂
M 41	Norin 8	r-ray (chronic)	<i>spl</i> ₃
M 114	"	"	<i>spl</i> ₄
M 87	"	"	<i>spl</i> ₅
CM 20	Kinmaze	Chemicals	<i>spl</i> ₆
M 64	Norin 8	r-ray (chronic)	<i>spl</i> ₇
CM 207	Kinmaze	Chemicals	<i>spl</i> ₈
LT 26	Norin 8	r-ray (acute)	<i>spl</i> ₉

¹⁾ Not original variety but name of mutant line.

induced by a chemical mutagen, N-nitroso-N-methylurea, at Kyushu University.

The phenotypic characteristics of the mutants are as follows.

*spl*₁: Large reddish brown spots on leaves and stems, of which appearance begins in the seedling stage and continues to heading time.

*spl*₂: Partial discoloration of leaves and stems. It appears from the seedling stage, but it is not so obvious in this stage and become more distinct in the tillering stage. Somewhat poor viability,

*spl*₃, *spl*₅ and *spl*₇: Relatively small reddish brown spots scattering over the whole surface of leaves. They appear from tillering stage to heading time. Their phenotypes are so resemble that it is difficult to distinguish each other.

*spl*₄: Relatively large reddish brown spots scattering on leaves, but not so much spots as *spl*₃, *spl*₅ and *spl*₇.

Table 2. List of marker genes and their linkage groups.

Linkage group	Gene symbol	Character	Reference
I	<i>wx</i> <i>dp</i> ₁	waxy endosperm depressed palea 1	Nagamatsu and Omura (1962) "
III	<i>eg</i> <i>lax</i> <i>d</i> A"	extra glume lax panicle tillering dwarf anthocyanin activator	Iwata and Omura (1971a) " " Nagao and Takahashi (1963)
IV	<i>d</i> ₆ <i>g</i> <i>Rc</i>	lop-leaved dwarf long empty glumes brown pericarp	" " "
IX	<i>al</i> _{K-2} <i>nl</i> ₁ <i>ri</i>	albino Kyushu-2 neck leaf 1 verticillate arrangement of rachis	Iwata and Omura (1978) Nagao and Takahashi (1963) "
X	<i>d</i> _w <i>gh</i> ₂	"Waisei-shirasasa" dwarf gold hull 2	Iwata and Omura (1971b) "
XI	<i>bc</i> ₁ <i>dl</i>	brittle culm 1 drooping leaf	" "

*spl*₆: Relatively large reddish brown spots on leaves and it is similar to *spl*₄.

*spl*₈: Fine striped spots of reddish brown on whole surface of leaves, it appears after tillering stage.

*spl*₉: Small blackish brown spots on leaves and stems but not so thick, and it appears after heading time.

The mutant strains were crossed with linkage testers having marker genes shown in Table 2, and the linkage relations were tested in F₂. In some cases, doubly recessive plants obtained from the above crosses were used for crossing. When the linkage was detected, the recombination value was estimated from the segregations in F₂ and in some cases in F₃ progenies by the method of maximum likelihood. The weighted mean was calculated when the recombination values were estimated from F₂ in both phase and F₃.

RESULTS AND DISCUSSION

*spl*₁: As shown in Table 3, the *spl*₁ exhibited trisomic segregation in a cross with A type of trisomics (Iwata and Omura, 1975). Namely, a observed ratio of normal to *spl*₁ in F₂ derived from trisomic F₁ plants was about 13: 1 that fitted well to a theoretical ratio of trisomic segregation being between 44: 1 and 3: 1. On the other hand, a segregation of F₂ derived from disomic F₁ plants of the same cross fitted well to 3: 1 ratio. So, it is concluded that the *spl*₁ composes a linkage group with three genes, *rl*₁, *d*_B and *nal*₂, reported previously, though the group does not correspond to any of the groups published by Nagao and Takahashi (1963).

Table 3. Trisomic and disomic segregations of *spl*₁ in F₂ of cross with A type of trisomics.

F ₁ plants	Observed number			χ^2 for 3 1	Ratio of domi.: rece.	
	Dominant	Recessive	Total		Theoretical	Observed
Trisomic	440	33	473	81.946***	8: 1-44: 1	13.3: 1
Disomic	365	118	483	0.084	3: 1	

*** Significant at 0.1% level.

*spl*₂: A linkage relation was observed between *spl*₂ and *gh*₂. From F₂ data shown in Table 4, the recombination values of *spl*₂-*gh*₂ were estimated at 17.5% from coupling phase, 15.2 % from repulsion phase and thus weighted mean of 16.8 % was obtained from them. It has been proved that *gh*₂ locates on the chromosome 8 corresponding to the linkage group X by the translocation method and that *gh* links with *d*_w with intensity of 31.2 % (Iwata and Omura, 1971 b). However, a clear linkage relation was not observed between *spl*₂ and *d*_w, showing the recombination value of 47.8%. Therefore, the sequence of the three genes at the map of the linkage group X may be *spl*₂-*gh*₂-*d*_w (Fig. 1).

Table 4. Linkage relations between *spl*₂ and genes belonging to the linkage group X.

Gene pair	Phase	No. of cross	Segregation mode in F ₂					Recombination value (%)	χ^2_{13}
			AB	Ab	aB	ab	Total		
<i>spl</i> ₂ - <i>gh</i> ₂	coup.	2	246 (244.6)	28 (29.2)	30 (29.2)	61 (62.4)	365	17.5±2.2	0.098
	Rep.	5	446 (421.8)	198 (203.7)	186 (203.7)	4 (8)	834	15.2±3.4	3.218
	Weighted mean							16.8±1.9	
<i>spl</i> ₂ - <i>d</i> _w	coup.	4	391 (387.0)	120 (123.8)	125 (123.8)	45 (46.5)	681	47.8±2.8	0.216
	Rep.	2	206 (198.4)	58 (68.6)	74 (68.6)	18 (20.4)	356	47.8±4.1	2.636
	Weighted mean							47.8±2.3

Fig. 1. Linkage map of the group X.

*spl*₃: The linkage relations were found in F₂ of crosses between *spl*₃ and such two genes as *dl* and *bc*, belonging to the linkage group XI. From F₂ data shown in Table 5, the recombination values of *spl*₃-*dl* were estimated at 15.8 % in coupling phase, 19.1% in repulsion phase and thus their weighted mean of 16.5%. The recombination value of *spl*₃-*bc*₁ was estimated at

Table 5. Linkage relations between *spl*₃ and genes belonging to the linkage group XI.

Gene pair	Phase	No. of cross	Segregation mode in F ₂					Recombination value (%)	χ^2_{13}
			AB	Ab	aB	ab	Total		
<i>spl</i> ₃ - <i>dl</i>	coup.	7	941 (914.1)	103 (98.4)	88 (98.4)	218 (239.1)	1,350	15.8±1.1	3.968
	Rep.	10	1,183 (1,139.0)	506 (539.5)	531 (539.5)	0	2,238	19.1±2.0	3.058
	Weighted mean							16.5±1.0	
<i>spl</i> ₃ - <i>bc</i> ₁	Rep.	1	101 (99.4)	28 (37.1)	45 (37.1)	8 (8.4)	182	43.0±6.0	3.960

Fig. 2. Linkage map of the group XI.

*spl*₄: Intimate linkage relations were observed between *spl*₄ and such two genes as *dp*₁ and *wx* belonging to the linkage group I. As shown in Table 6, linkage intensities of *spl*₄-*dp*₁ were estimated from F₂ in coupling phase and two kinds of F₃ lines from F₂ plants in repulsion phase showing such phenotypes as *spl*₄⁺*dp*₁ and *spl*₄*dp*₁⁺ at 3.0 %, 0.8 % and 3.4 %, respectively. The weighted mean was calculated at 2.5 % from these values. The linkage intensity of *spl*₄-*wx* was also estimated from F₂ in repulsion phase at 2.3 %. The linkage relation between *dp*₁ and *wx* with intensity of 2.2 % have been recognized previously (Nagamatsu and Omura, 1962). Therefore, it is obvious that the three genes, *spl*₄, *dp*₁ and *wx*, are nearly located each other at the map of the linkage group I, notwithstanding the sequence of them is not yet confirmed.

Gene pair	Items			Segregation mode				Total	Recombination value (%)	$\chi^2_{(3)}$		
	Phase	No. of cross	++	$+dp_1$	$spl_4 +$	$spl_4 dp_1$						
spl_4-dp_1	F ₂	Coup.	8	961 (950.5)	$\frac{27}{11}$ (19.2)	$\frac{27}{11}$ (19.2)	$\frac{294}{0}$ (304.0)	1,293	3.0±0.5	3.606		
		Rep.	2	205 (214.5)	102 (107.3)	122 (107.3)	(0)	429	≠0	2.706		
F ₃ from F ₂ plants				Seg.	Non-seg.		Total					
$(spl_4^+ dp_1)$				1	59		60	0.8f0.8				
$(spl_4 dp_1^+)$				5	71		76	3.4±1.5				
Weighted mean									2.5±0.4			
spl_4-wx	F ₂	Rep.	5	$\frac{wx^+ wx^+}{+ spl_4}$		$\frac{wx^+ wx}{+ spl_4}$		$\frac{wx wx}{+ spl_4}$	Total			
				+	spl_4	+	spl_4	+				spl_4
				8 (9.6)	176 (198.6)	441 (407.0)	9 (9.5)	198 (208.5)	1 (0.1)	833	2.3±0.5	6.234

Figure 1: A timeline diagram showing the sequence of events in the experiment. The timeline starts at d_0 , followed by g , then spl_5 , and ends at Re . The duration from d_0 to g is 5.7. The duration from g to spl_5 is 23.0. The duration from spl_5 to Re is 13.4. The total duration from d_0 to Re is 37.3. The duration from d_0 to spl_5 is 33.6.

Fig. 3. Linkage map of the group IV.

Table 7. Linkage relations between *spl*₅ and genes belonging to the linkage group IV.

Gene pair	Phase	No. of cross	Segregation mode in F ₂				Total	Recombination value (%)	$\chi^2_{(3)}$
			AB	Ab	aB	ab			
<i>spl</i> ₅ - <i>d</i> ₆	coup.	4	447 (434.8)	92 (78.2)	60 (44.2)	85 (92.2)	684	26.3±2.0	7.670
	Rep.	1	100 (100.9)	(47.6)	(47.6)	(1.9)	198	19.8±6.8	0.692
	Weighted mean							25.8±1.9	
<i>spl</i> ₅ - <i>g</i>	coup.	4	456 (442.9)	83 (70.1)	53 (70.1)	92 (100.9)	684	23.2±1.9	7.720
	Rep.	1	101 (101.0)	51 (47.5)	44 (47.5)	2 (2.0)	198	20.1±6.8	0.516
	Weighted mean							23.0±1.8	
<i>spl</i> ₅ - <i>Rc</i>	<i>coup.</i>	1	135 (136.1)	17 (12.4)	8 (12.4)	38 (37.1)	198	13.4±2.6	3.299

Table 8. Linkage relations between *spl*₆ and genes belonging to the linkage group III.

Gene pair	Phase	Items		Segregation mode				Total	Recombination value (%)	$\chi^2_{(3)}$
		No. of cross	++	+ <i>eg</i>	<i>spl</i> ₆ +	<i>spl</i> ₆ <i>eg</i>				
<i>spl</i> ₆ - <i>eg</i>	F ₂ Coup.	4	560 (550.0)	26 (29.0)	31 (29.0)	155 (164.0)		772	7.8±1.0	1.124
	Rep.	2	225 (220)	110 (110)	105 (110)	(0)		440	≠0	0.341
	F ₃ from F ₂ plants		Seg.	Non-seg.		Total				
				18	66	84		12.0±2.8		
				16	89	105		8.3±2.1		
Weighted mean									8.3±0.9	
<i>spl</i> ₆ - <i>lax</i>	Phase	No. of cross	++	+ <i>lax</i>	<i>spl</i> ₆ +	<i>spl</i> ₆ <i>lax</i>		Total		
	F ₂ Coup.	1	125 (130.8)	3 (1.9)	1 (1.9)	48 (42.3)		177	2.2±1.1	2.070
	Rep.	6	552 (556.0)	264 (275.0)	290 (275.0)	2 (2.0)		1,108	8.4±3.0	1.286
	F ₃ from F ₂ plants		Seg.	Non-seg.		Total				
				23	173	113	194	5.7±1.9, 2±1.9		
Weighted mean									4.9±0.7	
<i>spl</i> ₆ - <i>d</i> ₁₀	Phase	No. of cross	AB	Ab	aB	ab		Total		
	F ₂ Rep.	3	283 (279.0)	130 (138.0)	142 (138.0)	1 (38.0)		556	8.7±4.2	0.635
<i>spl</i> ₆ -A	F ₂ Coup.	3	287 (309.3)	126 (107.7)	105 (107.7)	31 (31.3)	5 5 6		52.6f3.3	6.220

data shown in Table 7, the recombination values of spl_5-d_6 , spl_5-g and spl_5-Rc were estimated at 25.8 %, 23.0 % and 13.4 %, respectively. The recombination values of d_6-g , $g-Rc$ and d_6-Rc have previously been calculated at 5.7 %, 33.6 % and 37.3 %, respectively (Iwata and Omura, 1971 b). Thus, the sequence of the four genes at the map may be $d_6-g-spl_5-Rc$ (Fig 3).

spl_6 : Regarding spl_6 , the linkage relations were observed with four genes of *eg*, *lax*, d_{10} and *A* belonging to the linkage group III (Table 8). The recombination values of spl_6-eg were estimated from F_2 in coupling phase and two kinds of F_3 lines from F_2 in repulsion phase at 7.8 %, 12.0 % and 8.3 %, respectively, and resulted in the weighted mean of 8.3 %. The recombination values of spl_6-lax were estimated at 2.2 % and 8.4 % from F_2 in both coupling and repulsion phase and at 5.7 % and 9.2 % from two kinds of F_3 's from F_2 in repulsion phase, respectively, and thus their weighted mean of 4.9% was obtained. The recombination values of spl_6-d_{10} and spl_6-A were also estimated from F_2 at 8.7 % and 52.6 %, respectively. The arrangement of $eg-lax-d_{10}-A$ on the group and linkage intensities between them have previously been confirmed (Iwata and Omura, 1971 a). Thus, the sequence of the five genes at the map of linkage group III would be $eg-spl_6-lax-d_{10}-A$ (Fig. 4).

Fig. 4. Linkage map of the group III.

Table 9. Linkage relations between spl_7 and genes belonging to the linkage group IX.

Gene pair	Items			Segregation mode				Recombination value	$\chi^2_{(3)}$
	Phase	No. of cross	No. of +	+ al_{K-2}	spl_7 +	$spl_7 al_{K-2}$	Total		
spl_7-al_{K-2}	F_2 Rep.	1	125	lethal	68	lethal	193	10.779***1)	
	F_3 from F_2 plants		al seg.	al non-seg.		Total			
				17					
	$(spl_7^+ al_{K-2}^+)$		107	52		124			
	$(spl_7 al_{K-2}^+)$		14			66			
Weighted mean								13.1±2.4	
	Phase	No. of cross	AB	Ab	aB	ab	Total		
spl_7-nl_1	F_2 Rep.	2	194 (194)	102 (97)	92 (97)	0 (0)	388	÷=0	0.515
spl_7-ri	F_2 Rep.	2	198 (195)	98 (96.0)	91 (96.0)	1 (1.0)	388	10.4±5.0	0.346

¹⁾ χ^2 in this cross was for 3: 1. and then degree of freedom was 1.

*** Significant at 0.1% level.

spl₇: The linkage relations were observed between *spl₇* and such three genes as *al₁*, *nl₁* and *ri* belonging to the linkage group IX (Table 9). Since homozygous plants for the *al_{K-2}* gene are lethal at the seedling stage (Iwata and Omura, 1978), only two of phenotypes survived in F₂ of the cross between *spl₇* and *al₁* were examined for segregation mode of *spl₇*. The mode deviated remarkably from 3: 1 ratio, suggesting a existence of linkage relation between them. The recombination values of 15.0 % and 11.9 % were estimated from the progeny test of F₂ plants having the phenotypes of *spl₇⁺al_{K-2}⁺* and *spl₇al_{K-2}⁺*, respectively, and their weighted mean of 13.1% was obtained. Between *spl₇* and *nl₁*, a very close linkage relation was assumed from the fact that non of the doubly recessive plant had been observed in F₂ in the cross of repulsion phase, though the recombination value could not be given. In the cross between *spl₇* and *ri*, only one of doubly recessive plant had been observed in F₂ in repulsion phase and thus a linkage intensity was estimated at 10.4 %. Although it needs additional data for confirming a accurate sequence of the four genes, the order of *al₁-nl₁-spl₇-ri* at the map of linkage group IX was adopted tentatively.

spl₈: Phenotypic characteristics of *spl₈* having fine striped spots of reddish brown on leaves is different clearly from that of any other *spl* genes described in this paper, and the linkage relation was observed between *spl₈* and *spl₇* with the intensity of 13.6 % from F₂ in the cross of repulsion phase (Table 10). Consequently, it is considered that *spl₈* also belongs to the linkage group IX.

Table 10. Linkage relation between *spl₈* and *spl₇*.

Phase	No. of cross	Segregation mode in F ₂					Recombination value (%)	$\chi^2_{(3)}$
		++	+ <i>spl₇</i>	<i>spl₈</i> +	<i>spl₈ spl₇</i>	Total		
Rep.	1	107 (107.5)	49 (52.3)	56 (52.3)	1 (0.9)	213	13.6±6.7	0.473

Table 11. Segregations for normal vs. physiological leaf spots (*spl₉*) in F₂ lines between linkage testers and a mutant strain (LT 26) showing physiological leaf spots.

+	<i>spl₉</i>	Total	χ^2 for 3: 1
151	44	195	0.617
146	49	195	0.002
147	47	191	0.393
141	40	188	0.000
148		188	1.390
150	45	194	0.557
146	39	191	0.211
152		191	2.138
1,181	352	1,533	3.397

spl₉: Blackish brown spots of this mutant is unique in feature. Segregation modes of normal to this character in F₂ were accordant with a ratio

of 3: 1, indicating that the character was controlled by a single recessive gene, *spl*₉ (Table 11). However, the linkage group to which *spl*₉ belongs is so far unknown.

As described above, the linkage studies with nine of *spl* genes expressing spotted leaf were made in this paper, and with eight of them the linkage groups to which they belonged were proved. Three genes, *spl*₃, *spl*₅ and *spl*₇ were so similar that they could not be distinguished by their phenotypic feature, nevertheless they were controlled by different genes and belonged to different linkage groups. The same was observed between *spl*₄ and *spl*₆. Therefore, it is well considerable that there are many mutants even if they have similar phenotype. A hundred and one of spotted leaf mutants have been collected in our laboratory, so the identification of genes and the linkage analysis are carrying on.

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