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INHERITANCE AND LINKAGE RELATIONSHIP ON ZEBRA CHLOROSIS AND ZEBRA NECROSIS IN RICE

—Genetical studies on rice plant, LXXXVIII[1,2]—

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Introduction

It is well known that the chlorophyll aberrations are governed by a single recessive gene or duplicate genes except in cases due to cytoplasmic inheritance. As to the zebra characters, at least five gene locus were known in the VIIIth, XIth and *su* linkage groups[9].

In this paper, the authors dealt with the inheritance of two kinds of zebra characters which show chlorosis and necrosis, respectively.

Materials and Methods

The nature of the mutants is explained as follows:

zebra chlorosis.... The mutant was first found in the population of a cultivar 'Do-hoku 21 go' after treatment with ethylene imine (EI) by Dr. Shinbashi in the Kamikawa branch, Hokkaido Prefectaral Agricultural Experiment Station. In the mutant character (Plate I), a heavy chlorosis occurs at the seeding stage and turns to the zebra bands around the fourth leaf stage. Though the green color recovers at the progressing stage, spikelets and anthers turn to whitish in the heading stage. The mutant strain, M-51 also behaves as a tillering dwarf together with the zebra character. Therefore it is estimated that both mutant characters were induced simultaneously by the chemical.

zebra necrosis.... The mutant was induced by gamma irradiation in the

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diploid strain, AC 581-3 which was raised by anther culture from F_1 plant of the cross, A-5 Akamuro × H-69. The mutant was chracterized by a kind of necrosis which appears as zebra bands of yellow or reddish color on the leaf blade with spacing of 3 or 4 mm in distance (Plate I). In zebra necrosis, the plant vigour was slightly reduced in comparison with normal plants.

The strains used in the expreiments are listed in Table 1. F_1 plants and F_2 populations were grown in the experimental paddy field or greenhouse in 1981 and 1982. Progeny tests using F_3 lines were carried out in the greenhouse during the winter. The recombination values were calculated by Immer's productive ratio or maximum likelihood methods.

TABLE 1. List of strains used in the experiments

a) mutants

Strain	Source	Mutant character
M-51	induced by EI treatment from 'Dohoku 21 go'	zebra chlorosis, tillering dwarf
M-52	induced by gamma irradiation from AC-581 : 3* (*C^{Br}, A, nl-1*)	zebra necrosis

* derived from F_1 hybrid, A-5×H-69 by anther culture.

b) Testers

Strain	Marker genes	Strain	Marker genes
A -5	*C^{Br}, A, Pr, I-Bf+, Rc, Rd*	H-839	*d-2, lg, bc-1*
H- 21	*bl-1, Rc, Sh*	H-840	*lax*
H- 79	*d-2, bc-1, lg, la*	H-841	*lg, tri, dl*
H-606	*C^{B}, A, Pl^{w}, I-Pl, Hg, lg*	N-105	*st-1, lg*
H-836	*bc-1, g-1*	N-110	*st-1, wx, lg*
H-837	*C^{B}, A, lg, g-1, la*	AC-7	*C^{Br}, A, Pr, I-Bf+, Rc, Rd*
H-838	*d-2, la*		

Results

1. Zebra chlorosis

In the cross combinations involving M-51, F_1 plants show a normal phenotype and the segregations for normal and zebra occur in all F_2 populations. As shown in Table 2, the segregation ratios of the crosses indicated a significant deviation from 3 : 1 or 15 : 1 ratios due to a single recessive or duplicate genes on the whole. Frequencies of zebra plants varied from 3.5% to 15.1% showing a considerable fluctuation.

TABLE 2. Segregations of zebra chlorosis in F_2 populations

Cross combination	Phenotype	Normal	Zebra chlorosis	Total	χ^2(3:1)	χ^2(15:1)	Freq. of zebra chlorosis (%)
H-836 × M-51	Obs.	309	21	330	61.13***	0.01	6.4
H-837 × "	"	186	10	196	41.39***	0.44	5.1
H-838 × "	"	248	44	292	15.36***	38.75***	15.1
H-839 × "	"	225	21	246	35.56***	2.20	8.5
H-840 × "	"	221	8	229	56.49***	2.97	3.5
H-841 × "	"	256	13	269	58.35***	0.92	4.8
N-105 × "	"	270	27	297	40.09***	4.09*	9.1
AC-7 × "	"	298	28	326	46.83***	3.04	8.6
M-51 × H-79	"	250	37	287	22.44***	21.61***	12.9
Total	"	2263	209	2472	360.91***	20.51***	8.5

*, *** Significant at the 5% and 0.1% level.

It is known that anomalous segregation of the crosses involving distantly related strains is caused by a linkage relation with the gene or genes for F_1 hydrid sterility[12]. Therefore, the possibility of the linkage with the sterility gene was first examined in this experiment. Although the average seed fertility of F_1 plants indicated 94.2% in the cross combination, M-51 × H-79, the frequency of zebra chlorosis resulted in 8.5%, showing a typical distorted ratio.

Thus, it is highly possible that a new gemetophyte gene is responsible for the occurrence of distortion interacting with marker genes. Assuming a new single recessive gene (*z-5*) for the zebra chlorosis, it was estimated that the distorted segregation of *z-5* is caused by a linkage relation between *z-5* and *ga-10 (t)* (tentatively designated) as well as in the cases of waxy endosperm[5], brittleness[11] and liguleless[10]. As to the other genes involved in the crossings, it was found that *lg* (liguleless) and *d (t)* (tentatively designated for tillering dwarf) also showed significant deviation from the monogenic ratio (Table 3). The frequencies of these recessive classes resulted in an excess of *lg* or shortage of *d (t)*. In addition, a close genetic association was found between *z-5* and *lg* as well as between *z-5* and *d-(t)* (Table 4 and 5). Therefore, it is probable that three genes, *z-5*, *lg* and *d (t)* belong to the second linkage group. Because of the excess of liguleless and shortage of zebra and tillering dwarf, it is probable that the mutant strain, M-51 possesses a recessive gametophyte gene *ga-10 (t)* which belongs to the second linkage group. The linkage phase between *ga-10 (t)* and *z-5* was a coupling

TABLE 3. Segregations of marker genes in F_2 populations of the crosses between M-51 and testers

Cross combination	Marker gene *A*	Linkage group		*A*	*a*	Total	χ^2 (3:1)	Freq. of *a* (%)
H-836 ×M-51	*g-1*	IV	Obs.	254	76	330	0.68	23.0
	bc-1	XI	"	251	79	330	0.20	23.9
	d(t)		"	308	22	330	59.16***	6.7
H-837 × "	*lg*	II	"	134	62	196	4.60*	31.6
	g-1	IV	"	150	46	196	0.24	23.5
	la	VIII	"	150	46	196	0.24	23.5
	d(t)		"	186	10	196	41.39***	5.1
H-838 × "	*d-2*	II	"	214	78	292	0.46	26.7
	la	VIII	"	215	77	292	0.29	26.4
	d(t)		"	241	51	292	8.84**	17.5
H-839 × "	*d-2*	II	"	184	62	246	0.01	25.2
	lg	II	"	167	79	246	6.64**	32.1
	bc-1	XI	"	177	69	246	1.22	28.0
	d(t)		"	219	27	246	25.80***	11.0
H-840 × "	*lax*	III	"	164	65	229	1.40	28.4
	d(t)		"	223	6	229	61.17***	2.6
H-841 ×M-51	*lg*	II	"	160	109	269	34.56***	40.5
	tri	X	"	216	53	269	4.03*	19.7
	dl	XI	"	202	67	269	0.00	24.9
	d(t)		"	254	15	269	54.13***	5.6
N-105 × "	*st-1*	I	"	223	74	297	0.00	24.9
	lg	II	"	171	126	297	48.09***	42.4
	d(t)		"	274	23	297	47.17***	7.7
AC-7 × "	*d(t)*		"	296	30	326	43.39***	9.2
M-51 ×H-79	*d-2*	II	"	214	73	287	0.03	25.4
	lg	II	"	204	83	287	2.35	28.9
	la	VIII	"	213	74	287	0.09	25.8
	bc-1	XI	"	223	64	287	1.12	22.3
	d(t)		"	248	39	287	19.93***	13.6

*, **, *** Significant at the 5%, 1% and 0.1% levels, respectively.

TABLE 4. Relationship between the segregations of zebra chlorosis, *z-5*, and tillering dwarf, *d (t)*

Cross combination		+	*z-5*	Total	Indepencence χ^2
H-836×M-51	+	308	0	308	313.98***
	d (t)	1	21	22	
H-837× »	+	186	0	186	196.00***
	d (t)	0	10	10	
H-838× »	+	241	0	241	244.81***
	d (t)	7	44	51	
H-839× »	+	219	0	219	186.23***
	d (t)	6	21	27	
H-840× »	+	221	2	223	170.21***
	d (t)	0	6	6	
H-841× »	+	254	0	254	231.31***
	d (t)	2	13	15	
N-105× »	+	269	5	274	226.03***
	d (t)	1	22	23	
AC-7× »	+	295	1	296	278.92***
	d (t)	3	27	30	
M-51 ×H-79	+	248	0	248	270.10***
	d (t)	2	37	39	
Total	+	2241	8	2249	2112.81***
	d (t)	22	201	223	

*** Significant at the 0.1% level.

TABLE 5. Relationship between the segregations of zebra chlorosis, *z-5*, and liguleless, *lg*

Cross combination		+	*z-5*	Total	Independence χ^2
H-837×M-51	+	124	10	134	4.87*
	lg	62	0	62	
H-839× »	+	146	21	167	10.86***
	lg	79	0	79	
H-841× »	+	147	13	160	9.31**
	lg	109	0	109	
N-105× »	+	144	27	171	21.88***
	lg	126	0	126	
M-51 ×H-79	+	167	37	204	17.28***
	lg	83	0	83	
Total	+	728	108	836	64.69***
	lg	459	0	459	

*, **, *** Significant at the 5%, 1% and 0.1% levels, respectively.

phase while the linkage between *ga-10* (*t*) and *lg* was a repulsion phase. Applying the formula advocated by IWATA *et al.*[5] recombination values between *z-5* and *ga-10*(*t*) and between *lg* and *ga-10*(*t*) were calculated as 1.4% and 26.6% respectively by using the data due to F_3 progeny test as shown in Tables 6 and 7. A fertilizing ability, K was calcuated by the formulas, $K=(p-2f)/(2f+p-1)$ in the coupling phase and $K=(1-p+2f)/(2f-p)$ in the repulsion phase. Inserting the average frequencies (f) of *z-5* or *lg* ($f_{z\text{-}5}=0.136$, $f_{lg}=0.289$), K was calculated as 0.3161 for the former and 0.500 for the latter. The discrepancy of K values might be caused from the fact that a small part of the zebra chlorosis died at seedling stage.

A linkage relation between *lg* and *z-5* was re-examined by using F_3 lines. From the segregations of *lg* and *z-5* within F_3 lines normal phenotypic

TABLE 6. Lnikage relationship between the gene for zebra and the gametophyte gene depending on the segregation mode in F_3 lines of the cross, M-51×H-79

Phenotype	*z-5*-shortage <22%	Normal 22-28%	*z-5*-excess >28%	Total	Goodness of fit	
Genotype	$\frac{z\text{-}5\ \ ga}{+\ \ +}$	$\frac{z\text{-}5\ \ ga}{+\ \ ga}$ $\frac{z\text{-}5\ \ +}{+\ \ +}$	$\frac{z\text{-}5\ \ +}{+\ \ ga}$		χ^2	p
Obs. no. of lines	136	2	1	139		
Expected freq.	$(1-p)^2$	$2p(1-p)$	p^2			
Cal. (p=1.4%)	135.135	3.838	0.027		35.95	<0.001

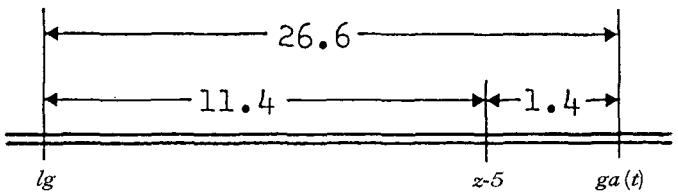
TABLE 7. Linkage relationship between *lg* and the gametophyte gene depending on the segregation mode in F_3 lines of the cross, M-51×H-79

Phenotype	*lg*-shortage <22%	Normal 22-28%	*lg*-excess >28%	Total	Goodness of fit	
Genotype	$\frac{lg\ \ ga}{+\ \ +}$	$\frac{lg\ \ ga}{+\ \ ga}$ $\frac{lg\ \ +}{+\ \ +}$	$\frac{lg\ \ +}{+\ \ ga}$		χ^2	p
Obs. no. of lines	14	49	82	145		
Expected freq.	p^2	$2p(1-p)$	$(1-p)^2$			
Cal. (p=26.6%)	10.26	56.62	78.12		2.58	0.3-0.2

TABLE 8. Linkage relationship between *z-5* and *lg*

Genotyse	++ ++	+++*lg*	+*z-5*++	+*z-5*+*lg*	Total	Goodness of fit χ^2	p
Obs.	0	20	14	125	159		
Cal. (1:2:2:4)	17.67	35.33	35.33	70.67		78.968	<0.001
Cal. (R.C.V.=11.4%)	1.027	15.956	15.956	126.061		2.301	0.6–0.5

plants in F_2 were classified as shown in Table 8. The recombination value between *lg* and *z-5* was estimated as 11.4% in the repulsion phase. The order of the three gene locus, *lg*, *z-5* and *ga-10(t)* were estimated as follows:



Thus, it is probable that the both genes, *z-5* and *d(t)* which were located in the second linkage group were induced simultaneously by the chemical mutagen and that the gamtophyte gene, *ga-10 (t)* was involved in the mutant strain M-51.

2. Zebra necrosis

There are various kinds of necrosis and discoloration of leaf blade and sheath. These genes are denoted as *bl* or *spl* series and the specific symbols such as *sl* (sekiguchi lesion) and *ysl* (yellow leaf spot). The mutant character resembled the zebra necrosis in maize[1,2]. Therefore the authors adopted the gene symbol, *zn*. In the cross combination ivolving the mutant strain M-52, F_1 plants showed a normal phenotype and F_2 segregations indicated a monogenic ratio, 3 : 1 for normal *vs.* zebra necrosis (Table 9). In two

TABLE 9. Segregations of zebra necrosis in F_2 populations of the crosses between M-52 and testers

Cross combination	Phenotype Genotype	Normal +	Zebra necrosis *zn*	Total	Goodness of fit χ^2(3:1)	p
H-21 ×M-52	Obs.	188	64	252	0.02	0.90–0.80
H-606× "	Obs.	226	73	299	0.05	0.90–0.80
Total	Obs.	414	137	551	0.01	0.95–0.90

Homogenity: χ^2=0.07, d. f.=1, p=0.8–0.7.

TABLE 10. Combined segregations between *zn* (zebra necrosis) and marker genes in F_2 populations

H-21×M-52

Gene pair A	Gene pair B	Linkage phase	R. C. V. (%)		F_2 segregation AB	Ab	aB	ab	Total	Goodness of fit Ratio	χ^2	p
C^{Br}	*zn*			Obs.	122	61	66	3	252	9:3:3:1	24.51	>0.001
		Rep.	20.2	Cal.	128.58	60.43	60.43	2.57	252.01		0.92	0.90–0.80
bl-1	"	Rep.	52.9	Obs.	140	45	48	19	252	9:3:3:1	0.81	0.90–0.80
nl-1	"	Coup.	49.2	Obs.	143	48	45	16	252	9:3:3:1	0.13	0.99–0.98
H-606×M-52												
Pl^w	*zn*	Coup.	55.7	Obs.	159	57	67	16	299	9:3:3:1	3.04	0.40–0.30
lg	"	Rep.	59.4	Obs.	179	48	47	25	299	9:3:3:1	5.45	0.20–0.10
nl-1	"	Coup.	43.8	Obs.	172	49	54	24	299	9:3:3:1	2.56	0.50–0.40
Hg	"	Coup.	52.8	Obs.	165	56	61	17	299	9:3:3:1	0.65	0.90–0.80

kinds of cross combinations, combined segregation ratios were examined to detect the linkage relations. As shown in Table 10, it is evident that the gene, *zn* has a linkage relation with the gene C^{Br} (Chromogen fo anthocyanin color) having a recombination value of 20.2% in the repulsion phase.

Discussion

As to the genes for zebra characters, two genes (*z-1* and *z-2*) are alloted to the eighth linkage group and *z-3* belongs to the eleventh linkage group[3,4,6,7,13]. In addition, it was found that *z-4* is located on chromosome 12 by trisomic analyses.[8] Since it was indicated that *z-5* belongs to the second linkage group, a new gene loci was alloted for the zebra chlorosis. However, the plants possessing a genotype of *z-5* indicates a heavy chlorosis at the seedling stage. There are two possibilities that these chlorophyll aberrations are caused by the pleiotropic action of *z-5* and the complete or close linkage with the gene of different kinds of chlorophyll aberration such as virescence. Further experiment is needed to discriminate the pleiotropic action and the linkage relation. It is already known that *ga-6* and *d-3* belong to the second linkage group (TAKAHASHI 1982). Since *ga-10* (*t*) and *d* (*t*) inserts their actions similar to *ga-6* and *d-3*, respectively, genic identification is now being conducted to elucidate the allelism.

It was shown that both zebra chlorosis and tillering dwarf were induced simultaneously by EI. It is an interesting fact that the different gene mutations occurred in the same linkage group.

It was found that the gene for zebra necrosis, *zn* belongs to the first linkage group. Some necrosis characters are caused by the gene or genes from *bl-* or *spl*-series. Though *spl-4* belongs to the first linkage group, relatively large reddish brown spots were different from those caused by *zn*. From the above finding, both characters can be used as markers for linkage analyses.

Summary

Two mutant characters, zebra chlorosis and zebra necrosis were induced by artifical mutations. Though the anomalous segregation ratios were obtained from the crosses involving the mutant strain of zebra chlorosis, it was estimated that the single recessive gene, *z-5* was linked with the gametophyte gene, *ga-10* (*t*) in the coupling phase. In addition, *z-5* linked with *lg* for liguleless and *d* (*t*) for tillering dwarf and belonged to the second linkage group. The order of gene locus was estimated as *lg-z-5-ga-10* (*t*) in the second linkage group.

Zebra necrosis was governed by a single recessive gene, *zn* which belongs to the first linkage group. Both of *z-5* and *zn* can be used as marker genes for linkage study.

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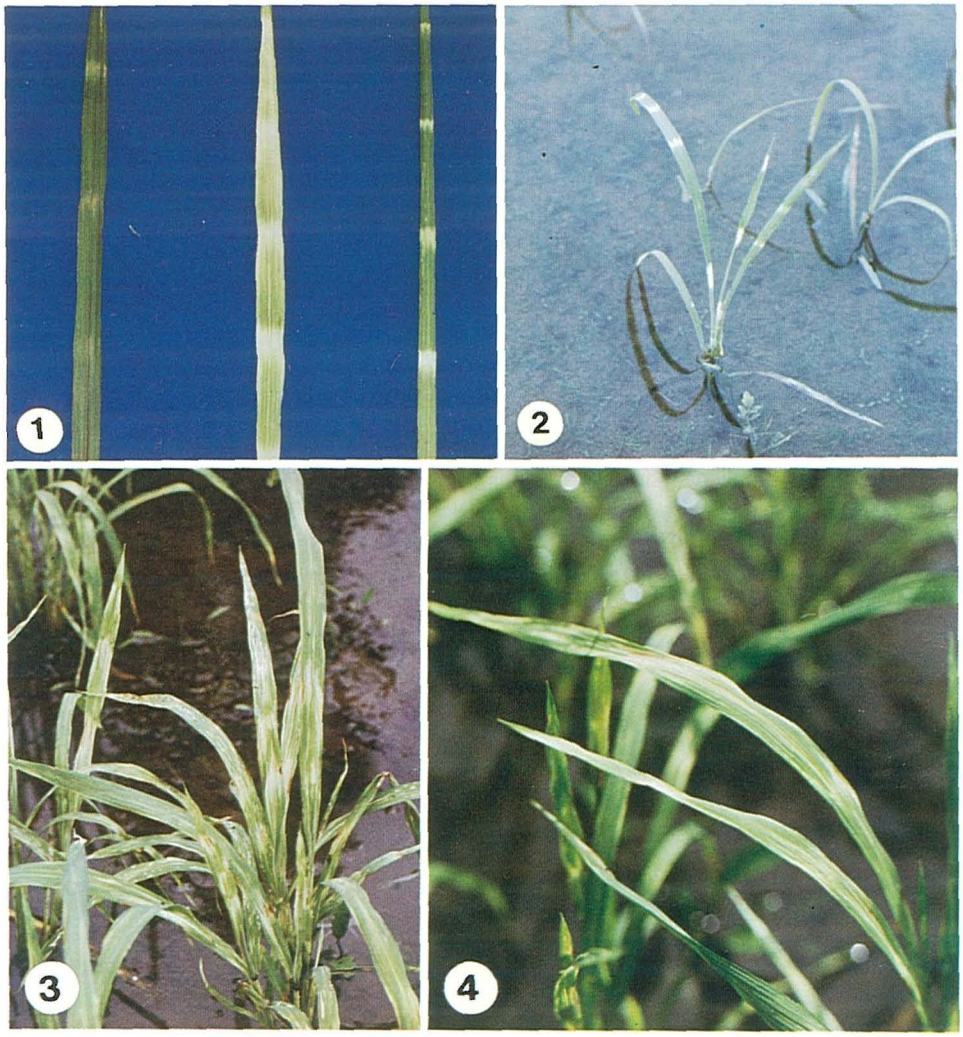
Legend for Plate 1

1. Zebra chlorosis (leaf blade of adult plant)
2. Zebra chlorosis (young plant in paddy field)
3. Zebra necrosis (plant in paddy field.)
4. Zebra necaosis (leaf blade of adult plant)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