



# HOKKAIDO UNIVERSITY

Title	Inheritance and Linkage Relationship on Zebra Chlorosis and Zebra Necrosis in Rice : Genetical studies on rice plant, LXXVIII
Author(s)	KINOSHITA, Toshiro; TAKAMURE, Itsuro
Citation	Journal of the Faculty of Agriculture, Hokkaido University, 61(4), 445-455
Issue Date	1984-03
Doc URL	<a href="https://hdl.handle.net/2115/13005">https://hdl.handle.net/2115/13005</a>
Type	departmental bulletin paper
File Information	61(4)_p445-455.pdf



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

— Genetical studies on rice plant, LXXXVIII<sup>1,2)</sup> —

**Toshiro KINOSHITA and Itsuro TAKAMURE**

(Plant Breeding Institute, Faculty of Agriculture,  
Hokkaido University, Sapporo, Japan)

(Received November 19, 1983)

## 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<sup>9</sup>.

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 Prefectural 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

---

[J. Fac. Agr. Hokkaido Univ., Vol. 61, Pt. 4, 1984]

- 1) Contribution from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.
- 2) This study was partly defrayed by Grant-in-Aid for Co-operative Research (A) 56360001 from the Ministry of Education, Scientific and Culture, Japan.

diploid strain, AC 581-3 which was raised by anther culture from F<sub>1</sub> plant of the cross, A-5 Akamuro × H-69. The mutant was characterized 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 experiments are listed in Table 1. F<sub>1</sub> plants and F<sub>2</sub> populations were grown in the experimental paddy field or greenhouse in 1981 and 1982. Progeny tests using F<sub>3</sub> 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* ( <i>C<sup>Br</sup></i> , <i>A</i> , <i>nl-1</i> )	zebra necrosis

\* derived from F<sub>1</sub> hybrid, A-5 × H-69 by anther culture.

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

## Results

### 1. Zebra chlorosis

In the cross combinations involving M-51, F<sub>1</sub> plants show a normal phenotype and the segregations for normal and zebra occur in all F<sub>2</sub> 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<sub>2</sub> populations

Cross combination	Pheno-type	Normal	Zebra chlo-rosis	Total	$\chi^2(3:1)$	$\chi^2(15:1)$	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
A C-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<sub>1</sub> hybrid sterility<sup>12</sup>. Therefore, the possibility of the linkage with the sterility gene was first examined in this experiment. Although the average seed fertility of F<sub>1</sub> 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 gametophyte 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<sup>5</sup>, brittleness<sup>10</sup> and liguleless<sup>10</sup>. 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		Obs.	A	a	Total	$\chi^2$ (3:1)	Freq. of a (%)
	A	Linkage group						
H-836 × M-51	<i>g-1</i>	IV	254	76	330	0.68	23.0	
	<i>bc-1</i>	XI	251	79	330	0.20	23.9	
	<i>d(t)</i>		308	22	330	59.16***	6.7	
H-837 × "	<i>lg</i>	II	134	62	196	4.60*	31.6	
	<i>g-1</i>	IV	150	46	196	0.24	23.5	
	<i>la</i>	VIII	150	46	196	0.24	23.5	
H-838 × "	<i>d(t)</i>		186	10	196	41.39***	5.1	
	<i>d-2</i>	II	214	78	292	0.46	26.7	
	<i>la</i>	VIII	215	77	292	0.29	26.4	
H-839 × "	<i>d(t)</i>		241	51	292	8.84**	17.5	
	<i>d-2</i>	II	184	62	246	0.01	25.2	
	<i>lg</i>	II	167	79	246	6.64**	32.1	
H-840 × "	<i>bc-1</i>	XI	177	69	246	1.22	28.0	
	<i>d(t)</i>		219	27	246	25.80***	11.0	
	<i>lax</i>	III	164	65	229	1.40	28.4	
H-841 × M-51	<i>d(t)</i>		223	6	229	61.17***	2.6	
	<i>lg</i>	II	160	109	269	34.56***	40.5	
N-105 × "	<i>tri</i>	X	216	53	269	4.03*	19.7	
	<i>dl</i>	XI	202	67	269	0.00	24.9	
	<i>d(t)</i>		254	15	269	54.13***	5.6	
M-51 × H-79	<i>st-1</i>	I	223	74	297	0.00	24.9	
	<i>lg</i>	II	171	126	297	48.09***	42.4	
	<i>d(t)</i>		274	23	297	47.17***	7.7	
A C-7 × "	<i>d(t)</i>		296	30	326	43.39***	9.2	
M-51 × H-79	<i>d-2</i>	II	214	73	287	0.03	25.4	
	<i>lg</i>	II	204	83	287	2.35	28.9	
	<i>la</i>	VIII	213	74	287	0.09	25.8	
	<i>bc-1</i>	XI	223	64	287	1.12	22.3	
	<i>d(t)</i>		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		+	<i>z-5</i>	Total	Independence $\chi^2$
H-836×M-51	+	308	0	308	313.98***
	<i>d(t)</i>	1	21	22	
H-837× "	+	186	0	186	196.00***
	<i>d(t)</i>	0	10	10	
H-838× "	+	241	0	241	244.81***
	<i>d(t)</i>	7	44	51	
H-839× "	+	219	0	219	186.23***
	<i>d(t)</i>	6	21	27	
H-840× "	+	221	2	223	170.21***
	<i>d(t)</i>	0	6	6	
H-841× "	+	254	0	254	231.31***
	<i>d(t)</i>	2	13	15	
N-105× "	+	269	5	274	226.03***
	<i>d(t)</i>	1	22	23	
A C-7× "	+	295	1	296	278.92***
	<i>d(t)</i>	3	27	30	
M-51 ×H-79	+	248	0	248	270.10***
	<i>d(t)</i>	2	37	39	
Total	+	2241	8	2249	2112.81***
	<i>d(t)</i>	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		+	<i>z-5</i>	Total	Independence $\chi^2$
H-837×M-51	+	124	10	134	4.87*
	<i>lg</i>	62	0	62	
H-839× "	+	146	21	167	10.86***
	<i>lg</i>	79	0	79	
H-841× "	+	147	13	160	9.31**
	<i>lg</i>	109	0	109	
N-105× "	+	144	27	171	21.88***
	<i>lg</i>	126	0	126	
M-51 ×H-79	+	167	37	204	17.28***
	<i>lg</i>	83	0	83	
Total	+	728	108	836	64.69***
	<i>lg</i>	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.*<sup>6)</sup> 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<sub>3</sub> progeny test as shown in Tables 6 and 7. A fertilizing ability, K was calculated 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* ( $f_{z-5}=0.136$ ,  $f_{lg}=0.289$ ), 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<sub>3</sub> lines. From the segregations of *lg* and *z-5* within F<sub>3</sub> lines normal phenotypic

TABLE 6. Linkage relationship between the gene for zebra and the gametophyte gene depending on the segregation mode in F<sub>3</sub> lines of the cross, M-51×H-79

Phenotype	<i>z-5</i> -shortage <22%		Normal 22-28%	<i>z-5</i> -excess >28%	Total	Goodness of fit	
Genotype	<u><i>z-5</i></u> +	<u><i>ga</i></u> +	<u><i>z-5</i></u> +	<u><i>ga</i></u> +		$\chi^2$	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

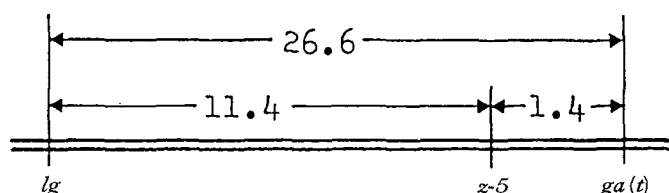
TABLE 7. Linkage relationship between *lg* and the gametophyte gene depending on the segregation mode in F<sub>3</sub> lines of the cross, M-51×H-79

Phenotype	<i>lg</i> -shortage <22%		Normal 22-28%	<i>lg</i> -excess >28%	Total	Goodness of fit	
Genotype	<u><i>lg</i></u> +	<u><i>ga</i></u> +	<u><i>lg</i></u> +	<u><i>ga</i></u> +		$\chi^2$	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*

Genotype	++ ++	+++ <i>lg</i>	+ <i>z-5</i> ++	+ <i>z-5</i> + <i>lg</i>	Total	Goodness of fit $\chi^2$	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<sup>1,20</sup>. Therefore the authors adopted the gene symbol, *zn*. In the cross combination involving 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 <i>zn</i>	Total	Goodness of fit $\chi^2(3:1)$	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

Homogeneity :  $\chi^2=0.07$ , d. f. = 1, p=0.8-0.7.

TABLE 10. Combined segregations between *zn* (zebra necrosis) and marker genes in F<sub>2</sub> populations

H-21×M-52												
Gene pair <i>A</i>	<i>B</i>	Linkage phase	R. C. V. (%)	F <sub>2</sub> segregation				Total	Goodness of fit			
				<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>		Ratio	$\chi^2$	p	
<i>CBr</i>	<i>zn</i>	Rep.	20.2	Obs.	122	61	66	3	252	9:3:3:1	24.51	>0.001
				Cal.	128.58	60.43	60.43	2.57	252.01		0.92	0.90-0.80
<i>bl-1</i>	"	Rep.	52.9	Obs.	140	45	48	19	252	9:3:3:1	0.81	0.90-0.80
<i>nl-1</i>	"	Coup.	49.2	Obs.	143	48	45	16	252	9:3:3:1	0.13	0.99-0.98
H-606×M-52												
<i>Pl<sup>w</sup></i>	<i>zn</i>	Coup.	55.7	Obs.	159	57	67	16	299	9:3:3:1	3.04	0.40-0.30
<i>lg</i>	"	Rep.	59.4	Obs.	179	48	47	25	299	9:3:3:1	5.45	0.20-0.10
<i>nl-1</i>	"	Coup.	43.8	Obs.	172	49	54	24	299	9:3:3:1	2.56	0.50-0.40
<i>Hg</i>	"	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<sup>Br</sup>* (Chromogen for 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 allotted to the eighth linkage group and *z-3* belongs to the eleventh linkage group<sup>3,4,6,7,13</sup>. In addition, it was found that *z-4* is located on chromosome 12 by trisomic analyses.<sup>9</sup> Since it was indicated that *z-5* belongs to the second linkage group, a new gene loci was allotted 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 artificial 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.

#### Acknowledgement

The authors wish to express the sincere thanks to Dr. N. SHINBASHI and Dr. M. MAEKAWA for their cooperation during the study.

#### Literature Cited

1. GIESHRECHT, J.: A second zebra-necrotic gene in maize. *J. Hered.* **56**: 183-185. 1965
2. HOROVITZ, S.: Zebra necrosis. *Maize Genet. Co-op. Newsletter* **22**: 42-43. 1948
3. IWATA, N. and T. OMURA.: Studies on the trisomics in rice plants (*Oryza stiva* L.) III Relation between trisomic and genetic linkage groups. *Japan J. Breed.* **25** (6): 363-368. 1975
4. IWATA, N. and T. OMURA.: Linkage studies in rice (*Oryza sativa* L.). On some mutants derived from chronic gamma irradiation. *J. Fac. Agr., Kyushu Univ.* **21**: 117-127. 1977
5. IWATA, N. NAGAMATSU, T. and T. OMURA: Abnormal segregation of waxy and apiculus coloration by a gametophyte gene belonging to the first linkage group in rice. *Japan. J. Breed.* **14** (1): 33-39, 1964 (in Japanese with English summary)
6. IWATA, N. OMURA, T. and H. SATOH.: Linkage studies in rice. The sequence of genes at the eighth and eleventh linkage groups. *Jap. J. Breed.* **28** (Suppl. 1): 170-171. 1978 (in Japanese)
7. IWATA, N., H. SATOH and T. OMURA.: Linkage studies in rice. New genes belonging to the 11th linkage group. *Jap. J. Breed.* **29** (Suppl. 2): 182-183. 1979 (in Japanese)
8. IWATA, N., H. SATOH and T. OMURA.: Linkage studies in rice. On some marker genes locating on chromosome 12. *Jap. J. Breed.* **33** (Suppl. 1): 114-115. 1983 (in Japanese)
9. KINOSHITA, T.: In Present Status of Genic Analyses in rice. Ed. T. OMURA, Fukuoka: 11-28. 1982 (in Japanese)
10. MAEKAWA, M., T. KINOSHITA and M. TAKAHASHI: A new gametophyte gene in the second linkage group of rice. *Genetical studies on rice plant, LXXVI.* *J. Fac. Agr. Hokkaido Univ.* **60** (2): 107-114. 1981
11. NAKAGAHARA, M.: Genic mechanism on the distorted segregation of marker genes belonging to the eleventh linkage group in cultivated rice. *Japan J. Breed.* **22**: 232-238. 1972
12. OKA, H.: Analysis of genes controlling F<sub>1</sub> sterility in rice by the use of isogenic lines. *Genetics* **77**: 521-534. 1974

13. OMURA, T. and N. IWATA.: Linkage studies in rice. On the linkage groups 8, 10 and 11. *Jap. J. Breed.* 22 (Suppl. 1): 43-44. 1972 (in Japanese)
14. TAKAHASHI, M.: Gene analyses and its related problems. Genetic studies on rice plant, LXXX. *J. Fac. Agr., Hokkaido Univ.* 61(1): 91-142. 1982

**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 necrosis (leaf blade of adult plant)

