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INHERITANCE STUDIES ON CYTOPLASMIC MALE STERILITY INDUCED BY NUCLEAR SUBSTITUTION[1)]

— Genetical studies on rice plant, LXX —

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Introduction

Since alloplasmic male sterility was induced in *Epilobium*[9)] and wheat[5)] by nuclear substitution, the source of male sterility was remarkably enlarged for the utilization of this character to produce hybrid seeds on a large scale. In rice plants, the effect of alien cytoplasm in the pollen and spikelet fertility in F_1 hybrids was noticed by several workers[6,7)] and male sterile strains were produced by means of nuclear substitution[2,11,14,15)]. In this study, the authors produced new male sterile lines by substitution of the cytoplasm and two kinds of pollen fertility restoration lines which are used for the *boro*-cytoplasm male sterile tester were found among the linkage testers. The genic analysis for the pollen restoration under the *boro*-cytoplasm was carried out by using crosses between the male sterile and the pollen restorer strains.

Materials and Methods

The strains used in this experiment are listed in Table 1. The breeding of male sterile strains started from the reciprocal crossings between Japanese and Indian varieties. After successive backcrossings, male sterility was bred true by the pollination of the Japanese varieties. Pollen fertility restorers were selected from 16 strains of linkage tester by the crossings with the *boro*-cytoplasm tester which were donated by SHINJO of Ryukyu Uni-

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TABLE 1. List of the strains used in this study

Strain		Genotype	Remarks
A- 5	Akamuro	C^{Br}, *A*, *Pr*, *Rc*, *Rd*	Linkage marker
A- 13	Chabo	C^{B}, A^{+}, *Pr*	"
A- 43	Hokkaimochi-1-go	C^{+}, A^{+}, *wx*	"
A-133	Norin-9-go	$rf_1 rf_1$	Nuclear parent for K-11 and K-13
I- 44	Bhutmuri-36		Indian variety
I- 88	Assam III		"
I- 34	Mushakdanti		"
I- 45	Charnock		"
I-127	SHINJO's tester	(*cms-boro*) $rf_1 rf_1$	Cytoplasmic male strility
I-128	Taichung-65-go	$rf_1 rf_1$	Maintainer for I-127
I-130	SHINJO's tester	(*cms-boro*) $Rf_1 Rf_1$	
K- 11	Nuclear substitution line		I-44×A-133 SB_7
K- 13	"		I-88×A-133 SB_{10}

versity. The mode of inheritance on pollen fertility restoration was studied using the hybrid populations derived from the crossings between the male sterile strain with *boro*-cytoplasm and the pollen fertility restorers. Besides that, the genetical nature of the cytoplasm in the newly induced strains was identified with those of *boro*-cytoplasm testers.

The effect of environmental condition was examined by growing the hybrid populations in a growth chamber under different temperature conditions. In order to protect against the effects of low temperatures during microsporogenesis, most of the materials were grown in geen or vinyl houses. For pollen counts, three out of five anthers were collected from the main panicles of each plant and pollens were macerated in a drop of 1-percent iodium potassium iodide. About 300-500 pollen grains per plant were counted. The discrimination between fertile and sterile pollens depended on the intensity of staining by the solution. Spikelet fertility was examined by using the early emerging panicles from each plant.

Results

1. Reciprocal differences of pollen and spikelet fertility in F_1 hybrids

Diallel crossings in reciprocal combinations were made between eight

kinds of Japanese and Indian varieties. As shown in Table 2, significant differences of both fertilities were recognized between the reciprocal hybrids of the crosses involving I-44 and I-88. It is reasonable that the effect of cytoplasm is responsible for the hybrid sterility as well as a nuclear gene or genes.

TABLE 2. Pollen (a) and spikelet (b) fertilities (%) in the F_1 hybrids of the reciprocal crossings between Japanese and Indian varieties and the test of singnificance of the reciprocal difference

a. Pollen fertility

Japanese var. \ Indian var.	I-44 Bhutmuri-36 ♀	I-44 Bhutmuri-36 ♂	I-88 Assam III ♀	I-88 Assam III ♂	I-34 Mushakdanti ♀	I-34 Mushakdanti ♂	I-45 Charnock ♀	I-45 Charnock ♂
A- 5 Akamuro	23	45	17	29	88	89	41	53
	t=16.94**		t=5.99**		t=0.42		t=2.72*	
A- 13 Chabo	13	43	17	28	88	89	11	14
	t=27.07**		t=7.26**		t=0.26		t=2.24*	
A- 43 Hokkaimochi-1-go	17	23	14	22	61	63	13	15
	t= 5.84**		t=3.78**		t=0.64		t=0.55	
A-133 Norin-9-go	11	34	19	27	74	73	18	18
	t=18.76**		t=9.22**		t=0.05		t=0	

* Significance at 5% level.
** Significance at 1% level.

b. Spikelet fertility

Japanese var. \ Indian var.	I-44 Bhutmuri-36 ♀	I-44 Bhutmuri-36 ♂	I-88 Assam III ♀	I-88 Assam III ♂	I-34 Mushakdanti ♀	I-34 Mushakdanti ♂	I-45 Charnock ♀	I-45 Charnock ♂
A- 5 Akamuro	14	26	4	32	93	93	69	58
	t= 5.81**		t= 7.31**		t=0		t=2.37*	
A- 13 Chabo	7	48	3	18	89	90	2	6
	t=24.87**		t=11.07**		t=0.54		t=2.30*	
A- 43 Hokkaimochi-1-go	11	27	8	23	62	61	2	3
	t= 6.96**		t= 4.47**		t=0.29		t=0.44	
A-133 Norin-9-go	11	42	23	36	76	77	19	13
	t=16.82**		t= 6.56**		t=0.29		t=1.80	

* Significance at 5% level.
** Significance at 1% level.

2. Induction of male sterile strains

Successive backcrossings of the reciprocal hybrids between I-44 and A-133 and between I-88 and A-133 were carried out to produce the substitution and restoration lines. As shown in Table 3, pollen sterility was intensified at the later generations in the substitution type of crossing, while pollen fertility was steadly increased in the restoration type of crossing. The substitution of the genome was attained around the SB_7 and SB_{10} in K-11 and K-13 strains. In order to confirm the function of the female organs, K-11 and K-13 were crossed with the pollens of the normal strains. The spikelet fertility of the crossed plants in K-11 and K-13 showed 85 and 77% respectively. The male sterile strains, K-11 and K-13 possessed the constitution of the cytoplasm from Indian varieties and genome of the Japanese variety, "Norin-9-go".

TABLE 3. Pollen fertility (%) at each generation in the two types of successive backcrossings

Generation	I-44×A-133		I-88×A-133	
	Substitution	Restoration	Substitution	Restoration
SB_1	6.9 (0-19)	43.5 (29-72)	—	—
SB_2	1.0 (0- 3)	57.7 (34-79)	—	—
SB_3	3.2 (0-16)	71.8 (51-79)	0.1 (0-0.4)	—
SB_4	0.9 (0- 8)	79.2 (70-89)	0	49.5
SB_5	—	—	0	94.4 (93-97)
SB_6	—	—	2.1 (0-4.8)	84.1 (79-89)

1) Parenthesis means the range of variation.
2) Substitution and restoration crossings mean the crossings to A-133 and I-44 or I-88, respectively.

3. Selection of pollen fertility restorer

Test crossings were carried out to select the pollen fertility restorer from the 16 tester strains by the use of the *boro*-cytoplasm male sterile tester, I-127. Pollen fertilities of the F_1 hybrids are shown in Table 4. Two linkage testers, H-103 and H-406 possesed the gene or genes for pollen restoration interacting with the *boro*-cytoplasm. Both strains had a relation with the foreign varieties and the marker genes, gl_1 and Pl^w were introduced from the varieties. Namely, H-103 is a progeny selected from the cross, H-69×Garumbalay and H-406 is a inbred line derived from the cross, A-5× H-121 in which H-121 is a linkage marker bred true from the cross,

A-13×Pirurutong.

Pollen and spikelet fertilities were examined for three years in the F_1 hybrids which showed pollen fertility restoration as shown in Table 5. Though the fluctuation of the fertility was observed in different years, the cross, I-127×H-406 showed a higher fertility than the other cross, I-127×H-103 in both the pollen and spikelet fertilities. In the experiment of 1978, the ability of pollen fertility restoration indicated an order of I-127>H-406>H-103 when crossed with I-127.

TABLE 4. Pollen and spikelet fertilities (%) of F_1 plants from the crossings between I-127 and linkage testers

Cross combination	Pollen fertility		Spikelet fert.*
	mean	range	mean
I-127×Taichung-65-go	0.79	0- 3.9	0
〃 ×A-23 Daikoku	0.20	0- 0.4	0
〃 ×A-58 Kokushokuto-2	0.15	0- 0.4	12.0
〃 ×A-136 Shiokari	0.72	0- 4.7	2.4
〃 ×N-65 Hosetsu-A	0.58	0- 2.2	13.6
〃 ×H-9 Linkage tester	0.04	0- 0.6	0
〃 ×H-21 〃	0.37	0- 8.1	1.4
〃 ×H-35 〃	0	0	0
〃 ×H-61 〃	0.11	0- 0.7	0
〃 ×H-69 〃	1.48	0- 4.0	1.4
〃 ×H-75 〃	0.36	0.2- 0.7	7.7
〃 ×H-79 〃	0.18	0- 1.6	0
〃 ×H-103 〃	42.78	30.3-48.8	66.1
〃 ×H-126 〃	2.39	0-11.5	0
〃 ×H-150 〃	0	0	10.0
〃 ×H-406 〃	44.45	34.0-51.4	72.7
〃 ×H-477 〃	0.14	0- 1.4	0
H-69×Taichung-65-go	95.88	94.3-97.1	90.9

* Fertility under open pollination.

TABLE 5. Pollen and spikelet fertilities (%) in the F_1 plants showing fertility restoration

a. Data in 1973 and 1974

Fertlity	Cross combination	1973		1974	
		Number of plants	Mean±S. D.	Number of plants	Mean±S. D.
Pollen fert.	I-127×H-103	42	42.8±3.76	35	32.0± 8.59
	I-127×H-406	22	44.4±8.41	35	49.4±10.41
	Difference	−1.6 (t=1.097)		−17.4** (t=7.617)	
Seed fert.	I-127×H-103	22	9.3±4.72	33	3.5± 2.67
	I-127×H-406	10	14.5±9.31	36	9.6± 4.77
	Difference	−5.2* (t=2.096)		−6.1* (t=6.418)	

b. Data in 1978

Cross combination	Pollen fertility		Spikelet fertility	
	Number of plants	Mean±S. D.	Number of plants	Mean±S. D.
(1) I-127×H-103	19	36.60± 9.49	19	17.47±13.23
(2) I-127×H-406	13	43.43±10.98	13	21.05± 8.61
(3) I-127×I-130	20	56.10± 5.49	20	74.68± 6.16
(1) — (2)	−6.83 (t=1.879)		−3.58 (t= 0.656)	
(1) — (3)	−19.50** (t=7.908)		−57.21** (t=13.265)	
(2) — (3)	−12.67** (t=4.404)		−53.63** (t=20.882)	

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

4. Mode of inheritance on pollen fertility restoration

a. Experiment in 1975

F_2 populations and progenies of the backcrossings and the various kind of three way crossings were grown in green houses in 1975. The weather condition was moderate for rice cultivation. However, the late flowering plants were affected slightly by low temperatures and resulted in the decrease of pollen fertility. As shown in Fig. 1 a, the frequency distributions of pollen fertility of both populations, I-127×H-103 F_2 and I-127×H-406 F_2, indicated

a continuous variation. The average pollen fertility of the former cross was lower than that of the later cross. The frequency distributions of pollen fertility in the progenies of the crosses between two kinds of female plants, I-127×H-103 F_1 and I-127×H-406 F_1 and the four kinds of male parents, A-133, H-103, H-406 and I-130 are shown in Fig. 2. In most of the progenies of the crosses in which the I-127×H-406 F_1 was used as the female parents, the average fertility was always higher than those in the progenies of the female parent, I-127×H-103 F_1. It is highly probable that the ability of pollen fertility restoration is different between the genotypes

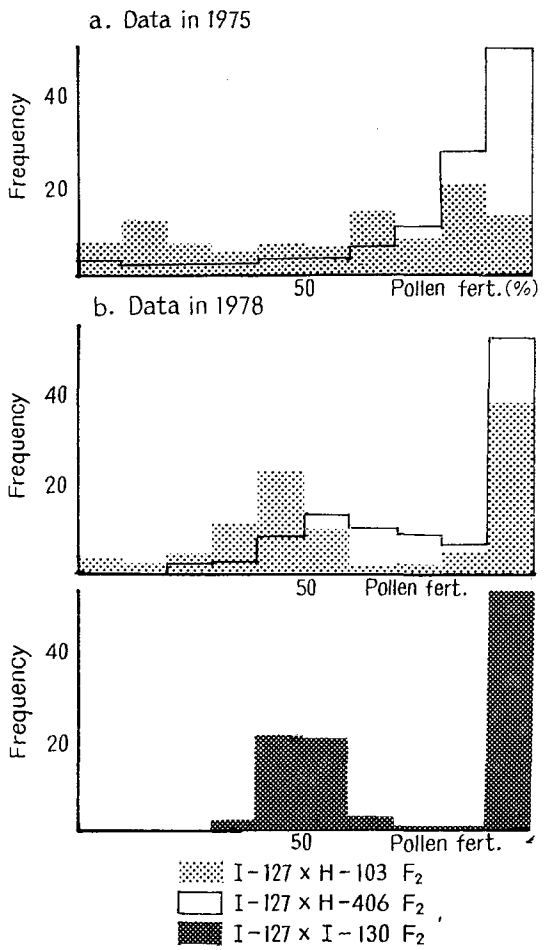

Fig. 1. Histograms of pollen fertility in F_2 populations of the crosses, I-127×H-103, I-127×H-406 and I-127×I-130. Population size of each cross is around 300 and frequency is expressed as %.

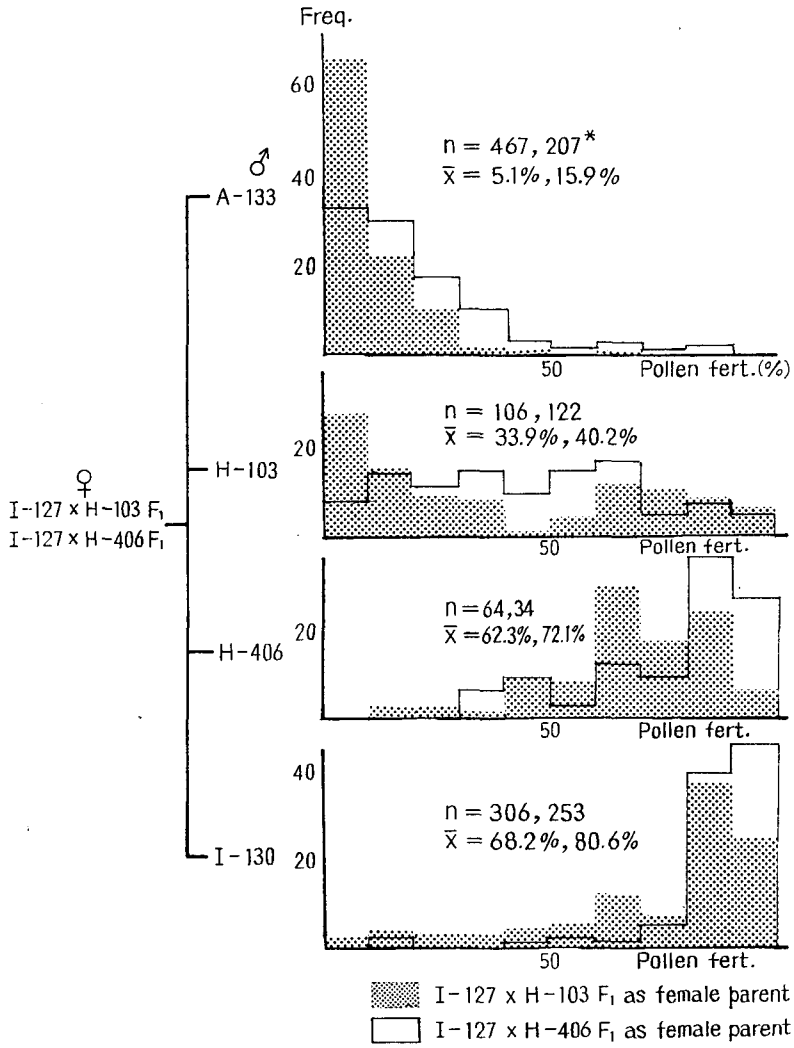

Fig. 2. Histograms of pollen fertility in the progenies derived from the various crossings with the two kinds of F_1 hybrids, I-127× H-103 and I-127×H-406 (data in 1975). *, Population size (n) and average ($\bar{x}$) in the crosses with I-127×H-103 F_1 (female) and I-127×H-406 F_1 (female) are shown, respectively.

of both strains, H-103 and H-406. In addition, low fertility plants appeared in the progenies of the crosses with the female plant, I-130 which possesses the homozygous genotype Rf_1 Rf_1 for pollen fertility restoration. Possibly, both strains, H-103 and H-406 may possess a gene or genes other than

Rf_1[13]. Thus, there are different genotypes on the pollen fertility restoration under the *boro*-cytoplasm as represented by the three strains, H-103, H-406 and I-130. As to the gametophytic action of the gene or genes for pollen fertility restoration, two kinds of backcross populations, (I-127 × H-103) × H-103 and (I-127 × H-406) × H-406 were compared with F_2 populations of the respective crosses. Though the frequency distributions were not completely identical, the average fertilities did not differ significantly between B_1 and F_2 populations in the cross, I-127 × H-406. The bimodal distribution which is expected by a single gene like Rf_1 was rare or not seen among the ten kinds of populations both in Figs, 1 a and 2. There is a possibility that the environmental conditions also affect the variation of pollen fertility even in the protected cultivation in a green house.

b. Experiment in 1978

In order to homogenize the effect by weather conditions, all populations at different generations up to F_5 were grown in a vinyl house protected from low temperatures. The weather condition in this year was suitable for cultivation. It is indicated that the average fertility of the two kinds

TABLE 6. Frequency distributions of pollen fertility in P_1, F_1 and F_2 populations of the crosses, I-127 × H-103, I-127 × H-406 and I-127 × I-130

Generation	Cross combination	Pollen fertility (%)											Total	Mean ± S.D.
		0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100		
P_1	I-127	16	4	—	—	—	—	—	—	—	—	—	20	0.40 ± 3.01
	H-103	—	—	—	—	—	—	—	—	—	—	19	19	97.88 ± 1.27
	H-406	—	—	—	—	—	—	—	—	—	—	20	20	97.55 ± 1.48
	I-130	—	—	—	—	—	—	—	—	—	—	20	20	99.99 ± 0.04
F_1	I-127 × H-103	—	—	—	6	6	6	1	—	—	—	—	19	36.60 ± 9.49
	" × H-406	—	—	—	1	4	6	1	1	—	—	—	13	43.43 ± 10.98
	" × I-130	—	—	—	—	—	2	13	5	—	—	—	20	56.10 ± 5.49
F_2	I-127 × H-103	—	8	6	14	36	72	32	7	6	16	120	317	65.27 ± 28.69
	" × H-406	—	—	—	5	7	26	46	34	27	21	178	344	80.15 ± 21.52
	" × I-130	—	—	—	—	4	56	52	9	1	1	140	263	76.73 ± 24.81

Fitness for high (over 81%) vs. low fertility groups = 1 : 1

I-127 × H-103 $\chi^2 = 6.388$, d. f. = 1, P = 0.01-0.02.

I-127 × H-406 $\chi^2 = 8.477$, d. f. = 1, P = 0.001-0.1.

I-127 × I-130 $\chi^2 = 1.373$, d. f. = 1, P = 0.2-0.3.

of F_2 populations in 1978 were considerably higher than those in 1975 because of the favourable weather conditions. As shown in Fig. 1 b, the bimodal distributions were evident both in the crosses, I-127×H-103 and I-127×H-406 and differed from the continuous variation in the same populations grown in 1975. Thus, the weather condition plays an important role for

TABLE 7. Frequency distributions of pollen fertility in F_3 lines of the crosses, I-127×H-103 and I-127×H-406 (data in 1978)

a. I-127×H-103

F_2 plant*		Pollen fertility (%) in F_3 line										Total	Mean±S. D.
No.	Poll. fert. (%)	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100		
1	90	—	—	—	—	—	—	—	—	1	19	20	97.62± 3.88
2	90	—	—	1	—	2	2	1	3	1	9	19	78.95±23.66
3	90	—	—	—	1	2	4	2	—	—	11	20	77.90±24.76
4	80	—	—	—	—	—	—	—	—	1	19	20	98.34± 2.89
5	80	—	—	—	—	—	—	—	—	—	20	20	99.58± 0.85
6	80	—	—	—	—	1	7	2	—	—	10	20	77.50±23.41
7	80	—	—	—	—	5	5	—	—	—	9	19	73.92±25.32
8	80	—	—	—	—	—	—	—	—	—	19	19	98.66± 2.25
9	80	—	—	—	—	—	—	—	—	1	17	18	97.52± 6.96
10	80	—	1	—	—	1	—	—	1	1	15	19	89.81±22.48
11	80	—	—	—	—	—	—	—	—	—	20	20	99.89± 0.38
12	80	—	—	—	—	—	—	—	—	1	19	20	98.69± 3.34
13	80	—	—	—	—	4	4	2	1	—	9	20	75.93±23.05
14	80	—	—	—	—	3	4	4	2	—	7	20	73.32±20.86
15	80	—	—	—	—	—	—	—	—	1	16	17	98.94± 2.86
16	80	—	—	—	—	—	—	—	—	—	20	20	99.74± 0.79
17	80	—	—	—	—	—	—	—	—	—	20	20	98.12± 2.70
18	70	—	—	—	—	—	—	—	—	—	19	19	98.18± 2.33
19	70	—	—	—	3	7	4	—	—	—	3	17	55.17±21.09
20	60	—	—	—	—	—	—	—	—	—	20	20	99.12± 2.22

* Data in 1975.

b. I-127×H-406

F_2 plant*		Pollen fertility (%) in F_3 line										Total	Mean±S. D.
No.	Poll. fert. (%)	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100		
1	90	—	—	—	—	—	—	—	1	3	16	20	94.34± 5.58
2	90	—	—	—	—	—	—	—	—	2	18	20	93.07± 3.14
3	90	—	—	—	—	—	—	—	1	6	13	20	91.72± 5.35
4	90	—	—	—	—	1	—	—	1	6	12	20	89.23±12.80
5	90	—	1	—	—	—	—	2	5	4	8	20	81.16±19.64
6	90	—	1	—	1	1	1	2	2	6	6	20	76.21±22.38
7	90	—	—	—	1	4	1	1	3	1	8	19	73.59±21.99
8	90	1	2	1	2	2	3	2	3	—	2	18	52.28±27.30
9	90	2	1	3	2	3	3	1	2	1	2	20	48.79±27.63
10	90	5	2	3	—	1	—	1	3	3	2	20	45.65±33.91
11	80	—	—	1	—	6	2	3	1	3	4	20	65.43±22.34
12	80	—	—	—	—	1	2	1	—	—	7	11	80.84±22.32
13	80	—	—	—	1	1	1	3	4	3	7	20	76.50±18.43
14	80	—	—	—	1	2	3	1	3	5	5	20	73.67±19.81
15	80	—	—	—	4	2	1	—	—	—	12	19	75.37±27.19
16	70	—	—	—	—	—	—	—	1	4	15	20	93.09± 6.94
17	70	—	—	—	—	—	1	4	2	3	10	20	83.17±13.73
18	70	—	2	—	—	—	2	3	5	6	2	20	71.23±22.77
19	70	—	—	1	1	4	2	2	3	2	5	20	68.29±23.60
20	70	1	—	—	2	4	3	2	2	2	3	19	61.48±24.05

* Data in 1975.

the variation of pollen fertility which was caused by the interaction between the nuclear gene or genes and the *boro*-cytoplasm.

The variations of pollen fertility in P_1, F_1 and F_2 populations in the three kinds of crosses are shown in Table 6. The segregation of low and high fertilities is expected to fit in a ratio of 1 : 1 if a single gene by gametophytic action was responsible for the segregation. The hypothesis was satisfied well in the F_2 population of the cross, I-127×I-130 depending on Rf_1. As the variation of pollen fertility was clearly different among the three

kinds of crosses, it is reasonable that the three strains possess differnt genotypes for the pollen fertility restoration. If a single gene was responsible for the pollen fertility restoration, it is expected that the progeneies of the high fertile plants in F_2 shall be bred true in F_3 lines. The variations of pollen fertility in F_3 lines which were selected from the high fertility group in the F_2s of the both crosses, I-127×H-103 F_2 and I-127×H-406 are shown in Table 7. About half of the lines were bred true in the high fertility in the cross, I-127×H-103, while most of the F_3 lines indicated a considerable range of variation containing low fertile plants. From the results in F_2 and F_3 generations, at least more than one gene were responsible for the pollen

TABLE 8. Means and coefficients of variability of pollen fertility (%) in F_4 and F_5 lines in the selection experiments for high pollen fertility using the two crosses, I-127×H-103 and I-127×H-406 (data in 1978)

a. I-127×H-103

F_3 plant*		F_4 line		F_4 plant*		F_5 line	
No.	Mean	Mean±S. D.	C. V.	No.	Mean	Mean±S. D.	C. V.
1	95.7	98.98± 0.91	0.92	1	92.9	98.51± 1.25	1.27
2	91.3	98.39± 2.05	2.09	2	58.4	97.56± 2.23	2.29
3	79.6	98.69± 0.93	0.94	3	54.4	99.13± 0.96	0.97
4	75.8	96.26± 2.50	2.60	4	54.4	96.43± 2.89	3.00
5	42.8	98.41± 1.01	1.02	5	34.8	91.86±17.43	18.97
6	37.6	97.24± 1.45	1.49	6	32.8	98.46± 0.71	0.72
7	35.5	81.37±27.81	34.18	7	27.7	98.87± 0.64	0.64
				8	24.1	98.98± 1.00	1.01

b. I-127×H-406

F_3 plant*		F_4 line		F_4 plant*		F_5 line	
No.	Mean	Mean±S. D.	C. V.	No.	Mean	Mean±S. D.	C. V.
1	90.7	97.57± 1.86	1.90	1	85.1	98.38± 1.31	1.33
2	90.0	84.09±13.63	16.21	2	84.8	95.58±11.30	11.83
3	80.8	97.30± 1.70	1.75	3	80.9	98.42± 1.22	1.24
4	79.9	89.94±13.57	15.09	4	80.5	98.49± 1.11	1.13
5	76.0	96.14± 3.32	3.46	5	79.9	98.34± 1.73	1.76
6	75.6	98.01± 1.52	1.55	6	77.7	98.09± 1.68	1.72
7	70.9	89.14±14.74	16.54	7	75.1	98.55± 1.03	1.04
8	70.0	85.91±15.23	17.73	8	71.2	98.50± 0.99	1.00
9	64.3	93.39± 4.70	5.03				

* Data in 1977.

fertility restoration in the both crosses, I-127×H-103 and I-127×H-406.

5. Selection experiments for high pollen fertility up to F_5 generation

The selection to the high pollen fertility was repeated in F_3 and F_4 generations. Outcomes in F_4 and F_5 generations are shown respectively in Table 8. Most of the lines indicated relatively low values of variation coefficient in F_4 of the cross, I-127×H-103 and in F_5 of the cross, I-127×H-406. Thus, it is probable that a more complicated genetic mechanism exists in both crosses, I-127×H-103 and I-127×H-406 than that in the cross, I-127×I-130 which is caused by the single gene *Rf*$_1$.

6. Relation between pollen and spikelet fertilities

The average spikelet fertility and the variation in P_1, F_1 and F_2 populations are shown in Table 9. Generally, the spikelet fertility was lower than the pollen fertility. Correlation coefficients were calculated in the populations at each generation (Table 10). Significant correlations existed in H-406 and the F_1 plants of the cross, I-127×H-103. In addition, the F_2 populations of both crosses, I-127×H-103 and I-127×H-406 showed a highly significant correlation while no correlation existed in the cross, I-127×I-130.

TABLE 9. Variations of spikelet fertility in P_1, F_1 and F_2 populations of the crosses, I-127×H-103, I-127×H-103, I-127×H-406 and I-127×I-130

Generation	Cross combination	Spikelet fertiliy (%)											Total	Mean±S.D.
		0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100		
P_1	I-127	20	—	—	—	—	—	—	—	—	—	—	20	0
	H-103	—	—	—	—	—	—	—	—	—	8	11	19	91.29± 3.72
	H-406	—	—	—	—	—	—	—	—	—	6	14	20	91.82± 4.20
	I-130	—	—	—	—	—	—	—	8	9	2	1	20	72.77± 7.84
F_1	I-127×H-103	1	8	4	2	1	1	2	—	—	—	—	19	17.47±18.23
	〃 ×H-406	—	1	4	6	2	—	—	—	—	—	—	13	21.05± 8.61
	〃 ×H-130	—	—	—	—	—	—	—	5	10	5	—	20	74.68± 6.16
F_2	I-127×H-103	2	57	37	40	40	47	36	33	22	3	—	317	36.11±22.88
	〃 ×H-406	2	17	21	35	42	43	46	48	44	29	17	344	51.47±24.28
	〃 ×I-130	—	1	1	2	10	10	15	36	61	92	35	263	75.34±16.31

TABLE 10. Correlation coefficients between pollen and spikelet fertilities in P_1, F_1 and F_2 populations of the crosses, I-127×H-103 and I-127×H-406

Generation	Population	Number of plants	Correlation coefficient
P_1	H-103	19	−0.1080
	H-406	20	0.5178*
F_1	I-127×H-103	19	0.6721**
	〃 ×H-406	13	0.4595
	〃 ×I-130	20	0.2838
F_2	I-127×H-103	317	0.5112**
	〃 ×H-406	344	0.5263**
	〃 ×I-130	263	−0.0062

* Significance at 5% level.
** Significance at 1% level.

TABLE 11. Frequency distributions of pollen fertility in F_1 plants between the three kinds of male sterile strains and the tester strains for the fertility restoration

Cross combination	Pollen fertility (%)											Total	Mean±S. D.
	0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100		
I-127× I-130	—	—	—	—	—	6	7	2	—	—	—	15	52.81± 5.42
K- 11× 〃	—	—	—	—	—	13	12	1	—	—	—	26	51.39± 4.61
K- 13× 〃	—	—	—	—	—	3	7	2	—	—	—	12	52.45± 5.86
I-127×H-406	—	—	—	—	—	6	6	—	—	—	—	12	49.48± 6.19
K- 11× 〃	—	—	—	—	—	4	5	3	—	—	—	12	54.83± 6.81
K- 13× 〃	—	—	—	—	—	—	5	8	—	—	—	13	62.65± 5.48
I-127×H-103	—	—	—	3	2	8	4	1	—	—	—	18	44.18±10.96
K- 11× 〃	—	—	—	4	7	3	—	—	—	—	—	14	32.63± 6.72
K- 13× 〃	—	—	—	—	4	9	—	—	—	—	—	13	41.71± 4.45
I-127× I-128	16	4	—	—	—	—	—	—	—	—	—	20	0.60± 1.27
K- 11× 〃	7	5	—	—	—	—	—	—	—	—	—	12	0.71± 1.00
K- 13× 〃	4	6	—	—	—	—	—	—	—	—	—	10	1.48± 2.18

* The genotypes of pollen fertility restoration are known as Rf_1Rf_1 for I-130 and rf_1rf_1 for I-128, respectively.

7. Identification of cytoplasms

As mentioned in the second section, two kinds of male sterile strains, K-11 and K-13 were induced by nuclear substitution. The two cytoplasmic donors, I-44 and I-88 belong to the so-called '*boro*' type of Indian variety as well as the variety 'Chisurah boro-II' which was used as the cytoplasmic donor in the tester strain, I-127. The three kinds of male sterile strains were crossed with the three kinds of tester strains possesing the gene or genes for pollen fertility restoration and the maintainer, I-128 for the male sterile strain, I-127. As shown in Table 11. the maintainer and the fertility restorer for I-127 exhibited an identical action for the three male sterile strains. Therefore it is reasonable that the cytoplasm of K-11 and K-13 is identical with the *boro*-cytoplasm. However, there were a little discrepancy among the pollen fertilities of three kinds of F_1 plants when they were crossed with H-103 and H-406. It may be caused by the difference in the genetic nature of the male sterile cytoplasms.

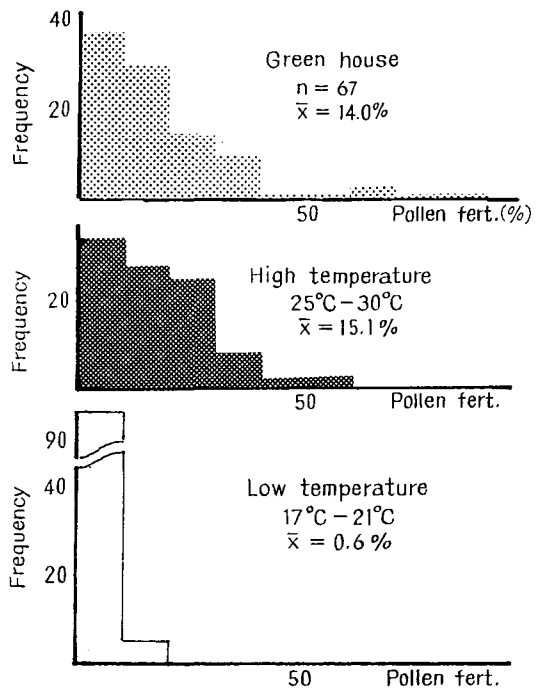

Fig. 3. Histograms of pollen fertility in the populations chosen from the cross, (I-127×H-406)×A-133 under the high or low temperature conditions kept in a growth chamber (data in 1975).

8. Effect of temperature conditions

As mentioned in the previous section, the weather conditions affect the variation of pollen fertility in the hybrid populations. Therefore, the effect of low or high temperatures was examined by growing the plants in a growth chamber conditioned by low or high temperatures. 67 plants chosen from the population of the cross, (I-107 × H-406) F_1 × A-133 were propagated asexually for the experimental materials. The condition in the growth cabinet were kept at 30°C in day time and 25°C at night (high temperature) and 21°C in day time and 17°C in night (low temperature) after the initiation of young panicle. As shown in Fig. 3, the variation of pollen fertility under the high temperature condition was eqivalent with that in the green house, while the plants grown under low temperature condition showed nearly complete sterility. Thus, the low temperature remarkably decreases the pollen fertility in the case of cytoplasmic male sterility.

Discussion

Practical use of hybrid rice became feasible with the development of a cytoplasmic male sterility and fertility restoration gene even though there are some problems to be solved[1,2,3,4]. The degree of heterosis was already examined in the F_1 plants of the crosses between the cultivated varieties in the northern part of Japan[10]. Recently, two kinds of cytoplasmic male sterile strains were induced by a combination between the cytoplasm from Indian or Burmuse varieties and the nucleus of the Japanese varieties[11,14]. Similar techniques of nuclear substitution was also applied for the induction of cytoplasmic male sterility in American[2] or Chinese[8] varieties and the variety of *Oryza glaberrima*[15]. In this report, the authors recognized the cytoplasmic effect on the hybrid sterility in some crosses between *Japonica* and *Indica* varieties and have bred the male sterile strains by successive backcrossings using the nuclear parent, 'Norin-9-go' and the cytoplasmic donors, 'Bhutmuri-36' and 'Assam III'. In the use of the cytoplasmic male sterility, the fertility restorer possesing a strong ability is necessary for the seed crops like rice. The authors made the experiment to select the fertility restorer among the linkage tester strains. Though the two strains, H-103 and H-406 were selected using the *boro*-cytoplasm tester, the spikelet fertility of F_1 plants decreased considerably than the pollen fertility. Therefore they belong to the weak restorer named by SHINJO[12]. The source of the pollen fertility restoration may possibly come from the Philippinese varieties, 'Garumbalay' and 'Pirurutong'. The mechanism of inhertiance was studied by the use of the fertility restorers. Though the gametophytic action of the

gene or genes was satisfied, the gene or genes other than Rf_1[13] was responsible for the pollen fertility restoration and took two or three generations for the true-breeding of the pollen fertility restoration. Detailed study on the number of genes and the genic interaction is being carried on. The effects of environmental factors for the variation of pollen fertility in hybrid populations also can not be negelected. Low temperatures during the microsprogenesis resulted in a remarkable decrease of pollen fertility. In the cytoplasm test using K-11, K-13 and the *boro*-cytoplasm tester, the three male sterile strains indicated a equivalent reaction when crossed with the testers, (*cms-boro*) $rf_1\,rf_1$ and $Rf_1\,Rf_1$. However, a minor discre pancy existed in the pollen fertility of F_1 hybrids when they were crossed with the weak restorers, H-103 and H-406. The problem of whether the diversity of the cytoplasm exists or not must be solved by examinations using the iso-plasmic lines on the respective cytoplasms.

In the northern part of Japan, the male sterile plants remain in nearly no seed setting under the open pollination. Therefore, it appears cross-pollination will be the limiting factor in the production of F_1 hybrid seeds as suggested by several workers[1,2,4]. The floral device to make easy the cross pollination must be induced by the mutation or introduce from related species.

Summary

1. Reciprocal difference of pollen and spikelet fertilities was recognized in F_1 hybrids between Japanese and Indian varieties and the hybrid sterility was caused by the genetic interaction between the cytoplasm and the nucleus in these crosses.

2. Two kinds of male sterile strains, K-11 and K-13 were bred true by the successive backcrossings of the two kinds of *Indica-Japonica* crosses with the recurrent parent, 'Norin-9-go'.

3. Two kinds of linkage testers, H-103 and H-406 possesed the ability of pollen fertility restoration under the *boro*-cytoplasm. However, the ability for pollen and spiklet fertility restoration showed an order of I-127 > H-406 > H-103. Therefore, both strains belong to the weak restorers in the group made by SHINJO.

4. Mode of inheritance was examined using the three kinds of fertility restoring strains. Variations of pollen fertility in F_2 populations, differed by the pollen parents and also the different years. It is highly probable that the gene or genes other than Rf_1 was responsible for the pollen fertility restoration in the crossings with the before mentioned weak restorers. The outcome of the selection experiments toward high fertility also sustained

the non-allelism with Rf_1. In addition, weather conditions affected remarkably the fluctuation of pollen fertility.

5. Identification test for the cytoplasm proved that the cytoplasms of K-11, K-13 and I-127 show an equivalent reaction to the *boro*-cytoplasm tester, (*cms-boro*) $Rf_1 Rf_1$. However, a minor discrepancy of the reaction may exist in the cytoplasms of K-11, K-13 and I-127 when crossed with the weak restorers, H-103 and H-406.

6. It was demonstrated that low temperaure conditions during microsporogenesis cause a severe decrease of pollen fertility in the plants possessing the *boro*-cytoplasm. In addition, there is also a problem on the cross-pollination to obtain the F_1 hybrid seeds under natural conditions in the northern part of Japan.

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