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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 Hreeding, National Institute of Agricultual 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	spl_1
HO 696	Katsumonbyo[1)]	〃	spl_2
M 41	Norin 8	r-ray (chronic)	spl_3
M 114	〃	〃	spl_4
M 87	〃	〃	spl_5
CM 20	Kinmaze	Chemicals	spl_6
M 64	Norin 8	r-ray (chronic)	spl_7
CM 207	Kinmaze	Chemicals	spl_8
LT 26	Norin 8	r-ray (acute)	spl_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	*wx*	waxy endosperm	Nagamatsu and Omura (1962)
	dp_1	depressed palea 1	〃
III	*eg*	extra glume	Iwata and Omura (1971a)
	lax	lax panicle	〃
	d	tillering dwarf	〃
	A"	anthocyanin activator	Nagao and Takahashi (1963)
IV	d_6	lop-leaved dwarf	〃
	g	long empty glumes	〃
	Rc	brown pericarp	〃
IX	al_{K-2}	albino Kyushu-2	Iwata and Omura (1978)
	nl_1	neck leaf 1	Nagao and Takahashi (1963)
	ri	verticillate arrangement of rachis	〃
X	d_W	"Waisei-shirasasa" dwarf	Iwata and Omura (1971b)
	gh_2	gold hull 2	〃
XI	bc_1	brittle culm 1	〃
	dl	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 F_2. 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 F_2 and in some cases in F_3 progenies by the method of maximum likelihood. The weighted mean was calculated when the recombination values were estimated from F_2 in both phase and F_3.

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 F_2 derived from trisomic F_1 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 F_2 derived from disomic F_1 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 F_2 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 F_2 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 AB	Ab	aB	ab	Total	Recombination value (%)	$\chi^2_{[3]}$
spl$_2$–*gh*$_2$	coup.	2	246 (244.6)	28 (29.2)	30 (29.2)	61 (62.1)	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	
spl$_2$–*d*$_W$	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	

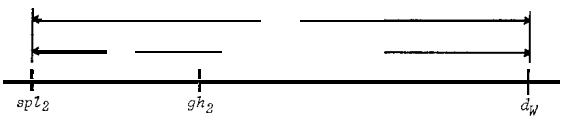

Fig. **1.** Linkage map of the group X.

spl$_3$: The linkage relations were found in F_2 of crosses between *spl*$_3$ and such two genes as *dl* and *bc,* belonging to the linkage group XI. From F_2 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 AB	Ab	aB	ab	Total	Recombination value (%)	$\chi^2_{[3]}$
spl$_3$–*dl*	coup.	7	941 (914.1)	103 (98.4)	88 (98.4)	218 (239.18.1)	1,350	15.8±1.1	3.968
	Rep.	10	1,183 (1,139.0)	506 (539.5)	531 (539.5)	(20.0)	2,238	19.1±2.0	3.058
	Weighted mean							16.5±1.0	
spl$_3$–*bc*$_1$	Rep.	1	101 (99.4)	28 (37.1)	45 (37.1)	8 (8.4)	182	43.0±6.0	3.960

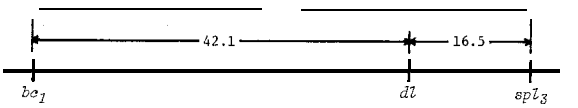

Fig. 2. Linkage map of the group XI.

43.0 % from the data of repulsion phase. Because the linkage intensity between *dl* and *bc,* is about 40 % (Iwata and Omura, 1971 b), the sequence of the three genes at the map of the linkage group XI is probably bc_1–*dl*–spl_3 (Fig. 2).

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 F_2 in coupling phase and two kinds of F_3 lines from F_2 plants in repulsion phase showing such phenotypes as $spl_4^+dp_1$ and $spl_4dp_1^+$ 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 F_2 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.

Table 6. Linkage relations between *spl,* and genes belonging to the linkage group I.

Gene pair	Items: Phase	No. of cross	Segregation mode: ++	$+dp_1$	spl_4 +	spl_4dp_1	Total	Recombination value (%)	$\chi^2_{(3)}$
spl_4–dp_1	F_2 Coup.	8	961 (950.5)	27 (19.2)	11 (19.2)	294 (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

	Phase	No. of cross	wx^+ wx^+ +	wx^+ wx^+ spl_4	wx^+ *wx* +	wx^+ *wx* spl_4	wx wx +	wx wx spl_4	Total		
spl_4–*wx*	F_2 Rep.	5	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

spl_5: The linkage relationships between the spl_5 and such three genes as *d,, g* and *Rc* belonging to the linkage group IV were observed. From F_2

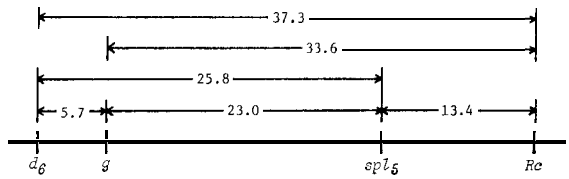

Fig. 3. Linkage map of the group IV.

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 AB	Ab	aB	ab	Total	Recombination value (%)	$\chi^2_{(3)}$
spl_5-d_6	coup.	4	447 (434.8)	92 (78.2)	60 (78.2)	85 (93.8)	684	26.3±2.0	7.670
	Rep.	1	100 (100.9)	52 (47.6)	44 (47.6)	2 (1.9)	198	19.8±6.8	0.692
	Weighted mean			83	53	92		25.8±1.9	
spl_5-g	coup.	4	456 (442.9)	(70.1)	(70.1)	(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			17	8	38		23.0±1.8	
spl_5-Rc	*coup.*	*1*	***135 (136.1)***	(12.4)	(12.4)	(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	Items: Phase	No. of cross	++	+ *eg*	spl_6 +	spl_6 eg	Total	Recombination value (%)	$\chi^2_{(3)}$
spl_6-*eg*	F_2 Coup.	*4*	***560 (550.0)***	26 (29.0)	31 (29.***0)***	155 (164.0 ***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		
	(spl_6^+ *eg*)		18		66		84	12.0±2.8	
	(spl_6 eg^+)		*16*		89		105	***8.3t2.1***	
	Weighted mean							8.3±0.9	
	Phase	No. of cross	++	+ lax	spl_6 + spl_6	lax	Total		
spl_6-*lax*	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		
	(spl_6^+ s_6alax)		21		173		194	5.7±1.9	
	(spl_6 l ux^+)		23		113		136	2±1.9	
	Weighted mean							4.9±0.7	
	Phase	No. of cross	AB	Ab	aB	ab	Total		
spl_6-d_{10}	F_2 Rep.	3	283 (279.0)	130 (138.0)	142 (138.0)	1 (1.0)	556	8.7±4.2	0.635
spl_6-A	F_2 Coup.	3	287 (309.3)	126 (107.7)	105 (107.7)	38 (31.3)	556	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).

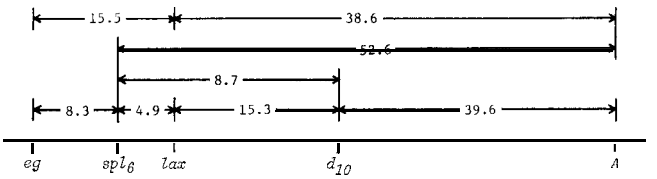

Fig. 4. Linkage map of the group III.

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	++	$+al_{K-2}$	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		
	(spl_7^+ al_{K-2}^+)			107		52	124	15.0±3.8	
	(spl_7 al_{K-2}^+)			14			66	11.9±3.1	
	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 (195.0)	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 begree of freedom was 1.
*** Significant at 0.1% level.

spl$_7$: The linkage relations were observed between *spl*$_7$ and such three genes as ***al,***$_2$, *nl*$_1$ 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 F_2 of the cross between *spl*$_7$ and ***al,***$_2$ were examined for segregation mode of *spl*$_7$. 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 F_2 plants having the phenotypes of *spl*$_7$$^+$*al*$_{K-2}$$^+$ and *spl*$_7$*al*$_{K-2}$$^+$, respectively, and their weighted mean of 13.1% was obtained. Between *spl*$_7$ and *nl*$_1$, a very close linkage relation was assumed from the fact that non of the doubly recessive plant had been observed in F_2 in the cross of repulsion phase, though the recombination value could not be given. In the cross between *spl*$_7$ and *ri*, only one of doubly recessive plant had been observed in F_2 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 ***al,***$_2$–*nl*$_1$–*spl*$_7$–*ri* at the map of linkage group IX was adopted tentatively.

spl$_8$: Phenotypic characteristics of *spl*$_8$ 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 *spl*$_8$ and *spl*$_7$ with the intensity of 13.6 % from F_2 in the cross of repulsion phase (Table 10). Consequently, it is considered that *spl*$_8$ also belongs to the linkage group IX.

Table 10. Linkage relation between *spl*$_8$ and *spl*$_7$.

Phase	No. of cross	Segregation mode in F_2				Total	Recombination value (%)	$\chi^2_{(3)}$
		+ +	+ *spl*$_7$	*spl*$_8$ +	*spl*$_8$ *spl*$_7$			
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 (*spl*$_9$) in F_2 lines between linkage testers and a mutant strain (LT 26) showing physiological leaf spots.

+	*spl*$_9$	Total	χ^2 for 3: 1
151	49	195	0. 617
146		**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

spl$_9$: Blackish brown spots of this mutant is unique in feature. Segregation modes of normal to this character in F_2 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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