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LINKAGE STUDIES BY THE USE OF BACKCROSS DATA IN RICE

— Genetical studies on rice plant, XCV[1] —

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Introduction

The construction of an elaborate chromosome map is an important basis of rice breeding. In the current maps, 119 kinds of marker genes are located on twelve chromosomes[3]. As regards linkage intensities, most of the recombination values are calculated depending on the data of F_2 and F_3, since the production of backcross seeds needs a lot of work and time for emasculation and pollination procedures. Recently the development of cytoplasmic male sterile (CMS) strains makes it possible to exclude the emasculation stage to obtain crossed seeds in large quantities.

In the work reported in this paper, the authors raised the CMS multiple marker stocks and used them for linkage analysis between the known marker genes.

Before going further, the authors are very grateful to Professors emeritus, S. NAGAO and M. TAKAHASHI, Hokkaido University for the use of their F_2 data. The authors also wish to express their sincere appreciation to Dr. C. SHINJO, Faculty of Agriculture, University of Ryukyus for the seeds of the male sterile strain and its maintainer, and to Dr. M. MAEKAWA for his valuable aid in many ways.

Materials and Methods

The genetic stocks of marker genes used in these experiments are shown in Table 1. Both the male sterile strain and its maintainer possess a nuclear genotype of Taichung 65, and the cytoplasm of Taichung 65 CMS is derived from an Indica variety 'Chinsurah boro II'.

First the cytoplasm of Taichung 65 CMS denoted as [*ms-bo*][7] was trans-

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1) Contribution from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Sapporo, Japan

TABLE 1. List of the strains used

	Strain	Marker gene	Source
A- 5	Akamuro	C^{Br},*A,P,Pr,Rc,Rd,I-Bf*$^{+}$	Hokkaido variety
A- 58	Kokushokuto-2	C^{B},*A,P,Pr,Pn,Ph,wx*	do.
A- 77	Murasaki-ine	C^{Bp},*A,P,Pl*	do.
A-133	Norin-9	C^{Bm},A^{d},*P*	do.
A-136	Shiokari	C^{Bm},A^{d},*P*	do.
H- 21	Linkage tester	*bl-1,Rc,sh*	
H- 23	do.	*spr-1*	
H- 59	do.	C^{B},A^{d},*P,lg,wx*	
H- 61	do.	*d-2*,C^{Bp},*A*,P^{+},*Pn,I-Bf*$^{+}$	
H- 68	do.	*ri,bl-1*	
H- 69	do.	*nl-1,fs-1*,C^{Bp},A^{+}	
H- 79	do.	*d-2,bc-1,lg,la*	
H- 94	do.	C^{B},*A,P,Pn,gl-1*	
H-103	do.	C^{Bp},*A,P,nl-1,gl-1*	
H-121	do.	C^{Bp},*A,P*,Pl^{w},*I-Pl,I-Bf*$^{+}$	
H-126	do.	*d-6*,C^{Bp},*A,P,Pl,Hg*	
H-131	do.	*Er*	
H-135	do.	*d-1,gh-1,g-1*	
H-136	do.	*d-1,gh-1,g-1*	
H-138	do.	C^{B},A^{d},*P,d-6,g-1*	
H-143	do.	*st-2,gh-1,Rc*	
H-326	do.	*bl-2*	
H-339	do.	*Cl*	
H-479	do.	*st-1,sp*	
485	do.	*d-6,Hg,spr-1*	
I -127	Taichung 65 CMS		CMS line having [*ms-bo*] cytoplasm
I -128	Taichung 65		Maintainer for I-127

TABLE 2. Substitution of male sterile cytoplasm, [*ms-bo*] for multiple marker stocks

No.	Name	Backcross	Donor	
			Cytoplasm	Nucleus
A- 5 CMS	Akamuro	B_7	[*ms-bo*]	A- 5
A -58 CMS	Kokushokuto-2	B_{11}	[*ms-bo*]	A- 58
A-133 CMS	Norin-9	B_5	[*ms-bo*]	A-133
A-136 CMS	Shiokari	B_{11}	[*ms-bo*]	A-136
H- 21 CMS	Linkage tester	B_9	[*ms-bo*]	H- 21
H- 61 CMS	do.	B_7	[*ms-bo*]	H- 61
H- 68 CMS	do.	B_4	[*ms-bo*]	H- 68
H- 69 CMS	do.	B_{11}	[*ms-bo*]	H- 69
H- 79 CMS	do.	B_7	[*ms-bo*]	H- 79
H-126 CMS	do.	B_{11}	[*ms-bo*]	H-126
H-135 CMS	do.	B_6	[*ms-bo*]	H-135
H-143 CMS	do.	B_6	[*ms-bo*]	H-143
H-339 CMS	do.	B_{10}	[*ms-bo*]	H-339

ferred to the multiple marker stocks by successive backcrossings from 4 to 11 times. By using the CMS multiple marker stocks F_1 and backcross populations were produced without manual emasculation. F_2 data corresponding to linkage relations confirmed in the backcross data were quoted from the papers by NAGAO and TAKAHASHI[6] and their collaborators[5,8].

Results

The cytoplasm of 13 kinds of multiple marker stocks was replaced by that of Taichung 65 CMS, [*ms-bo*] by successive backcrossings from 4 to 11 times as shown in Table 2. Since most Japonica rice possesses the recessive genotype for pollen fertility restoration, F_1 plants indicated complete male sterility. These F_1s were crossed to euplasmic marker stocks to produce a large quantity of backcross seeds without artificial emasculation.

The linkage relations between the known marker genes were examined in several combinations of backcross, and the recombination values were compared with the corresponding data obtained from F_2 as shown in Table 3.

There were no conspicuous differences in recombination values between F_2 coupling and backcross, while the values in the F_2 repulsion data in *A—Pn* and *d-6—g-1* were higher than those of the backcross data [In the latter case these may have been some overestimation].

TABLE 3. Linkage relations between known marker genes in backcross and F_2 data

Gene pair	population	Linkage phase (R.C.V.)	Segregation mode AB	Ab	aB	ab	Total	Recombination value	χ^2	d.f.	p	Cross[1] or literature*
Group I ("*wx*" group)												
C–st-1	B	c (27.6%)	10 10.5	4 8.0	4	11 10.5	29	27.6 ±5.60	0.048	2	0.95 -0.98	(1)
	F_2	c (25.5%)	239 238.3	46 41.5	37 41.5	51 51.8	373	25.5 ±1.81	0.99	3	0.80 -0.90	8*
Cl–fs-1	B	c (0.6%)	154 160.5	1	166 160.5		321	0.6 ±0.30	0.38	1	0.50 -0.70	(2)
	F_2	c (0.8%)	617 622.1	2 6.9	6	209 205.1	834	0.8 ±0.21	0.30	2	0.80 -0.90	5*
fs-1–Ur-1	B	c (27.3%)	91 92.0	24 34.5	45 34.5	93 92.0	253	27.3 ±1.89	6.41	3	0.05 -0.10	(3)
	F_2	c (27.5%)	414 433.8	80 81.5	90 81.5	103 90.3	687	27.5 ±1.39	3.61	3	0.30 -0.50	6*
Group II ("*Pl*" group)												
d-2–lg	B	c (50%)	27 22.5	22 22.5	27 22.5	14 22.5	90	54.4 ±3.54	5.02	3	0.10 -0.20	(4)
	F_2	c (47.2%)	920 886.5	297 280.6	243 280.6	96 108.5	1556	47.2 ±2.65	8.69	3	0.02 -0.05	6*
d-2–Pr	B	c (43.7%)	51 49.0	34 38.0	42 38.0	47 49.0	174	43.7 ±2.65	1.01	3	0.70 -0.80	(5)
	F_2	c (50%)	341 325.1	121 108.4	90 108.4	26 36.1	578	52.8 ±2.16	8.19	3	0.02 -0.05	6*
d-2–P	B	c (50%)	62 65.75	72 65.75	66 65.75	63 65.75	263	52.5 ±2.08	0.92	3	0.80 -0.90	(6)
Pl–lg	B	c (25.6%)	92 93.0	31 32.0	33 32.0	94 93.0	250	25.6 ±1.86	0.084	3	>0.99	(7)
	F_2	c (30.9%)	1325 1344.4	289 271.0	267 271.0	272 260.8	2153	30.9 ±0.84	2.02	3	0.50 -0.70	6*
P–Pr	B	c (32.2%)	61 59.0	24 28.0	32 28.0	57 59.0	174	32.2 ±2.39	1.28	3	0.70 -0.80	(5)

Gene pair population		Linkage phase	Segregation mode AB	Ab	aB	ab	Total	Recombination value	χ^2	d.f.	p	Cross[1] or literature*
lg–Pr	B	c (26.7%)	46 44.0	16 16.0	16 16.0	42 44.0	120	26.7 ±2.72	0.18	3	0.98 –0.99	(4)
	F_2	c (28.2%)	486 497.4	93 95.8	103 95.8	109 101.9	791	28.2 ±1.31	1.37	3	0.70 –0.80	6*
Group III ("*A*" group)												
A–Pn	B	c (20.0%)	96 96.8	24 24.2	122 121.0		242	20.0 ±1.73	0.017	2	>0.99	(4)
	F_2	r (29.9%)	333 358.9	173 156.4	162 156.4	19 15.4	687	29.9 ±2.13	4.69	3	0.10 –0.20	6*
Group IV ("*g-1*" group)												
d-6–g-1	B	c (7.7%)	24 24.0	1 4.0	3	24 24.0	52	7.7 ±2.49	0.00	2	1.00	(8)
	F_2	r (14.0%)	383 363.5	178 176.5	156 176.5	3 3.5	720	14.0 ±2.45	3.42	2	0.10 –0.20	6*
Group VI+IX ("*d-1*" group)												
gh-1–st-2	B	c (48.2%)	97 107.5	104 100.0	96 100.0	118 107.5	415	48.2 ±1.65	2.37	3	0.30 –0.50	(9)
	F_2	r (40.9%)	282 275.9	106 105.9	101 105.9	20 21.4	509	40.9 ±2.43	0.45	3	0.90 –0.95	6*
gl-1–An-2	B	c (31.7%)	34 29.9	6 5.6	21 23.4	10 12.1	71	31.7 ±6.52	1.21	3	0.70 –0.80	(10)
	F_2	c (39.5%)	272 271.0	68 71.7	76 71.7	41 42.5	457	39.5 ±2.08	0.51	3	0.90 –0.95	6*

1) Cross combination:

(1) (H-69CMS×H-479)×H-479
(2) (H-69CMS×H-139)×H-139
(3) (H-69CMS×H-131)×H-131
(4) (A-58CMS×H-79)×H-79
(5) (H-61CMS×A-58)×H-61
(6) (H-61CMS×A-77)×H-61
(7) (H-126CMS×H-59)×H-59
(8) (H-21CMS×H-138)×H-138
(9) (A-58CMS×H-143)×H-143
(10) (H-69CMS×H-94)×H-103

2) Rocombination values with probable errors were calculated by IMMER's productive ratio method.[2]

The new recombination values were calculated by the method of maximum likelihood[1] from the combined data as shown in Table 4. Thus the accuracy of the recombination values was improved by the addition of backcross data.

In addition, a new linkage relation was found between *Pl* (Purple leaf) and *spr-1* (spreading panicle-1) with the recombination value, 27% in a coupling phase (Table 5).

The independent relations among the known marker genes are shown in Table 6. The results obtained indicated consistency with the data from F_2.

TABLE 4. Recombination values calculated from the combined data by maximum likelihood

Linkage group	Gene pair	Recombination value (%)	Homogeneity χ^2	d.f.	p
I	*C–st-1*	26.2±1.74	0.052	1	0.80–0.90
	fs-1–Ur-1	27.4±1.11	0.002	1	0.95–0.98
	d-2–lg	48.1±1.18	1.648	1	0.10–0.20
	d-2–Pr	48.9±1.61	3.137	1	0.05–0.10
II	*Pl–lg*	29.4±0.75	2.066	1	0.30–0.50
	Pr–lg	28.4±1.18	0.062	1	0.80–0.90
IV	*d-6–g-1*	11.2±1.59	0.934	1	0.30–0.50
VI+IX	*gh-1–st-2*	46.0±1.35	2.477	1	0.10–0.20

1) Probable error.
2) *Homogeneity test between backcross and F_2 data.

TABLE 5. A new linkage relation found between *Pl* (Purple leaf) and *spr-1* (spreading panicle) in the second linkage group

Cross combination: (H-126 CMS×H-23)×485

Gene pair		F_2 segregation Ab	Ab	aB	ab	Total	Linkage phase	Recombination value (%)
Pl–spr-1	Obs.	35	10	16	34	95	c	27.4±3.09
	Cal. (27.4%)	34.5	13.0	13.0	34.5			

$\chi^2=1.398$, d.f=3, p=0.70–0.80.

Discussion

In the linkage maps constructed hitherto, F_2 and F_3 data were mostly used for the calculation of recombination values[3,6]. However, it is profitable to add the backcross data from the stand point of the reliability of linkage intensities. Therefore, backcross populations were raised by the use of CMS genetic stocks in which the cytoplasm was replaced with [*ms-bo*] from Taichung 65 CMS by successive backcrossings.

It was demonstrated that the recombination values obtained from backcross data were in near accordance with those from F_2 coupling data, while they differed from those from F_2 repulsion data. Theoretically, the reliability of recombination values is exceedingly high in the backcross data compared with low recombination values calculated from F_2 repulsion data, 8 kinds of recombination values were recalculated from the combined data from backcross and F_2 data, and the accuracy of the linkage intensities was considerably improved. A new linkage relation was found between *spr-1* and *Pl*, both genes belonging to the second linkage group.

TABLE 6. Recombination values calculated between marker genes belonging different linkage groups

Gene pair (linkage group)	Recombination value (%)	
	Backcross	F_2
C(I)–*st-2*(VI+IX)	45	49
C(I)–*st-2*(VI+IX)	50	51
d-2(II)–*A*(III)	52	50
d-2(II)–*la*(VIII)	51	50
d-2(II)–*bc-1*(XI)	53	52
Pl(II)–*A*(III)	46	48
lg(II)–*A*(III)	53	49
lg(II)–*la*(VIII)	50	48
lg(II)–*bc-1*(XI)	51	51
Pr(II)–*A*(III)	45	47
Pr(II)–*la*(VIII)	50	53
Pl(II)–*Hg*(XII)	50	54
A(III)–*Hg*(XII)	53	51
A(III)–*st-2*(VI+IX)	45	51
A(III)–*gh-1*(VI+IX)	50	50
A(III)–*la*(VIII)	46	51
A(III)–*bc-1*(XI)	49	48
Dn(VII)–*nl-1*(VI+IX)	55	52
la(VIII)–*bc-1*(XI)	49	50

Furthermore, CMS multiple marker stocks are already used for the genic analysis of anthocyanin characters,[4] and they are useful for producing F_1 hybrids and backcross populations on a large scale. Thus it is valuable to use them for gene analysis and linkage studies.

Summary

13 kinds of cytoplasmic male sterile genetic stocks containing various marker genes were developed by the method of cytoplasmic substitution. The cytoplasm, [*ms-bo*] of Taichung 65 CMS was transferred to the respective marker stocks by successive backcrossings from 4 to 11 times.

By using these CMS genetic stocks, F_1 hybrid and backcross seeds were

produced efficiently without artificial emasculation. The linkage relations between the known marker genes were confirmed by backcross data. There was close proximity of the recombination values between F_2 coupling and backcross data, while the recombination values estimated from F_2 repulsion data exceeded those from backcross data.

Combined data of F_2 and backcross populations were used for the recalculation of recombination values by the method of maximum likelihood.

In addition, a new linkage relation was found between *spr-1* (spreading panicle) and *Pl* (Purple leaf), both genes belonging to the second linkage group. Furthermore, CMS multiple markers can be used for gene analysis of new mutant characters.

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