A NEW GAMETOPHYTE GENE IN THE SECOND LINKAGE GROUP OF RICE[1)]

— Genetical studies on rice plant, LXXVI —

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Introduction

It is well known that the several gametophyte genes are located in the first and eleventh linkage groups[1,4,2)]. These genes are responsible for the occurrence of distortion of segregation ratios by marker genes which are linked with them. In the present report, the authors found a new gametophyte gene in the second linkage group through the distorted segregation on liguleless character.

Materials and Methods

Strains and marker genes used in this experiment are shown in Table 1. A mutant line, M-533 was induced by the irradiation of the cultivar, 'Nohrin 8 go' by Dr. Tanaka, Institute of Radiation Breeding, NIAS MASS.

TABLE 1. List of the strains and marker genes

Strain	Marker gene	Source
M-533	lg^+	Mutant induced by the gamma irradiation from the variety 'Nohrin 8 go'
H- 79	$lg\ d_2\ bc\ la$	
H-152	$lg^+\ C^{Bp}\ A\ Pl^w\ I\text{-}Pl\ Hl_a$	
No. 1500	$lg^+\ d_2\ C^{Bp}\ A\ P^+\ Pn$	
No. 1501	lg^+	
No. 1502	lg	Progeny of the cross, M-533×H-79
A-136	lg^+	Promising variety 'Shiokari' in Hokkaido
H-79 MS	$lg\ d_2\ bc\ la$	Cytoplasmic male sterile line of H-79

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Crossings were made between the M-533 and linkage markers. Most of F_1 hybrids indicated sound pollen and seed fertilities. The liguleless character was screened at the seedling stage in the segregating populations of the crosses involving M-533. The recombination values were calculated by the maximum likelihood method.

Results

(1) Aberrant segregation of the liguleless character

It is recognized that the liguleless character is governed by a single recessive gene, *lg*, belonging to the second linkage group[3]. As shown in Table 2, two cross combinations, No. 1500 × H-79 and No. 1501 × H-79, showed a monogenic segregation (3 : 1) showing the percentages of the liguleless plants as 27.6 and 26.8, respectively.

TABLE 2. Abnormal segregation of liguleless brought in F_2 of the cross between M-533 and H-79 as compared with the normal segregation in F_2

Cross comb.	Phenotype		Total	Goodness of fit (3 : 1)		Percentage of liguleless (%)
	Normal	liguleless		χ^2	P	
Normal segregation						
No. 1500 × H-79	202	77	279	1.00	0.50-0.30	27.6
No. 1501 × H-79	218	80	298	0.54	0.50-0.30	26.8
Abnormal segregation						
M-533 × H-79	110	75	185	23.83	<0.001	40.5
〃 × 〃	851	557	1408	159.19	<0.001	39.6
Total	961	632	1593	182.93	<0.001	39.7

In contrast with this, two F_2 populations from the cross, M-533 × H-79, indicated a significant deviation from the monogenic ratio and the percentages of the liguleless plants increased to 40.5 and 39.6, respectively. The aberrant type of segregation resembled those of waxy endosperm and brittleness caused by gametophyte gene or genes[1,2,4].

In F_1 plants of the cross, M-533 × H-79, pollen and seed fertilities was fairly high showing 96.2 and 91.8% respectively. Therefore, there is but a limited possibility for the aberrant segregation caused by genes for gametic development in hybrid sterility.

When F_1 plants of the cross, M-533 × H-79, were backcrossed to H-79,

TABLE 3. B_1F_1 segregation of liguleless in reciprocal crossings between (M-533×H-79) F_1 and H-79

Cross comb.	Phenotype / Genotype	Normal $\frac{+\ +}{lg\ +}, \frac{+\ ga_6}{lg\ +}$	liguleless $\frac{lg\ +}{lg\ +}, \frac{lg\ ga_6}{lg\ +}$	Total	Goodness of fit (1:1) χ^2	P	Percentage of liguleless (%)
(M-533×H-79) × H-79	Obs.	324	370	694			53.3
	Cal.	347	347		3.05	0.10-0.05	
H-79 MS × (M-533×H-79)	Obs.	9	26	35	8.26	0.10-0.001	74.3
H-79 × (M-533×H-79)	Obs.	11	39	50	15.68	<0.001	78.0
Total	Obs.	20	65	85	23.82	<0.001	76.5
	Cal.*	19.97	65.03		0.00		

* The recombination value between *lg* and ga_6 was estimated as 4.2% and fertilizing ability was calculated as 0.2669.

a monogenic ratio of 1 : 1 was satisfied as shown in Table 3. However, the shortage of normal (liguled) plants was prominent in the two progenies of the reciprocal crossings such as H-79×(M-533×H-79). Thus, it was ascertained that the pollen certation caused by a gametophyte gene, ga_6 caused the distorted ratio of *lg* segregation both in B_1F_1 and F_2 populations. The genotypes of M-533 and H-79 or H-79 MS were estimated to be $lg^+\ lg^+\ ga_6\ ga_6$ and $lg\ lg\ ga_6^+\ ga_6^+$, respectively.

(2) **Recombination value between *lg* and ga_6**

Because of the excess of liguleless plants both in the B_1F_1 and the F_2 populations, the linkage relation between *lg* and ga_6 was estimated as a repulsion phase. Inserting p $(0<p<0.5)$ as a recombination value and K $(0<K<1)$ as a fertilizing ability, then the theoretical frequencies of four genotypes such as $+ga_6/lg+$, $++/lg+$, $lg\ ga_6/lg+$ and $lg+/lg+$ in B_1F_1 generation were expected as $(1-p)/2$, $p/2$, $p/2$ and $(1-p)/2$, respectively. When B_1F_2 progenies were produced from the heterozygous genotypes of *lg*, two kinds of segregation types showing a monogenic segregation and the excess of liguleless segregants were produced. This is as shown in Fig. 1. If the normal segregation is restricted to the percentages of liguleless plants from 22 to 28%, the normal and the excess types segregated in the ratio of 11 : 252 (Table 4). The recombination value between *lg* anf ga_6 was calculated as 4.2%.

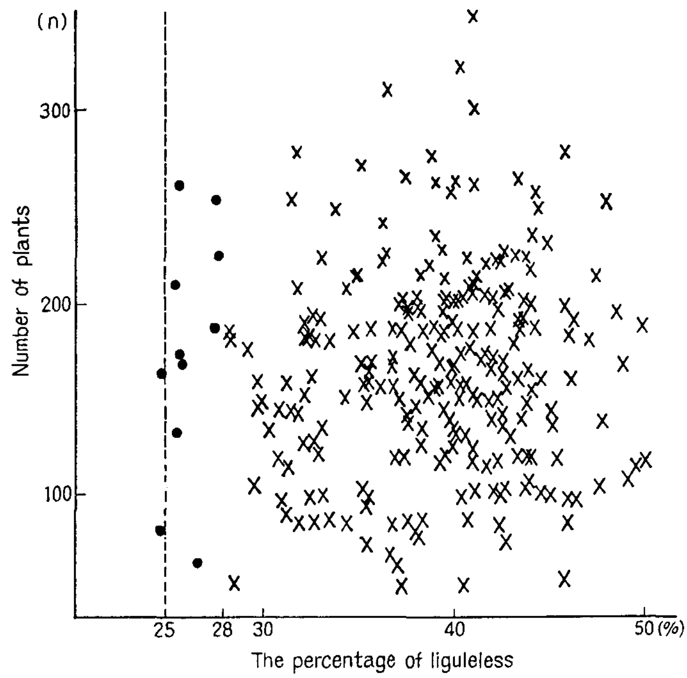

Fig. 1. Frequencies of relative percentages for liguleless plants obtained in B_1F_2 progenies of the backcross, (M-533×H-79)×H-79.
Note; ● Normal type of segregation of *lg*.
× Excess type of segregation of *lg*.

TABLE 4. Segregation of normal and distorted types for liguleless obtained from self-pollination of heterozygous genotype, $+lg$ in B_1F_2 of the cross, (M-533×H-79)×H-79, and in F_3 of the cross, M-533×H-79

Type of *lg*-segregation	Shortage (1-22)*	Normal (22-28)	Excess (28-60)	Total	Goodness of fit	
Genotype	$\frac{lg\ \ ga_6}{+\ \ +}$	$\frac{lg\ \ ga_6}{+\ \ ga_6}$ $\frac{lg\ \ +^{**}}{+\ \ +}$	$\frac{lg\ \ +^{**}}{+\ \ ga_6}$		χ^2	P
(M-533×H-79)×H-79 B_1F_2 progenies						
Obs. no. of lines		11	252	263		
Theor. freq.		p	(1-p)			
Cal. no. of lines (p=0.042)		11.05	251.95		0.00	>0.99
M-533×H-79 F_3 progenies						
Obs. no. of lines	1	12	36	49		
Theor. freq.	p^2	2p(1-p)	$(1-p)^2$			
Cal. no. of lines (p=0.143)	1.00	12.01	35.99		0.00	>0.99

* Percentage of liguleless plants.
** Genotypes in B_1F_1 of the cross, (M-533×H-79)×H-79.

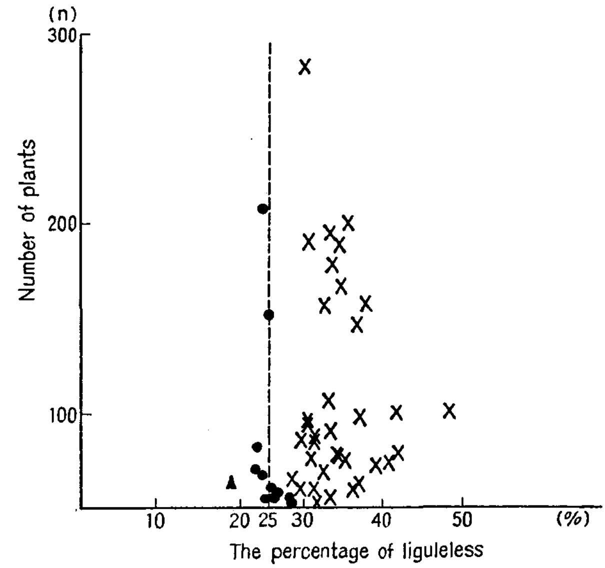

Fig. 2. Frequencies of relative percentages for liguleless plants obtained in F_3 progenies of the cross between M-533 and H-79.

Note; ▲ Shortage type of segregation of *lg*.
● Normal type of segregation of *lg*.
× Excess type of segregation of *lg*.

TABLE 5. F_2 segregation of liguleless of the cross between A-136 and No. 1502, a progeny derived from the cross, M-533×H-79

Cross comb.	Genotype of cross		Phenotype: Normal	Phenotype: liguleless	Total	Ratio	Goodness of fit: χ^2	Goodness of fit: P	liguleless (%)
A-136 × No. 1502	+ + + + × *lg lg* ga_6 ga_6	Obs.	994	99	1093	3:1	148.16	<0.001	9.1
		Cal.*	972.35	120.65			4.37	0.05–0.02	

* Based on the recombination value (p)=4.2% between *lg* and ga_6 and fertilizing ability (K)=0.2425.

TABLE 6. F_2 segregation of marker genes belonging to the second linkage group in the two crosses, M-533×H-79 and M-533×H-152

Cross comb.	Marker gene	Obs. no. of F_2 plants: Dominant	Obs. no. of F_2 plants: Recessive	Total	Goodness of fit: Ratio	χ^2	P
M-533×H-79	d_2	140	45	185	3: 1	0.05	0.90–0.80
M-533×H-152	Pl^w	222	202	424	27:37	17.98	<0.001

To estimate the recombination value by another method, the F_3 lines were grown from the selfing of the heterogeneous genotype of *lg*. In this case, another type of segregation showing the shortage segregants of liguleless plants was expected besides the said two types. As shown in Fig. 2 and Table 4, one line of shortage type appeared out of the other two types. By using F_3 data, the recombination value of 14.3% was calculated.

Since the liguleless percentages in F_2 and B_1F_1 populations are theoretically expected to be $(1-p+pK)/2(1+K)$ and $(1-p+pK)/(1+K)$, the fertilizing ability of K was calculated as 0.2181 and 0.2669, respectively.

A liguleless line, No. 1502, was selected from F_4 progenies of the cross, M-533 × H-79 and crossed with a cultivar 'Shiokari'. In the F_2 population, the liguleless plants showed a pronounced shortage from the expected number. Therefore, it was estimated that No. 1502 was bred true from a crossover plant which possessed both genes in a coupling phase. Substituting $p=4.2\%$, $K=0.2425$ in this case, expected numbers of normal and liguleless plants in F_2 population were calculated to be 972.35 and 120.65, as shown in Table 5. Although the fitness was not satisfactory, the observed number approached closer to the expected numbers than those obtained from 3 : 1 ratio.

In F_2 of the crosses, M-533 × H-79 and M-533 × H-152, the segregation of d_2 (ebisu dwarf) fitted in a monogenic ratio, while the segregation of leaf blade coloration which depends on the complementary action of *C*, *A* and Pl^w indicated a significant deviation from the expected ratio (27 : 37). Although both genes, *lg* and Pl^w belong to the second linkage group, Pl^w is much closer to *lg* than d_2. Possibly the distorted segregation of Pl^w depends on the linkage relation between Pl^w and ga_6, while the effect of ga_6 for the d_2 segregation was considerably reduced because of the distant locus from ga_6.

Discussion

Three gametophyte genes, ga_1, ga_2, ga_3 were found in the first linkage group and the other genes, ga_4 and ga_5 were located in the eleventh linkage group[1,2,4,5]. In this study, a new gametophyte gene, ga_6 was found out in the second linkage group. A close linkage relationship of this gene with *lg* in the recombination value of 4.2%, caused an excess or shortage of liguleless plants in the segregating populations. As to the segregation of liguleless character, a digenic segregation of 15 normal : 1 ligueless was reported by SASTRY[7] in addition to the monogenic segregation which is prominent in many experiments. There is a possibility that the distortion of the segregation by the gametophyte gene bring about a similar ratio by the digenic segregation in the coupling phase of *lg* and ga_6.

Among the six kinds of gametophyte genes, ga_1 and ga_6 were induced by the mutation of atomic bomb and gamma ray irradiation, respectively[1]. The other genes were found from the crossings between *Japonica* and *Indica*. Thus, there is a possibility that many loci of the gametophyte genes are responsible for the aberration of genic segregations. In a similar respect, OKA[6] found that the many sets of gametic-development genes for F_1 hybrid sterility caused the abnormal segregation of genes which are linked with the said genes. As to the liguleless character, there is a distortion of the segregation by the linkage with gene, s_2. In the future studies, the authors intend to elucidate the relation between the genes responsible for gametophyte and gametic-development, comparing the action of ga_6 with that of s_2.

Summary

A distorted segregation of liguleless character occurred in the segregating populations in the cross combinations involving the line M-533 which was induced by gamma irradiation. Namely, F_2 population of the cross, M-533 × H-79 showed a percentage of liguleless plants (*lg*%) of 39.7% which was significantly different from the monogenic ratio (*lg*% = 25). In the reciprocal crossings between (M-533 × H-79) F_1 and H-79, *lg*% was remarkably increased when F_1 plants were used as pollen parents, while liguleless character segregated into 1 : 1 in the B_1F_1 populations of the cross, F_1 × H-79. In addition, F_1 and F_2 plants showed sound pollen and seed fertilities. Therefore it was proven that the linkage relation between ga_6 and *lg* was responsible for the abnormal segregation of liguleless character. The genotypes of M-533 and H-79 were estimated as $+\ ga_6/+\ ga_6$ and $lg\ +/lg\ +$, respectively.

Based on the frequency of *lg*% in B_1F_2 progenies, the recombination value between *lg* and ga_6 was calculated to be 4.2% in a repulsion phase and fertilizing ability of ga_6 against ga_6^+ pollen grain was estimated to be 0.2425. Since M-533 carried a gametophyte gene, ga_6, it is probable that ga_6 was induced by gamma irradiation from the original cultivar, 'Nohrin 8 go'.

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