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Linkage Studies in Rice (*Oryza sativa* L.)
On Some Virescent and Chlorina Mutants

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Linkage analyses on five virescent and four chlorina mutants were carried out. Six of these mutants, v_1, v_2, v_5, ch_1, ch_2 and ch_3, were found to belong to the eleventh linkage group. Also. it was found that v_3 and ch, belonged to the first linkage group, and v_4 to the eighth group. The sequences of the genes in respective linkage groups were made clear, though a few of the loci were undetermined.

INTRODUCTION

Various kinds of chlorophyll mutants are known in rice, however, only a few of the linkage studies on the mutants had been made (Jodon, 1940; Nagao and Takahashi, 1960; Nagamatsu and Omura, 1962) before the authors reported some of them (Iwata and Omura, 1971, 1975, 1978). Most of the chlorophyll mutants are modified their character manifestation by environmental conditions, being clearly distinguishable from the normal in certain conditions but indistinguishable in other conditions. Therefore, the mutants are usable in linkage analysis, when the conditions suitable to character manifestation of the mutants are known.

Recently, many chlorophyll mutants have been obtained, then the authors are carrying on the studies on their character manifestation as shown in some papers (Omura and Tanaka, 1959; Omura *et al., 1977;* Satoh *et al.,* 1977) on the one hand, and their linkage analyses on the other hand. The present paper described the results of linkage analyses on some of the virescent and chlorina mutants.

MATERIALS AND METHODS

The materials used were five virescent seedlings, v_1, v_2, v_3, v_4 and v_5, and four chlorina, ch_1, ch_2, ch_3 and ch_4.

The virescent mutants sprout out white leaves under low temperature condition, but pale green or nearly normal green leaves under high temperature condition, though the mutants have different threshold temperature for chlorophyll accumulation. Contrary, the chlorina mutants sprout out yellowish green leaves, their typical characteristics, under high temperature condition. Their main characteristics and sources are as follows.

v_1: The threshold temperature is 22°C (Omura et *al.,* 1977). When **it is** sown in late May, ordinary sowing time in Fukuoka, young seedlings are almost whole white but leaves emerging at or after transplanting are pale green with white midrib. Emerging panicles are white in color. It was introduced from Dr. Jodon of U. S. Department of Agriculture.

v,: The threshold temperature is about 20°C. Differing from v_1, midrib and panicles are not white but green. Other characteristics are almost the same as v_1. A spontaneous mutant from a Japanese cultivar "Yaeho".

v_3: The threshold temperature is about 30°C. An induced mutant from a Japanese cultivar "Kinmaze" by *N*-nitroso-*N*-methylurea treatment.

v_4: The threshold temperature is not examined. It is an induced mutant from "Norin 8" by irradiation and introduced from Division of Genetics, National Institute of Agricultural Science (LT 3).

v,: Except the threshold temperature is probably higher than v_3, it resembles v_3 in other characteristics. An induced mutant from "Kinmaze" as same as v_3.

ch,: When it is sown in late May, the distinction from the normal seedling is rather difficult, but after transplanting the chlorina chatacter of yellowish green leaves is clearly manifested. A spontaneous mutant stocked in our laboratory (HO 718-721).

ch_2: Leaves of young seedling exhibit orange in color but leaves emerging thereafter are yellowish pale green and finally green (LT 4). The same source as v_4.

ch_3: It manifested yellowish pale green leaves at just before heading. A spontaneous mutant stocked in our laboratory (HO 717).

ch_4: It is characterized by yellowish green leaves at tillering stage and by fewer culm and somewhat lower viability than the normal. It was induced in gamma field and introduced from Institute of Radiation Breeding, National Institute of Agricultural Science (No. 646).

Linkage was detected in F_2 by the trisomic and conventional methods. Trisomics used were some types described by Iwata and Omura (1975). Marker genes used in conventional method are listed in Table 1. Recombination

Table 1. List of marker genes used and their linkage groups.

Linkage group	Gene symbol	Character	Reference
I	*wx*	waxy endosperm	Nagamatsu and Omura (1962)
	dp_1	depressed palea 1	〃
	C	chromogen for anthocyanin	〃
	ws	white striped leaf	〃
	Cl	clustered spikelets	Nagao and Takahashi (1963)
VIII	*la*	lazy	Iwata and Omura (1971)
	sp	short panicle	〃
XI	ch_1	chlorina 1	〃
	fc	fine culm, tillering	Iwata and Omura (1977)
	bc_1	brittle culm 1	Iwata and Omura (1971)
	dl	drooping leaf	〃

values were calculated in F_2 and F_3 by the method of maximum likelihood. The F_2 segregation for ch_4 was disturbed by differential viability. It is known, however, that the differential viability does not influence the estimation of recombination value but influence the expected numbers of four phenotypes (Bailey, 1961). Therefore, the expected number was calculated in consideration of the parameter of differential viability (u), which is defined for the relative excess of ch_4^+ phenotypes over ch_4. The value of u is estimated from numbers of four phenotypes, a, b, c and d, by the following equation,

$$u=(a+b)/3(c+d).$$

Table 2. Linkage relations between four genes belonging to the eleventh linkage group.

Gene pair	Items		Segregation mode					Recombination value (%)	$\chi^2_{(3)}$
	Phase	No. of cross	++	$+fc$	ch_1+	ch_1fc	Total		
ch_1-fc	F_2 Coup.	4	461 (469.9)	90 (98.6)	111 (98.6)	96 (90.9)	758	30.8±2.1	2.763
	Rep.	6	587 (556.3)	246 (253.0)	232 (253.0)	14 (16.8)	1,079	24.9±2.8	4.087
	F_3 from F_2 plants		Seg.	Non-seg.			Total		
	(ch_1^+ fc)		37	47			84	28.2±4.5	
	(ch_1 fc^+)		36	47			83	27.7±4.4	
	Weighted mean							28.5±1.5	
	Phase	No. of cross	AB	Ab	aB	ab	Total		
ch_1-bc_1	F_2 Coup.	2	413 (404.1)	135 (144.2)	147 (144.2)	36 (38.6)	731	54.1±2.9	1.008
	Rep.	3	419 (425.5)	131 (146.8)	167 (146.8)	46 (44.0)	763	48.0±2.8	4.675
	Weighted mean							50.9±2.0	
ch_1-dl	F_2 Rep.	4	628 (629.5)	204 (215.0)	227 (215.0)	67 (66.5)	1,126	48.6±2.3	1.240
$fc-bc_1$	F_2 Coup.	1	119 (109.7)	22 (26.8)	27 (26.8)	14 (18.7)	182	35.8±4.6	2.829
	Rep.	7	751 (702.0)	258 (282.7)	268 (282.7)	36 (45.5)	1,313	37.2±2.3	8.339
	Weighted mean							37.0±2.1	
$fc-dl$	F_2 Rep.	9	1,014 (970.4)	324 (346.6)	338 (346.6)	80 (92.4)	1,756	45.9±1.9	5.309
bc_1-dl	F_2 Coup.	18	2,335 (2,283.0)	641 (648.8)	627 (648.8)	306 (328.5)	3,909	42.0±1.1	3.547
	Rep.	4	647 (616.1)	204 (207.4)	190 (207.4)	57 (67.1)	1,098	49.4±2.3	4.586
	Weighted mean							43.4±1.0	

RESULTS AND DISCUSSION

As it was found that ***ch*****,** belonged to the eleventh linkage group by the translocation method (Iwata and Omura, 1971), it was used as one of the marker genes of this group. The interrelation of the marker genes are shown in Table 2. The sequence of $ch_1-fc-bc_1-dl$ is suggested from respective recombination values, though that of $bc_1-dl-ch_1$ was previously reported (Iwata and Omura, 1971). The recombination values of v_1-fc and v_1-bc_1 were both 17.9 % (Table 3), and that of ***fc-bc*****,** was 37.0 % (Table 2). Consequent-

Table 3. Linkage relations between v_1 and genes belonging to the eleventh linkage group.

Gene pair	Items: Phase	Items: No. of cross	Segregation mode: ++	Segregation mode: $+fc$	Segregation mode: v_1+	Segregation mode: v_1fc	Segregation mode: Total	Recombination value (%)	$\chi^2_{(3)}$
v_1-fc	F_2 Rep.	4	340 (315.4)	140 (151.8)	140 (151.8)	3 (3.9)	623	15.9±3.9	3.916
	F_3 from F_2 plants		Seg.		Non-seg.		Total		
	(v_1^+ fc)		29		60		89	19.5±3.5	
	Weighted mean							17.9±2.7	
	Phase	No. of cross	++	$+bc_1$	v_1+	v_1bc_1	Total		
v_1-bc_1	F_2 Rep.	3	353 (320.8)	142 (154.0)	135 (154.0)	3 (4.3)	633	16.5±3.8	6.886
	F_3 from F_2 plants		Seg.		Non-seg.		Total		
	(v_1^+ bc_1)		27		57		84	19.1±3.6	
	Weighted mean							17.9±2.6	

Table 4. Linkage relations between v_2 and genes belonging to the eleventh linkage group.

Gene pair	Phase	No. of cross	Segregation mode in F_2: AB	Ab	aB	ab	Total	Recombination value (%)	$\chi^2_{(3)}$
v_2-bc_1	coup.	15	1,952 (1,910.0)	396 (397.7)	382 (397.7)	347 (371.5)	3,077	30.5±1.0	3.171
	Rep.	3	388 (359.6)	151 (169.9)	162 (169.9)	5 (6.6)	706	19.3±3.6	5.103
	Weighted mean							29.7±1.0	
v_2-dl	coup.	11	1,411 (1,388.5)	244 (245.0)	238 (245.0)	285 (299.5)	2,178	25.8±1.1	1.271
	Rep.	9	1,142 (1,118.9)	474 (501.9)	509 (501.9)	36 (38.4)	2,161	26.7±2.1	2.274
	Weighted mean							26.0±1.0	

Table 5. Linkage relation between v_5 and ch_1 belonging to the eleventh linkage group.

Items		Segregation mode					Recombination value (%)
Phase	No. of cross	++	$+ch_1$	v_5+	v_5ch_1	Total	
F_2 Rep.	2	193	93	59	0	345	≑0
F_3 from F_2 plants		Seg.		Non-seg.		Total	
(v_5^+ ch_1)		3		83		86	1.8±1.0
(v_5 ch_1^+)		2		35		37	2.8±2.0
Weighted mean							2.0±0.9

Table 6. Linkage relations between two genes, ch_2 and ch_3, newly described and marker genes belonging to the eleventh linkage group.

Gene pair	Phase	No. of cross	Segregation mode in F_2					Recombination value (%)	$\chi^2_{(3)}$
			AB	Ab	aB	ab	Total		
ch_2-bc_1	Rep.	2	212 (201.6)	92 (84.9)	69 (84.9)	9 (10.6)	382	33.4±4.5	4.350
ch_2-v_2	Rep.	2	175	106	101	0	382	≑0	
ch_2-dl	Rep.	3	303 (305.7)	139 (135.3)	134 (135.3)	12 (11.7)	588	28.2±3.7	0.145
ch_3-ch_1	Rep.	2	181 (178.6)	93 (84.6)	74 (84.6)	3 (3.1)	351	18.9±5.1	2.199
ch_3-fc	coup.	2	220 (227.4)	35 (35.8)	39 (35.8)	57 (51.9)	351	23.1±2.6	1.039
	Rep.	2	193 (188.2)	93 (90.8)	84 (90.8)	2 (2.2)	372	15.2±4.9	0.703
	Weighted mean							21.3±2.3	

ly, it is concluded that v_1 is located between *fc* and bc_1. Jodon (1940) found that a virescent gene (*v*) linked with gu (wx in our symbol), *as (C)* and *Cl*. This fact indicates that v belongs to the first linkage group, however, Takahashi and Morimura (1968) could not find the linkage relations, using a virescent gene introduced from Dr. Jodon. It is uncertain whether v_1 is identical with v or not, though v_1 is also introduced from Dr. Jodon.

Two virescent genes, v_2 and v_5, were found to belong to this group. The recombination values of v_2-bc_1 and v_2-dl were 29.7 and 26.0 %, respectively (Table 4). Then, the sequence of bc_1-v_2-dl is reliable. The recombination value of v_5-ch_1 was calculated in F_3 from two kinds of singly dominant F_2 at 2.0% (Table 5). This value shows that v_5 is located near by ch_1.

Two chlorina genes, ch_2 and ch_3 belonged also to this group. The recombination values of ch_2-bc_1 and ch_2-dl were estimated at 33.4 and 28.2 %, respectively (Table 6). The recombination value of 43.4 % have already been obtained between bc_1 and *dl* (Table 2). From these results, the sequence of bc_1-ch_2-dl was confirmed.

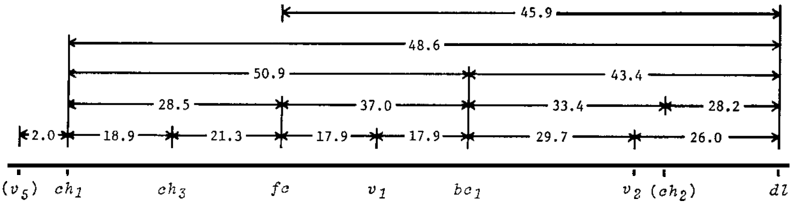

Fig. 1. Sequence of the nine genes at the eleventh linkage group.

Table 7. Linkage relations between v_3 and genes belonging to the first linkage group.

Gene pair	Items		Segregation mode							Recombination value (%)	$\chi^2_{(3)}$
	Phase	No. of cross	++	$+dp_1$	v_3+	v_3dp_1			Total		
v_3-dp_1	F_2 Rep.	1	192 (183.3)	85 (88.2)	83 (88.2)	2 (2.3)			362	16.0±5.1	0.874
	F_3 from F_2 plants		Seg.	Non-seg.					Total		
	(v_3^+ dp_1)		19	61					80	13.5±3.1	
	(v_3 dp_1^+)		1	25					26	2.0±2.0	
	Weighted mean									6.3tl.6	
	Phase	No. of cross	+			v_3			Total		
			++	+*wx*	wxwx	++	+*wx*	wxwx			
v_3-wx	F_2 Rep.	1	12	92	40	39	4	0	187	8.6t2.1	
	F_3 from F_2 plants		Seg.	Non-seg.					Total		
	(wx v_3^+)		9	29					38	13.4±4.4	
	Weighted mean									9.5rtrl.9	
	Phase	No. of cross	AB	Ab	aB	ab			Total		
v_3-C	F_2 Coup.	2	246 (254.3)	31 (24.7) 23	20 (24.7) 30	75 (68.3) 10			372	14.3±2.0	3.430
v_3-Cl	F_2 Coup.	1	113 (102.5)	(29.5)	(29.5)	(14.5)			176	42.7±5.2	3.915

The close linkage relation was observed between ch_2 and v_2, however, the recombination value was not calculated, because none of the doubly recessive plant was segregated in F_2 in repulsion phase (Table 6). As mentioned above, v_2 was also located between *bc,* and *dl,* therefore, the loci of *ch,* and v_2 should be adjacent each other, although accurate distance of them is so far uncertain. The recombination values of 18.9 and 21.3 % were calculated in ch_3—ch_1 and ch_3—fc, respectively (Table 6). As the value of ch_1—fc was 28.5 % (Table 2), *ch,* is located between ch_1 and fc. Based on these results, the sequence of the nine genes at the eleventh linkage group is tentatively drawn as shown in Fig. 1.

The other virescent gene (v_3) linked with marker genes of the first linkage group (Table 7). The recombination values between v_3 and wx, dp_1, *C* and

Table 8. Trisomic segregation of ch_4 in F_2 of a cross with B type of trisomics.

Portion of population	Observed number			χ^2		
	Dominant	Recessive	Total	Disomic 3 : 1	Trisomic 8 : 1 for 2x	Trisomic 44 : 1 for 2x+1
2x	139	1	84		*0.596*	
2x+1	83	15	237			*0.412*
Total	222			44.063***		

*** Significant at 0.1% level.

Table 9. Linkage relations between ch_4 and genes belonging to the first linkage group.

Gene pair	Items		Segregation mode					Recombination value (%)	$\chi^2_{(3)}$
	Phase	No. of cross	AB	Ab	aB	ab	Total		
ch_4-dp_1	F_2 Rep.	4	506 (497.1)	203 (189.9)	170 (189.9)	37 (39.1)	916	41.3±2.7	3.261
ch,-C	F_2 Coup.	1	106 (111.3)	36 (26.7)	20 (26.7)	22 (19.3)	184	35.3C4.6	5.549
	Phase	No. of cross	++	+ws	ch_4+	ch_4ws	Total		
ch_4-*ws*	F_2 Rep.	4	418 (416.9)	189 (190.1)	142 (141.9)	9 (9.1)	758	24.5t3.4	0.010
	F_3 from F_2 plants		Seg.		Non-seg.		Total		
	(ch_4^+ *ws*)		37		115		152	13.9±2.3	
	Weighted mean							17.2 ± 1.9	
	Phase	No. of cross	+*Cl*	++	ch_4Cl	ch_4 +	Total		
ch_4-*Cl*	F_2 Coup.	2	244 (246.6)	49 (46.4)	15 (16.6)	20 (18.4)	328	27.5±3.0	0.466

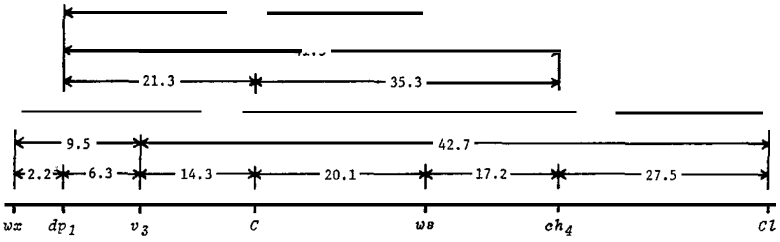

Fig. 2. Sequence of the seven genes at the first linkage group.

Table 10. Trisomic and disomic segregations of v_4 in F_2 of a cross with G type of trisomics.

F_1 plants	Observed number			χ^2 for 3 : 1	Ratio of domi.: rece.	
	Dominant	Recessive	Total		Theoretical	Observed
Trisomic	183	6	189	48.016***	8 : 1-44 : 1	30.5 : 1
Disomic	367	129	496	0.269	3 : 1	

*** Significant at 0.1% level.

Table 11. Linkage relations between v_4 and genes belonging to the eighth linkage group, and between la and *sp*.

Gene pair	Items		Segregation mode					Recombination value (%)	$\chi^2_{(3)}$
	Phase	No. of cross	+ +	+ *la*	v_4 +	v_4la	Total		
v_4-la	F_2 Coup.	3	343 (342.7)	33 (27.0)	21 (27.0)	96 (96.2)	493	11.6±1.6	0.024
	Rep.	2	215 (224.2)	118 (108.1)	107 (108.1)	3 (2.7)	443	15.5±4.6	1.337
	F_3 from F_2 plant		Seg.		Non-seg.		Total		
	(v_4^+ *la*)		14		75		89	8.5±2.3	
	(v_4 *la*$^+$)		14		64		78	9.9±2.6	
	Weighted mean							10.8±1.1	
	Phase	No. of cross	AB	Ab	aB	ab	Total		
v_4-*sp*	F_2 Rep.	1	54 (63.4)	37 (29.6)	31 (29.6)	2 (1.4)	124	21.1±8.5	3.569
la-*sp*	F_2 Coup.	24	2,831 (2,884.5)	455 (443.3)	448 (443.3)	703 (666.0)	4,437	22.5±0.7	4.723
	Rep.	7	669 (642.5)	266 (285.3)	281 (285.3)	21 (24.0)	1,237	27.8±2.6	2.831
	Weighted mean							22.9±0.7	

Cl were 9.5, 6.3, 14.3 and 42.7 %, respectively, so the gene sequence was thought to be $wx-dp_1-v_3-C-Cl$. It was confirmed that ch_4 belonged to the first linkage group by means of the trisomic segregation in F_2 of a cross with B type of trisomics (Table 8). The recombination values between ch_4 and dp_1, C and ws were calculated at 41.3, 35.3, 17.2 and 27.5 %, respectively (Table 9). Combining these values and the value of 30.6 % between ws and Cl reported by Iwata and Omura (1971), it is concluded that ch_4 is located between ws and Cl. Therefore, the sequence of these genes at the first linkage group is as shown in Fig. 2.

Takahashi and Morimura (1968) reported the linkage between a chlorina gene ***(chl)*** and *C*, Cl and wx with the recombination values of 33.0, 39.5 and 34.5 %, respectively. This chlorina gene (*chl*) sent by us is identical with ***ch,***. As above mentioned, ch_1 belongs to the eleventh linkage group and never links with marker genes of the first linkage group.

Lastly, v_4 was found to belong to the eighth linkage group, showing the trisomic segregation in F_2 of a cross with G type of trisomics (Table 10). The recombination values of v_4-la, v_4-sp and *la-sp* were 10.8, 21.1 and 22.9 %, respectively (Table 11). Therefore, their sequence is either $la-v_4-sp$ or $v_4-la-sp$. Linkage relations between v_4 and the other marker genes of this group are under examination.

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