イネにおける細胞質雄性不稔と稔性回復の遺伝学的研究(農学部)

Genetical Studies of Cytoplasmic Male Sterility and Fertility Restoration in Rice, *Oryza sativa* L.*

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I INTRODUCTION

When the practical importance of heterosis was demonstrated in corn and the hybrid corn was developed by pulling out immature tasseles of maternal parent, plant breeders naturally considered hopefully the possibility for utilization of hybrid vigor in other crops. Shortly after the heterosis in corn was demonstrated in America, practical hybrid onion was reported by utilization of cytoplasmic male-sterility. The commercial use of hybrid corn, onion, sorghum and pearl millet is a well-recognized achievment of mordern plant breeding. The cytoplasmically male sterile plants in other many crops are detecting and the possibility for utilization of male sterility is investgating in many countries.

Rice, *Oryza sativa* L., generally recognized as the most important basic food crop grown, is the main source of nourishment for over half the world's population. Thus, any acceptable system for increasing rice yields has world-wide implications. Rice has many publications impossibly counted for genetical and breeding problems, but little has been known about the cytoplasmic male-sterility before 1957. Recently, five male sterility-inducing cytoplasms in rice have been reported by Katsuo and Mizushima (46), Watanabe *et al.* (87), Erickson (two different cytoplasms, 23), and Athwal and Virmani (3). Though, unfortunately any effective fertility-restoring gene or genes for five above male sterility-inducing cytoplasms have not been reported.

The present author had detected a male sterility-inducing cytoplasm and its fertility-restoring gene from an Indica variety, Chinsurah Boro II, and both factors were introduced into a Japonica variety, Taichung 65, by means of successive backcrsses. He has been studying to develop hybrid rice utilizing above factors. Therefore in the present investigation the mordern trends to pave the way for hybrid rice breeding will be dealt systematically by the some genetic explorations of those hurdles lited below.

II REVIEW OF LITERATURE

A. Cytoplasmic male sterility and fertility restoration in higher plants

Correns (15) was the first reporter concerning maternal effect in the development of male organs in plants according to the reviews by Sirks (80), Caspari (10), Edwadson (21) and Duvick (18). Correns (15 and 16) crossed *Crisium oleraceum* used as maternal parent to *C. canum*. After six generations of backcrosses to *C. canum*, in which the majority of the genes must have been replaced by *C. canum* genes, the offspring was still exclusively female. He reached to the conclusion that the cytoplasm of maternal parent *C. oleraceum* possesses some properties which permit only the female sex organs to develop.

Bateson and Gairdner (4) reported the segregation of fertile and sterile plants in a 3 : 1 ratio in the F_2 generation of a cross between a common tall and procumbent flax (*Linum usitatissimum* L.). Male sterile plants were obtained only when the procumbent flax was used as the maternal parent. They attributed the unusual results to "anisogeny". Later on Chittenden and Pellow (13), and Gairdner (27) confirmed that the male sterility was due to the interaction of sterility-inducing cytoplasm of procumbent flax and a homozygous recessive gene.

From the results of .the inheritance of male sterile corn, Roades (70 and 71) reached to the conclusion that the transmission of the character was solely through the maternal cytoplasm and that nuclear factors exserted no influence; at the same time no indication of the male sterility being caused by a virus disease was found out. Recently, Duvick (19) discussed and summarized the intensive works on 84 different sources of cytoplasmic male sterility and the genetics of fertility restoration as well as other retarding effects of alien cytoplasms on the morphological characters other than pollen fertility.

In *Nicotiana*, East (20) reported a male sterility resulting interaction between certain *S* genes from *N. sanderae* and cytoplasm derived from *N. langsdorfii*, although offsprings from the reciprocal cross produced fertile pollens. He also reported that the phenotypic expression of certain characters in *langsdorfii* cytoplasm was different from that in *Sanderae* cytoplasm. Clayton (14) observed male sterile plants in the first backcross of (*Nicotiana debneyi* x *N. tabacum*) x *N. tabacum* and completely male sterile plants were obtained at the third backcross generation. Whereas in the reverse cross male sterile plants were not observed.

Michaelis (56) made reciprocal crosses between *Epilobium hirsutum* and *E. luteum*. The F_1 plants of *hirsutum* x *luteum* is completely male sterile, whereas in the reciprocal cross hybrid *luteum* x *hirsutum* no plant was completely male sterile. Later on Michaelis (57) summarized the results of his extensive works of several years on inheritance mode of cytoplasmic male sterility in *Epilobium*. Clear-cut morphological, physiological and

genetical differences were demonstrated.

In sugar beet, Owen (67) reported in detail on the mode of inheritance of cytoplasmic male sterility. According to his genic assumption, the degree of male sterility was controlled by the interaction of two nuclear genes *X* and *Z* in male sterility-inducing cytoplasm *S*. The genic constitution of the phenotypes are summarized as follows, (*S*)*xx zz* is responsible for comletely male sterile, (*S*)*Xx zz* or (*S*)*xx Zz* is for semi-male-sterile, usually without viable pollen, and (*S*)*Xx Zz* is for semi-male-sterile, usually with some viable pollen, and sometimes indistinguishable in normal plants. Later on Owen (68) recognized the difficulty in distinguishing (*S*)*xx zz* and (*S*)*xx Zz* phenotypically because of the minor effect of *Z* factor under certain evironmental conditions.

Stephens and Holland (81) reported that some partially male-sterile plants occurred in the F_2 population derived from the cross of milo x kafir in *sorghum*. Completely male-sterile plants were obtained in the second backcross generation. The F_1 plants restored their fertility when male-sterile plant was crossed with milo as pollen parent. The results confirmed that the male sterility occurred by interaction between milo cytoplasm and kafir nuclear factors. Cytoplasmic male sterility in onion, *Allium Cepa*, was reported in 1937 by Jones and Emsweller. The mode of inheritance of this character and its potential use in commercial hybridization were reported by Jones and Clarks (38), and Jones and Davis (39). Pollen abortion occurred only in the presence of a cytoplasmic factor (*S*) and the homozygous recessive gene *ms*. All other nuclear genotype plants produced normal pollen grains unless cytoplasmic factor, (*S*) or (*N*). This male sterile plant lends itself to commercial hybridization on a field scale. Large amounts of pollen-sterile seed can be produced if inbred maintainer line, (*N*)*ms ms*, are used to paternal parent. Fortunately there is no restriction in ovule fertility in onion.

Male sterility was known in carrot (88). Incomplete data suggested a few nuclear genes, possibly one dominant gene being involved. Peterson (69) observed male sterility in pepper (*Capsicum*) caused by male sterility-inducing cytoplasm and a nuclear recessive gene *ms*.

In the nucleus substitution backcross experiments in wheat, Kihara (47) reported cytoplasmic male sterility in the intergeneric hybrid, *Aegilops caudata* x *Triticum aestivum* where *T. aestivum* was recurrent paternal parent in the succeeding backcrosses. The plants with the complete genome of *T. aestivum* and cytoplasm of *Ae. caudata* were sterile. Fukasawa (24) observed completely male sterility in *Triticum durum* plants with *Aegilops ovata* cytoplasm. Later on, he (26) reported the nucleus of cmmon wheat (Norin 26) in the cytoplasm of *Ae. ovata* exhibited male sterility. Wilson and Ross (89, 90) reported completely male sterility in *T. aestivum* with *Ae. ovata* and *T. timopheevi* cytoplasms, respectively. Effective fertility restorers for three male sterility-inducing cytoplasms, *i. e. caudata*, *ovata* and *timopheevi* were reported by Kihara and Tsunewaki (49), and Tahir (82).

B. Cytoplasmic male sterility and fertility restoration in rice

Katsuo and Mizushima (46) observed completely male-sterile plants in the first backcrossed progeny from cross of (*Oryza sativa* f. *spontanea* x *O. sativa* cult. Fujisaka 5) x Fujisaka 5. Whereas in the reciprocal cross, any male sterile plant was not observed. Pollen grains of male sterile plants as well as normal ones were well stained with iodine and potassium iodide solution (73), but former pollen grains did not germinate on the normal rice stigma (44) or artificial culture media (73). The results may suggest that the male sterility caused by interaction between male sterility-inducing cytoplasm and recessive fertility restoring gene(s).

Kitamura (50, 51) reported a slightly lower seed fertility than the parental varieties in the Indica-Japonica hybrid. Variety Tadukan x Norin 8, where Norin 8 was the recurrent paternal parent in the succeeding backcrosses. He investigated slighly seed sterility in the test progenies. No abortion was seen in both male and female gametes but due to interruption of anther dehiscence the flowers became sterile.

Shinjyo and Omura (75) were the first researchers in the world to report male sterility conditioned by the interaction of the cytoplasm and gene in cultivated rice, *Oryza sativa* L. When an Indica variety, Chinsurah Boro II, was used as the maternal parent in the cross with a Japonica, Taichung 65, the F_1 plants produced about 20 percent pollen fertility, while the reciprocal cross produced about 40 percent pollen fertilty. Completely male sterile plants were obtained from B_1F_1 generation with backcross of Taichung 65 into Chinsurah Boro II cytoplasm.

Shinjyo (76) investigated the inheritance of above male sterility. The male sterility-inducing cytoplasm (*ms-boro*) and the fertility-restoring gene *Rf* were derived from Chinsurah Boro II and the experiments were made by the isogenic lines having genetic background of Taichung 65. The effect of fertility-restoring gene *Rf* was found to be of gametophytic type in the male sterility-inducing cytoplasm (*ms-boro*).

Shinjyo (77, 78) also investigated the distributions of the male sterility-inducing cytoplasm and fertility-restoring genes using Japanese lowland-rice and foreign rice varieties. As a rule, non-restorer varieties were mostly concentrated in the temperate countries where Japonica rice was mainly grown. On the other hand, the effective restorer varieties were mainly distributed in the tropics where Indica rice was exclusively grown.

Watanabe *et al.* (87) reported cytoplasmically male sterility with Lead Rice cytoplasm. Lead Rice which was introduced from Burma was a non-recurrent maternal parent and Fujisaka 5 was a recurrent paternal parent in the backcrosses. The offsprings of the reciprocal cross were fertile.

Erickson (23) reported two different male sterilities conditioned by the interaction of cytoplasm and genes in rice. When variety Bir-Co (PI 279120) was used as the maternal parent in the crosses with California varieties, the F_1 plants were almost completely sterile, while the reciprocal crosses produced about 50 percent seed set. The three California varieties, Calrose, Caloro, and Colusa, when used as recurrent paternal parent,

always gave higher sterility in the Bir-Co cytoplasm than in their own. The sterility increased with succeeding backcrosses of California Japonica varieties into Bir-Co cytoplasm and the third backcrossed plants became completely male sterility (9).

When *Oryza glaberrima* Steud. was used as maternal parent in the crosses with California Japonica varieties, the F_1 hybrid failed to set any seed upon natural selfing. The hybrid plants with *Oryza glaberrima* cytoplasm backcrossed four times to California varieties gave from zero to five percent selfed seed set under bag pollination.

Athwal and Virmani (3) obtained male sterile plants with Taichung Native 1 cytoplasm from following backcross. The F_1 plants derived from the cross of Taichung Native 1 x Pankhari 203 were backcrossed as maternal parent to Pankhari 203 and Taichung Native 1, respectively. The former B_1F_1 hybrid plants produced more than 33 percent higher pollen sterility compared with the latter backcross. Continuous backcrossing of selected sterile plants to Pankhari 203 progressively increased the pollen sterility of the progeny. In the second backcross generation, the most of the progeny were nearly 100 percent pollen sterile. On the other hand, backcrossing to Taichung Native 1 gradually restored fertility. The results indicate that the Taichung Native 1 is a source of both male sterility-inducing cytoplasm and fertility-restoring gene or genes, and that Pankhari 203 has a normal cytoplasm and acts as a maintainer of male sterile line.

Unfortunately, any effective fertility-restoring gene or genes for five above male sterility-inducing cytoplasms except for one by the author have not been reported.

C. Cytoplasmic effect on quantitative characters

Grogan and Sarvella (29) studied inbred lines of maize and reported that the Texas-type male sterility-inducing cytoplasm was effective in reducing the stalk length above ear, the length of internode above ear and the length of tassel culm, while no effect was detected on the internode number in stalk, the length of stalk below ear and leaf sheath length. They also found significant interaction between the nuclear genotype, cytoplasm and environment.

Chaplin and Ford (12) noted a differential cytoplasmic effect on various quantitative characters of tobacco using six different cytoplasms in *Nicotiana*.

Hori and Tsunewaki (31) reported effect of three male sterility-inducing cytoplasms, *i. e. Aegilops caudata*, *Ae. ovata* and *Triticum timopheevi*, on six quantitative characters of hexaploid wheats. *Ae. ovata* cytoplasm delayed heading date about 15 days and reduced plant height about 11 cm. *Timopheevi* cytoplasm caused a reduction of dry matter weight.

D. Gene analysis by the use of trisomics

Trisomic series are being produced in an increasing number of crops to localize genes on specific chromosome, to identify chromosomes with their respective linkage groups and to determine phenotypic effects of individual chromosomes. The earlier work in this

field was carried out with *Datura stramonium* (5, 6 and 8), maize (55), tomato (52), *Nicotiana* (28), and barley (85). Hermsen (30) reported the basic information needed for primary trisomics in localizing single and complementary dominant and recessive genes, accounting for random complete chromatid and random chromosome association, for heterozygosity of the trisomic and for female and male transmission rate.

Twelve types of primary trisomics possibly found in rice, *Oryza sativa*, were developed by Iwata *et al.* (34), and studied their morphological features at maturity. Every type of their trisomics was easily recognized by their special morphological features except for one line. Iwata *et al.* (34), and Iwata and Omura (35) are endeavouring to make more complete linkage map by means of trisomic analysis in addition to the conventional genetic methods. Nagao *et al.* (61) reported tentatively twelve linkage groups in Japonica rice by means of the conventional genetic methods.

E. Cyto-histological investigation of pollen degeneration in anther of cytoplasmic male sterile plants

In cytoplasmic male sterile plants, meiosis in PMC's has been reported to be normal by Artschwager (2) in *Beta vulgaris*, Tatebe (83) in *Allium Cepa*. Fukasawa (25) also concluded in *Aegilops ovata* cytoplasm that the degeneration of pollen grains began at the stage of first division of pollen nucli. Katsuo (45) and Watanabe *et al.* (87) reported that the cytoplasmically male sterile plants had been obtained from the succeeding backcrosses of Japonica rice varieties to a wild rice, *Oryza sativa* f. *spontanea*, and a Burmese variety, Lead Rice, respectively. These two cytoplasmic male-sterile plants produce the pollen which can be stained by iodide solution as those of parent varieties. No abortion can be found during the microspore development. Furthermore, Katsuo (45) indicates that no particle of polysaccharide is found in his cytoplasmic male sterility.

In cytoplasmic male sterile sugar beet, pollen abortion was associated with tapatum hypertrophy or plasmodium (2, 32). Similar phenomena was also observed by Sakai (72) in rice plants damaged by low temperature and by Shibuya (74) in semi-sterile rice.

III MATERIALS AND METHODS

This research work was conducted from April 1960 to October 1974 at the Laboratory of Plant Breeding, College of Agriculture, University of the Ryukyus, Naha, Okinawa, Japan. A work of cyto-histological investigation of pollen degeneration in anther of cytoplasmic male sterile rice was carried out at the Plant Institute, Academia Sinica, Taipei, Taiwan, China.

The materials used and the techniques employed for each experiment are given separately, and the practice common in all the experiments are recorded together under general procedure.

A. Inheritance of male sterility

Strain (*ms-boro*)*Rf*-Taichung 65 with the male sterility-inducing cytoplasm and fertility-restoring gene *Rf* derived from an Indica variety, Chinsurah Boro II, twenty-five Japonica and six Indica varieties of rice (*Oryza sativa* L.) were used for the present investigation. Indica varieties including Chinsurah Boro II were introduced from the National Institute of Agricultural Sciences, Hiratsuka, Kanagawa, Japan.

The strain (*ms-boro*)*Rf*-Taichung 65 was derived from the progeny of succeeding backcrosses as follows : When F_1 hybrid plant of Chinsurah Boro II x Taichung 65 was backcrossed as maternal parent to Taichung 65, the progeny segregated into partially male fertile and completely male sterile classes in a ratio of 1 : 1. Then the partially male-fertile plants in each backcrossed generation were backcrossed repeatedly with Taichung 65 as the recurrent paternal parent. The results in the successive backcrossed generations are given in Table 1. Completely male-fertile strain was selected from selfed progeny of partially male-fertile plant being in B_6F_1 generation (Table 2). This is the strain (*ms-boro*)*Rf*-Taichung 65 which has presumably the same genetic background as that of Taichung 65 except for the male sterility-inducing cytoplasm and dominant fertility-restoring gene *Rf*. On the other hand, ten male-sterile plants from each of the successive backcrossed generations (Table 1) were picked up at random and ovule fertility was examined. There is no restriction in ovule fertility as shown in Table 3.

Six Indica rice varieties and their F_1 hybrid plants were grown under short-day condition (nine hours) from fifth leaf stage to emergence of frag leaf. Cross combinations among their varieties and lines were critically given in the results. Pollen and spikelet fertilities of the tested progenies were observed.

Table 1. Segregation of partially male-fertile (10–80%) and completely male-sterile classes in each F_1 generation under successive backcrosses.

Generation	Pollen fertility [%]									Number of plants	χ^2-value
	0	10	20	30	40	50	60	70	80		1 : 1
B_1F_1	58		14	14	12	16				114	0.035
B_2F_1	259	1	14	26	64	112	46	23	1	546	1.435
B_3F_1	263	1	27	55	62	83	31	1		523	0.017
B_4F_1	240		4	7	41	151	30	1		474	0.076
B_5F_1	261			4	55	170	46	1		537	0.419
B_6F_1	84				3	71	4			162	0.222

Note : Original cross combination was Chinsurah Boro II × Taichung 65, and partially male-fertile plants in each backcross generation in this table were used as maternal parent and were crossed to Taichung 65.

Table 2. F_2 segregation of partially male-fertile and completely male-fertile classes by selfing partially male-fertile plants in B_6F_1 generation.

Generation and line	Pollen fertility (%)								Number of plants	χ^2-value
	30	40	50	60	70	80	90	100		1 : 1
B_6F_2 - 1		1	143	1			3	143	291	0.003
B_6F_2 - 2		1	150	1			1	143	296	0.215

Note : Two partially male-fertile plants showing 50 percent pollen fertility in Table 1 were selfed.

Table 3. Degree of ovule fertility in completely male-sterile plants in each backcross generation*

Maternal parent (male-sterile)			Ovule fertility by cross-pollination**
Generation	Pollen fertility	Bagged seed fertility	
B_1F_1	0.0%	0.01%	92.5 ± 4.56%
B_2F_1	0.0	0.02	93.4 ± 3.63
B_3F_1	0.0	0.01	93.1 ± 4.83
B_4F_1	0.0	0.00	93.1 ± 3.59
B_5F_1	0.0	0.03	96.5 ± 5.63
B_6F_1	0.0	0.03	92.4 ± 7.45

* : Ten male-sterile plants in each backcross generation showing Table 1 were used as maternal parent.

** : Pollinator was Taichung 65.

B. Seven quantitative characters of six isogenic lines

Six isogenic lines were used in this experiment. The genotype, generation and origin of each line are given in Table 4.

The experiment was carried out in the first and the second crop seasons in 1970. In the first crop season, seeds of those lines were sown in flats at 1.5 x 3 cm seed spacing on March 1, and when the seedlings grown in a green house reached to fourth leaf stage, they were transplanted in an experiment field at 15 x 25 cm plant spacing with one seedling per hill on March 20. A randomized block design with five replications was adopted, and five plants representing each of six lines were placed in every plot. In the second crop season, seeds were sown on July 20, and the seedlings grown in the natural temperature condition were transplanted in the field on August 2. The other methods used in the second crop season were closely resembled to the first ones.

Quantitative characters studied were heading date, ear number, number of leaves, culm length and each of three internodes (first, second and third) counted from the uppermost internode to lower. Heading date had been expressed in the form of number of days taken to ear emergence from the flag leaf sheath after sowing. As to ear number, only the tillers bearing ear including main culm in each plant were counted. Number of leaves of the main culm per plant were counted by methods of Katayama (42). Culm length of the tallest tiller per plant was measured from the ear neck to the ground level at maturity. The uppermost internode including from the ear neck to the next lower node having flag leaf sheath of the tallest tiller per plant was named as the first internode in this experiment and was measured, and the other ones were counted and measured gradually to lower one.

An analysis of variance was applied for the data, in which my interest was focused on the general effects of the cytoplasm, genotype and their interaction for fertility restoration.

Table 4. Six isogenic lines used for studies on quantitative characters.

Line	Generation	Genotype	Origin
A	B_9F_3	(*ms-boro*) *Rf Rf*	Chinsurah Boro II × Taichung 65[10]
B	$B_{10}F_1$	(*ms-boro*) *Rf rf*	Chinsurah Boro II × Taichung 65[11]
C	$B_{10}F_1$	(*ms-boro*) *rf rf*	Chinsurah Boro II × Taichung 65[11]
X	B_9F_3	(*n-boro*) *Rf Rf*	Taichung 65 × Line A (B_8F_2)
Y	$B_{10}F_1$	(*n-boro*) *Rf rf*	Taichung 65 × Line A (B_9F_2)
Z	B_9F_3	(*n-boro*) *rf rf*	Taichung 65 × Line A (B_8F_2)

C. Linkage analysis for fertility-restoring gene *Rf*

(1) Trisomic analysis

The following three kinds of materials were used in this experiment.

(*ms-boro*) *Rf*-Taichung 65: It is a carrier of a dominant fertility restoring gene *Rf* with male sterility-inducing cytoplasm derived from Chinsurah Boro II. It restores the pollen fertility of the male steriles partially (about 50 percent) in the F_1 hybrid plant, but their spikelet fertility is complete.

(*ms-boro*) *rf*-Taichung 65: It is a completely male sterile strain with male sterility-inducing cytoplasm derived from Chinsurah Boro II.

Trisomic series : Eleven trisomic lines (line A to K) were introduced from Dr. Nobuo Iwata, the Faculty of Agriculture, Kyushu University, and another one (line L) from Dr. Yoshiaki Koga, the National Institute of Agricultural Sciences, Hiratsuka. The trisomic lines developed by Iwata *et al.* (34) were derived from variety Aikoku and line L by Watanabe and Koga from Norin 15. Aikoku and Norin 15 are non-carriers of dominant fertility-restoring gene to the present male sterility-inducing cytoplasm.

For the analysis of fertility-restoring gene *Rf*, a modified method of trisomic analy-

sis was employed as follows: All trisomic lines were crossed as maternal parent to (*ms-boro*) *Rf*-Taichung 65. In the F_1 generation, at least two trisomic and two disomic plants were selected cytologically from each cross combination. The selected F_1 trisomic and disomic plants were crossed again as the paternal parent to (*ms-boro*) *rf*-Taichung 65 (male sterile strain).

The hybrid seeds of all the 12 trisomic as well as 12 disomic families derived from three way cross were sown in flats, and seedlings were transplanted to field. At the time of head emergence three panicles in each plant were covered with paraffin paper bags before anthesis to prevent cross pollination. All the bagged panicles on each plant were harvested separately at the time of maturity and their spikelet fertility (%) was estimated, and the tested plants were divided into fertile (70—100%) and sterile (0—5%) classes. The data were tested to fit the expected ratio of fertile and sterile plants in trisomic and disomic families of the three way cross, according to chi-square test.

The plants of each trisomic family derived from three way cross were divided into trisomic and disomic ones based on their morphological features at maturity.

(2) Linkage test for *Rf* gene

The following two kinds of materials were used in this investigation.

Genetic testers: There are only two genes carrying by the extra chromosome of trisomic C line, according to the report by Iwata and Omura (35). The tester Ho-775 involved *pgl* gene (pale green leaves) and F_4-6 involved *fl* gene (faded leaves) were introduced from above members. Both testers are carriers of recessive fertility-restoring gene *rf* with normal cytoplasm, according to the results of cytoplasm and nuclear gene tests for fertility restoration.

(*ms-boro*) *Rf*-Taichung 65: This strain contains dominant *Fl*, *Pgl* and *Rf* genes with male sterility-inducing cytoplasm, (*ms-boro*).

The gene analysis for above three genes was carried out by the conventional methods used first backcross or F_2 progenies. Two genetic testers were crossed as paternal parent to (*ms-boro*) *Rf*-Taichung 65. To make test progenies, at least four F_1 plants in each cross were backcrossed as maternal parent to their testers, respectively. At the same time, two genetic testers were crossed in each other, and F_2 seeds were obtained from eight F_1 plants. Their recombination values among the three genes were calculated by the method of maximum likelihood (1).

D. Cyto-histological investigation of pollen degeneration in anther of male sterile strain, (*ms-boro*) *rf*-Taichung 65

Taichung 65 and (*ms-boro*) *rf*-Taichung 65 were grown in an experimental field at the Institute of Botany, Academia Sinica, Taipei, Taiwan, China in 1970 at the first (March to July) and second (August to November) crop season, and cytological materials were collected and were fixed Carnoy's solution. The development of microspores was observed by aceto-carmine staining method. For observing tapetal cells, anthers containing microspores at two nucleate stage were paraffin-sectioned at a six micron thickness and stained

with Heidenhain's iron-alum hema-toxylin.

E. Distributions of male sterility-inducing cytoplasm and fertility-restoring genes in rice

(1) Commercial lowland-rice cultivated in Japan

Four rice varieties, namely, Honenwase, Manryo, Oirase and Taichung 65, which are known to contain non-fertility-restoring gene *rf rf* and normal cytoplasm, were used as the cytoplasm tester (C-tester); and four their male sterile lines, (*ms-boro*) *rf*-Honenwase, (*ms-boro*) *rf*-Manryo, (*ms-boro*) *rf*-Oirase and (*ms-boro*) *rf*-Taichung 65 developed by the present author, were used as the nuclear gene tester (N-tester) for fertility restoration. When each N-tester was crossed as the maternal parent to four C-testers, these sixteen F_1 progenies revealed completely male sterility. Consequently, these four N-testers were regarded to retain *rf rf* genes and the male sterility-inducing cytoplasm, and four C-testers to retain *rf rf* and the normal cytoplasm.

About 600 lowland-rice varieties were recommended by forty-seven prefectural authorities in 1962. These varieties were introduced into the University of the Ryukyus, Okinawa, in the same year and the breeding history of each variety was checked by two pubulications (17, 41). When the same variety was recommended by two or more authorities, the prefecture bred it or cultivated mainly was checked, and either material was used in the present investigation.

Out of 245 different varieties, 150 succeeded in both test crosses between one of four N-testers which used as the maternal parent and between one of four C-testers as the paternal parent.

Ten F_1 seedlings of each test cross were grown in the paddy field with the standard of cultivation. Pollen and spikelet fertilities in each plant were observed.

The method of determining the cytoplasmic type and nuclear genotype of a variety under the investigation was based on known facts in the following. When a variety possessed normal cytoplasm (*n-boro*) and fertility-restoring gene *Rf Rf*, its F_1 progenies were exhibit complete male-fertility in the cytoplasm test cross (C-testcross) and partially male fertility (ca. 50 percent) in the nuclear gene test cross (N-testcross), respectively; whereas completely male fertiles and completely male steriles were to be produced with the progenies in the respective test crosses when the variety contained (*n-boro*) and *rf rf*. Only partially male fertility was to appear on progenies in both testcrosses when their parent variety possessed the male sterility-inducing cytoplasm (*ms-boro*) and *Rf Rf*. When the variety contained (*n-boro*) and *Rf rf*, its progenies were to produce both partially male fertiles and completely male steriles at a ratio of 1 : 1 in N-testcross and only completely male fertiles in C-testcross; whereas only partially male fertiles in N-testcross and both of partially male fertiles and completely male steriles at a ratio of 1 : 1 in C-testcross when the variety possessed (*ms-boro*) and *Rf rf*. An illustrative aid is also given in Table 5. If the variety possessing other fertility-restoring gene or genes is present, the information will be given from the degree of pollen and spikelet fertilities of the N-testcross progeny.

Table 5. Pollen fertility F_1 progenies derived from testcrosses between cytoplasm or nuclear gene testers and five genotypes possibly found in rice varieties.

Cross combination		Pollen fertility (%) of F_1 progenies											χ^2-value
Strain* and C- and N-tester**	Genotype	0	10	20	30	40	50	60	70	80	90	100	1 : 1
A × C-tester	(*ms-boro*) *Rf Rf* × (*n-boro*) *rf rf*					1	13	1					
N-tester × A	(*ms-boro*) *rf rf* × (*ms-boro*) *Rf Rf*					1	12						
B × C-tester	(*ms-boro*) *Rf rf* × (*n-boro*) *rf rf*	15				1	10	1					0.319
N-tester × B	(*ms-boro*) *rf rf* × (*ms-boro*) *Rf rf*						24						
X × C-tester	(*n-boro*) *Rf Rf* × (*n-boro*) *rf rf*											16	
N-tester × X	(*ms-boro*) *rf rf* × (*n-boro*) *Rf Rf*						15						
Y × C-tester	(*n-boro*) *Rf rf* × (*n-boro*) *rf rf*											19	
N-tester × Y	(*ms-boro*) *rf rf* × (*n-boro*) *Rf rf*	15					18						0.273
Z × C-tester	(*n-boro*) *rf rf* × (*n-boro*) *rf rf*											21	
N-tester × Z	(*ms-boro*) *rf rf* × (*n-boro*) *rf rf*	20											

* : Respective strains had the same genetic background as the of Taichung 65

** : Taichung 65 was used as cytoplasm tester (C-tester) and completly male-sterile Taichung 65 as the nuclear gene tester (N-tester).

(2) Foreign rice varieties

Six C-testers, which are completety male fertile, have contained the normal cytoplasm (*n-boro*) and the non-fertility-restoring gene *rf rf* as given in Table 6. Whereas six N-testers, which are completely male sterile, have possessed (*ms-boro*) cytoplasm and genotype *rf rf*. The N-testers were developed by the present author. Each of the N-testers is in B_8F_1 generation and has the same features in all respects except for male sterility as that of their recurrent paternal parents.

Table 6. Some characters in two different testers for screening out the male sterility-inducing cytoplasm and fertility-restoring genes.

Testers	Genotype	Fertilities (%)		Grain type
		pollen	Seed	
Cytoplasm testers :				
Caloro	(*n-boro*) *rf rf*	99.0	93.2	A
Taichung 65	(*n-boro*) *rf rf*	99.0	94.0	A
Blue Rose	(*n-boro*) *rf rf*	99.0	90.5	B
Sesia	(*n-boro*) *rf rf*	98.0	90.5	B
Rexark	(*n-boro*) *rf rf*	99.0	89.6	C
Zenith	(*n-boro*) *rf rf*	99.0	93.2	C
Nuclear gene testers :				
(*ms-boro*) - Caloro	(*ms-boro*) *rf rf*	0.0	0.1	A
(*ms-boro*) - Taiching 65	(*ms-boro*) *rf rf*	0.0	0.0	A
(*ms-boro*) - Blue Rose	(*ms-boro*) *rf rf*	0.0	0.0	B
(*ms-boro*) - Sesia	(*ms-boro*) *rf rf*	0.0	0.1	B
(*ms-boro*) - Rezark	(*ms-boro*) *rf rf*	0.0	0.1	C
(*ms-boro*) - Zenith	(*ms-boro*) *rf rf*	0.0	0.0	C

One hundred and fifty-three rice varieties have been introduced into the University of the Ryukyus, Okinawa, from the National Institute of Genetics, Mishima, Shizuoka ; the National Institute of Agricultural Sciences, Hiratsuka, Kanagawa; the Kyushu University, Fukuoka ; and from the Taiwan Agricultural Research Institute, Taipei, Taiwan, China, with the record about their collection site. The collected varieties were of natives to sixteen different countries.

The length and width of ten unhulled grains of each variety, including the testers, were measured, and they were divided into three grain types, A, B, and C, by the method of Matsuo (53). For the test crossing a given variety as well as two different testers of the same grain type were chosen in order to avoid the blurring effect caused by intervarietal hybrid sterility as reported by Kato (43), Terao and Mizushima (84), Oka (62,

63, 64, 65), Morinaga and Kuriyama (58, 59), Nagamatsu and Shinjyo (60), and Chang (11). Each variety was crossed with C-tester used as the paternal parent and the N-tester used as the maternal one. Out of 153 varieties, 146 were successful in raising hybrids from both crosses, but the remaining seven failed in the cross with the C-testers. Ten F_1 hybrid plants in each test cross were grown in the paddy field, and pollen and spikelet fertilities were observed. As to the nuclear gene test of 153 progenies, the genotypes of 110 were easily determined, but the remaining 43 were difficult for the occurrence of intervarietal hybrid-sterility. The latter 43 progenies were backcrossed as the maternal parent with their paternal parents or self-pollinated, and the genotype was estimated from the spikelet and pollen fertilities of their progenies. The methods of the determination of the cytoplasm type and the genotype regarding fertility-restoration of the varieties under the investigation were based on the definite fact as given in Table 5 in this paper.

F. General procedure

Crossing technique: The maternal rice panicles were immersed in water contained in a electrical water bath at 43 degrees centigrade for period of six minutes. The panicles in the third or forth bay of blooming were chosen as the maternal parents. An hour or just before blooming normally began, the tillers were bent over carefully to avoid breaking and inserted into the water. The florets which had to open in that day began blooming in the hot water or soon after the treatment. Open florets were pollinated just after all of the closed florets of the panicles were cut off.

Pollen fertility: Five young florets expectedly blooming in the next day were collected from each plant, were fixed in Carnoy's solution, and were preserved in 75 percent alcohol. The pollen grains were stained with iodine and potassium iodide solution for pollen observation. Pollen grains were easily classified into two classes, fertile and sterile, as shown in Fig. 1. Fertile pollen grains were globular in shape and were deeply stained, while the sterile ones, though globular in shape, were smaller in size and were stained slighter. More than 500 pollen grains of each plant were observed, and fertile and sterile ones were counted separately. Pollen fertility (%) of each plant was calculated from the data.

Spikelet fertility: Two methods were employed in this experiment, but spikelet fertility was showed with either method. Two or three panicles of each plant grown at 15 x 25 cm plant spant spacing in the paddy field were covered with paraffin paper bags before flowering in order to prevent natural cross-pollination. Or the seedlings were transplanted under one plant per pot, and were replaced at 5 x 5 meter pot spacing before flowering. In the former case, the spikelet fertility was usually lower about 20 percent than that of the latter. The former method was used only in order to test whether or not the plant was recessive homozygous for fertility-restoring genes, while the latter one was used in order to know critical grade of spikelet fertility. All the panicles tested on each plant were harvested separately at the time of maturity and their spikelet fertility (%) was calculated.

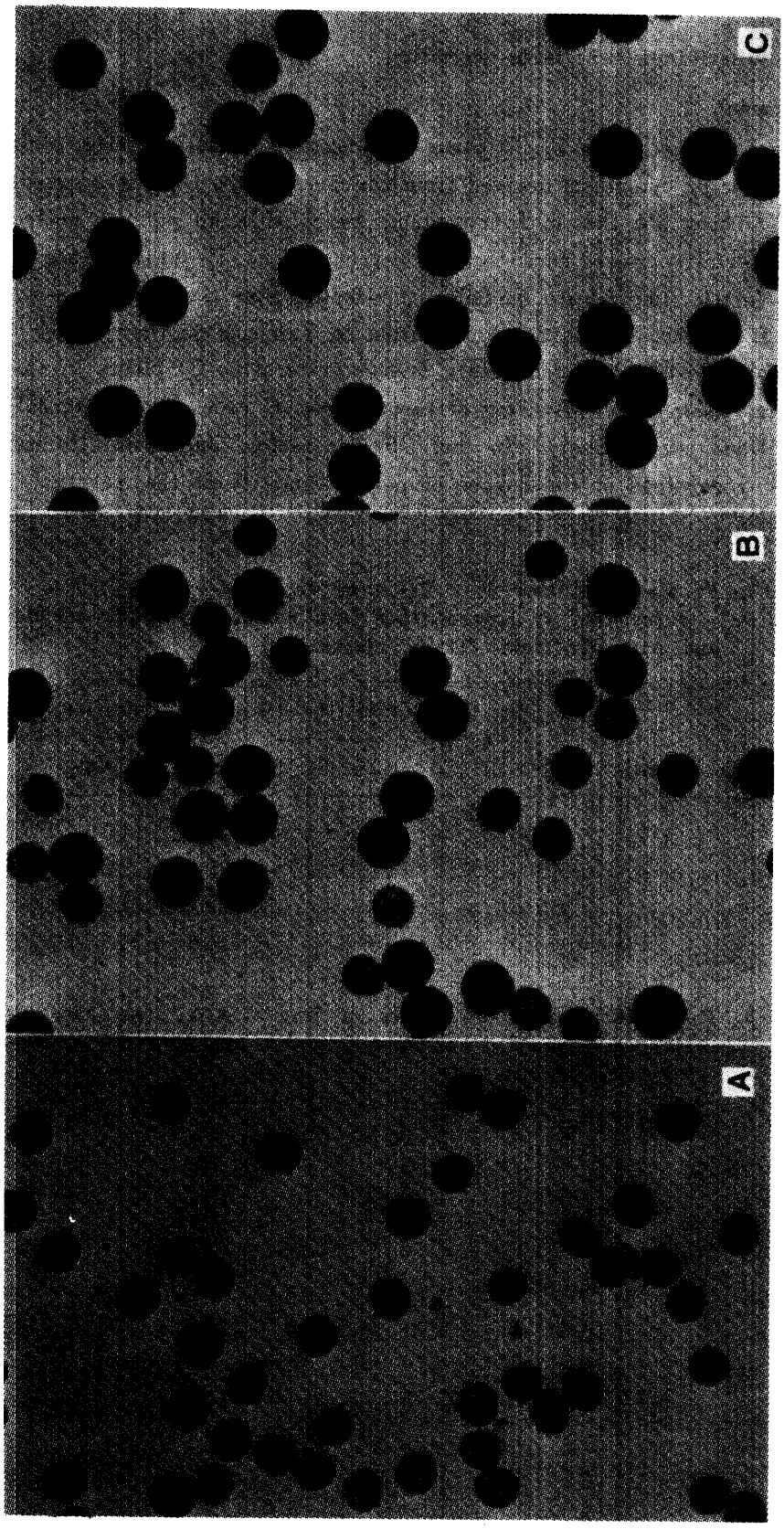

Fig 1. Pollen grains of three different phenotypic plants with male sterility inducing cytoplasm
A : Completely male-sterile. B : Partially male-fertile C : Completely male-fertile.

IV RESULTS

A. The inheritance of male sterility

Experiment 1.

When the strain (*ms-boro*) *Rf*-Taichung 65, completely male fertile, which had the male sterility-inducing cytoplasm derived from Chinsurah Boro II, was crossed as maternal parent to Taichung 65, the F_1 hybrid plants revealed a 50 percent pollen fertility though spikelet fertility were more than 90 percent. The F_2 progeny segregated into completely male-fertile (line A) and partially male-fertile (line B) plants in a 1 : 1 ratio. When the line B was crossed as maternal parent to Taichung 65, the progeny segregated into partially male-fertile and completely male-sterile (line C) plants in the ratio of 1 : 1 again, as given in Table 7. Out of three lines, C crossed as maternal parent to both A and B lines, respectively. In their F_1 generation, only partially male fertile plants appeared, regardless of the degree of pollen fertility of the paternal parent, as given in Table 8.

Table 7. F_1 plants of line B × Taichung 65, (*ms-boro*) *Rf rf* × (*n-boro*) *rf rf*, segregating into completely male-sterile and partially male-fertile classes.

Line and cross	Family	Pollen fertility (%) 0	10	20	30	40	50	60	Number of plants	χ^2-value 1 : 1
Line B × Taichung 65	1	49				1	49	1	100	0.040
	2	45					40	1	86	0.186
	3	65				1	60	1	127	0.071

Table 8. Pollen fertility of F_1 plants of line C × A, (*ms-boro*) *rf rf* × (*ms-boro*) *Rf Rf*; and line C × B, (*ms-boro*) *rf rf* × (*ms-boro*) *Rf rf*.

Line and cross	F_1 family	Pollen fertility (%) 30	40	50	60	70	Number of plants
Line C × line A	1		1	80	2		83
	2		3	92	3		98
	3		2	69	1		72
	4		2	98	1		101
Line C × line B	1		5	106	2		113
	2		1	96	3		100
	3		2	120	1		123

Above three lines were each selfed and the pollen fertility of the progenies were observed. The line A produced only completely male-fertile plants, while the line B selfed segregated into comletely male-fertile and partially male-fertile classes in the 1 : 1 ratio as shown in Table 9. Another line C produced only completely male-sterile plants.

On the other hand, in the reciprocal cross where Taichung 65 was used as maternal parent and strain *(ms-boro)Rf*-Taichung 65 as paternal one, the F_1 hybrid plants were completely male-fertile. The F_2 progeny revealed no segregation for pollen fertility, and all of the plants were completely male-fertile. The spikelet fertility was also high. From this F_2 progeny, eleven plants were picked up at random and were crossed as the paternal parent to line C. The pollen fertility of the F_1 hybrids of above eleven cross combinations was observed. Three of them showed a partially male fertility only, two showed completely male sterility, and the remaining six segregated into partially male-fertile and completely male-sterile classes in the ratio of 1 : 1. The paternal parent of the cross combinations showing partially male fertility was named as line X; that of combinations segregating into partially male fertility and completely male sterility, line Y; that of combinations showing only completely male sterility, line Z, as given in Table 10. When the X, Y and Z lines were selfed, respectively, all of the progenies were completely male-fertile.

Considering the results up to this point, the difference in pollen fertility of the three lines, A, B, and C lines, similarly having the male sterility-inducing cytoplasm derived from Chinsurah Boro II, might be due to difference in the nuclear genotype of pollen fertility-restoring genes. In the X, Y and Z lines which possessed the cytoplasm of Taichung 65 (normal cytoplasm), no pollen degeneration occurred that it was not related to the nuclear genotypes. These facts made it clear that the cytoplasm was an important factor in determining pollen fertility as well as nuclear gene. The cytoplasm derived from variety Chinsurah Boro II could be considered as male sterility-inducing cytoplasm, *(ms-boro)*, and that of Taichung 65 as normal one, *(n-boro)*, and the dominant fertility-restoring gene was symboled by *Rf* and its recessive allele, *rf*.

The cytoplasmic types and the nuclear genotypes for fertility restoration of these six lines were thus assumed to be as that shown in Table 11. The genotypes of A, B and C lines were considered as *(ms-boro)Rf Rf*, *(ms-boro)Rf rf* and *(ms-boro)rf rf*, respectively. On the other hand, the line X, Y and Z were considered as *(n-boro)Rf Rf*, *(n-boro)Rf rf* and *(n-boro)rf rf*, respectively.

Table 9. Segregation of partially male-fertile and completely male-fertile classes in the selfed progeny of line B, *(ms-boro) Rf rf*.

Line	Family	Pollen fertility (%)							Number of plants	χ^2-value 1 : 1
		40	50	60	70	80	90	100		
Line B (selfed)	1		71				2	72	145	0.062
	2	1	72	1			1	71	146	0.027

Table 10. Pollen fertility of F_1 plants of line C × X, *(ms-boro) rf rf* × *(n-boro) Rf Rf*; line C × Y, *(ms-boro) rf rf* × *(n-boro) Rf rf*; and line C × Z, *(ms-boro) rf rf* × *(n-boro) rf rf*.

Line and cross	Family	Pollen fertility (%)							Number of plants	χ^2-value 1 : 1
		0	10	20	30	40	50	60		
Line C	1					1	89	1	91	
×	2					2	86	2	90	
Line X	3					1	90	1	92	
Line C	1	56				1	47	1	105	0.467
	2	44				2	41	3	90	0.045
×	3	55				2	41	2	105	1.000
	4	49				1	30	2	82	3.122
	5	36				1	30	2	69	0.130
Line Y	6	54				2	42	3	101	0.485
Line C	1	105							105	
× Line Z	2	108							108	

Table 11. Genotypes of six lines and their fertilities.

Line	Genotype *	pollen fertility	Spikelet fertility
A	*(ms-boro) Rf Rf*	99.8 %	94.1 %
B	*(ms-boro) Rf rf*	49.6	93.5
C	*(ms-boro) rf rf*	0.0	0.01
X	*(n-boro) Rf Rf*	99.9	94.1
Y	*(n-boro) Rf rf*	99.9	92.5
Z	*(n-boro) rf rf*	99.8	91.6

* *(ms-boro)* ..... Male sterility-inducing cytoplasm derived from Chinsurah Boro II.
(n-boro) ..... Normal cytoplasm of Taichung 65.
Rf and *rf* ... Dominant and recessive fertility-restoring genes.

These experimental results suggested that the genotype of *(ms-boro)Rf*-Taichung 65 as well as of its maternal parent Chinsurah Boro II was *(ms-boro)Rf Rf*, and that of Taichung 65 was *(n-boro)rf rf*. Therefore, the pollen and spikelet fertilities of the two varieties as well as of *(ms-boro)Rf*-Taichung 65 were expected to be completely male-

fertile; the pollen fertility was more than 98 percent and spikelet fertility was more than 90 percent. These results suggested that the above-mentioned hypothesis proved to be true.

Experiment 2.

To confirm whether the principles obtained from experiment 1 also hold for other varieties, twenty-one Japonica varieties were crossed as paternal parent to *(ms-boro)Rf*-Taichung 65, respectively. Pollen fertility in their F_1 hybrid plants were around 50 percent and spikelet fertility was higher than 95 percent. The segregation ratio of completely male-fertile to partially male-fertile classes in the F_2 was a 1 : 1 as shown in Table. 12. The segregation ratio seen in the experiment 2 was the same as in the experiment 1 where line B was self-pollinated (Table 9). Thus the mode of inheritance seen in the experiment 1 was confirmed in the experiment 2.

Table 12. F_2 segregation for pollen fertility.

Paternal parent *	pollen fertility (%)							χ^2-value
	40	50	60	70	80	90	100	1 : 1
Aikoku 5	2	41	2			2	35	0.780
Eiko		37				2	35	0.000
Fujiminori	1	39	2			2	40	0.000
Fujisaka 5	4	35	5			3	42	0.428
Hamayu	2	39	2			2	34	0.620
Haya Norin	1	29	2			2	39	0.048
Honenwase	2	29	2			6	32	0.390
Hoyoku	3	30	2			6	39	1.250
Koshihikari	2	39	2			9	30	0.342
Manryo	2	40	1			1	35	0.620
Miyoshi	5	40	5			4	40	0.383
Nan - ei	2	39	4			5	35	0.294
Naruho	1	40	1			4	35	0.111
Norin 15	2	45	2			3	45	0.010
Norin 17	3	40	4			5	30	1.956
Norin 20	1	43	1			4	44	0.097
Norin 22	3	29	3			5	40	1.250
Norin 24	1	39	2			6	32	0.200
Oirase	1	40	1			2	45	0.281
Toyonishiki	1	40	2			3	38	0.106
Yutakawase	4	30	4			6	31	0.013

* : Maternal parent was *(ms-boro) Rf* - Taichung 65 having *(ms-boro) Rf Rf* genotype. The genotype of the paternal paternts was *(n-boro) rf rf*.

Therefore, the cytoplasmic types and nuclear genotypes for fertility restoration of these twenty-one Japonica varieties may presumably be the same as those of Taichung 65, *(n-boro) rf rf*. For confirmation, above twenty-one varieties were analyzed by the test of cytoplasmic type and nuclear genotype for fertility restoration. The results showed that all of them hab the genotype *(ms-boro) rf rf*. These also verify the pollen fertility variation shown in Table 12.

Experiment 3.

Six Indica varieties were used in order to test whether or not the fertility-restoring gene from Chinsurah Boro II and those from these varieties differed in action. The male sterile line (line C) was crossed as maternal parent to six Indica varieties, respectively. All of the F_1 hybrids were partially male-fertile. When F_1 hybrids of these six cross combinations were crossed as maternal parent to Japonica varieties, the B_1F_1 hybrids segregated into partially male fertile and completely male sterile classes in a ratio of 1 : 1. Moreover, when the partially male fertile plants in each hybrid generation were repeatedly back-crossed as maternal parents to their recurrent Japonica varieties, the generations successively segregated into the 1 : 1 ratio. The segregation pattern observed in the B_4F_1 generation of these six cross combinations is given in Table 13. When the fertility-restoring gene was introduced into the male sterility-inducing cytoplasm derived from Chinsurah Boro II, the heterozygous plants revealed partially male fertility. This was similar to that observed in the F_1 hybrid plants of line B x Taichung 65 in the experiment 1 (Table 7). No difference in pollen fertility restoration was seen between the genes originating from Chinsurah Boro II and the other six varieties tested.

Table 13. Segregation of completely male-sterile and partially male-fertile classes in B_4F_1 generation of different cross combinations, [(*ms-boro*) *rf rf* × *Rf Rf*] × (*n-boro*) *rf rf*[5]

Line or variety and cross *	Pollen fertility (%)		χ^2-value
	Male-sterile	Partially fertile	1 : 1
(line C × Salak) × Taichung 65[5]	45	44	0.011
(line C × Lati Soil) × Akibae[5]	44	49	0.269
(line C × Bandang putih) × Norin 24[5]	43	42	0.017
(line C × patnai 23) × Taichung 65[5]	49	54	0.243
(line C × Liu-tou-tu) × Taichung 46[5]	15	15	0.000
(line C × Tadukan) × Taichung 46[5]	19	21	0.100

* : Line C is male sterile with male sterility-inducing cytoplasm derived from Chinsurah Boro II. Non-recurrent paternal parents are Indica rice varieties. Partially male-fertile plants were selected in each backcrossed generation and were succesively backcrossed for four times.

B. Effects of male sterility-inducing cytoplasm and nuclear genotype for fertility restoration on main quantitative characters

(1) Lengths of culm and upper level internodes in plants

The analyzed results of variances on seven morphological characters of the isogenic lines tested are given in Table 14. Length of the first and second internodes were highly significant in the line factor, respectively, and culm length was also highly significant. Each variance mentioned above of line factor which was highly significant was analyzed again and divided into three factors of genotype, cytoplasm and interaction of genotype x cytoplasm as shown in Table 15. The analyzed results of the variances indicated that highly significant differences of both culm and the first internode were mainly attributed to cytoplasm, genotype and interaction of genotype x cytoplasm factors, but that of the third internode length was mainly due to genotype factor. Shorter culm and its related three shorter internodes were appeared alone on the male sterile line, but were not related in those of the other lines as given in Table 16.

Table 14. results of the analysis of variance.

Source of variation	Degree of freedom	Mean square for						
		heading date	ear number	main culm		internode length of maim culm		
				leaf number	length	first	second	third
Line (L)	5	0.11	0.33	0.01	92.68**	28.76**	0.54	2.04**
Season (S)	1	4724.16**	0.02	16.64**	1.32	0.04	0.57	5.44**
Block	8	0.74	0.21	0.02	4.70	0.49	0.89**	0.42
L × S	5	0.45	0.13	0.01	4.94	2.13	0.33	0.13
Error	40	0.44	0.25	0.01	4.21	0.68	0.28	0.52

** : Significant at 1% level.

Table 15. Mean Squares for three characters.

Source of variation	Degree of freedom	Length of main culm	Internode length	
			first	third
Genotype (G)	2	63.57**	16.15**	3.25**
Cytoplasm (C)	1	112.34**	50.78**	0.97
G × C	2	111.96**	30.37**	1.36
Error	40	4.21	0.68	0.52

** : Significant at 1% level.

Table 16. Mean values of seven characters of six lines.

Genotype	Heading date (day)	Ear number	Main culm		Internode length of main culm (cm)		
			leaf number	length (cm)	first	second	third
(*ms-boro*) *Rf Rf*	90.3	9.0	16.9	102.0	42.2	24.3	19.8
(*ms-boro*) *Rf rf*	90.2	9.3	16.9	102.0	41.7**	24.5	19.8
(*ms-boro*) *rf rf*	90.4	9.4	16.9	94.8**	38.3**	23.8*	18.7**
(*n-boro*) *Rf Rf*	90.4	9.5	16.9	102.1	42.2	24.3	20.0
(*n-boro*) *Rf rf*	90.5	9.3	16.9	101.9	42.6	24.2	19.6
(*n-boro*) *rf rf*	90.3	9.4	16.9	103.0	42.9	24.3	19.6
5% l. s. d.	0.60	0.45	0.09	1.85	0.74	0.48	0.65
1% l. s. d.	0.80	0.60	0.12	2.48	1.00	0.64	0.87

* and ** : Significant at 5% and 1% level, respectively, for (*n-boro*) *rf rf* line.

(2) Heading date, ears and leaves

Both characters of heading date and leaf number of the main culm revealed highly significant difference to seasonal factor. The heading date was later about 15 days and leaf number was increased one more in the first crop season than in the second one. The ear number in both seasons was mostly identical. As mentioned above, heading date and leaf number of the main culm subjected their selves to strong influence of seasonal factor, while each of line source of their characters was not significant (Table 14 and 16). Nevertheless the male sterile line had shorter culm including three shorter internodes, the leaf number, heading date and ear number of the male sterile line were the same degree as those of the other lines tested. These facts may be the most desirable factors for cross pollinated system in hybrid rice breeding.

C. Linkage analysis for fertility-restoring

(1) Trisomic analysis

The progenies of 12 disomics of the three way cross derived from (*ms-boro*)*rf*-Taichung 65 x F_1 disomic plants from Aikoku or Norin 15 trisomic lines x (*ms-boro*)*Rf*-Taichug 65 were classified into sterile and fertile classes based on their bagged spikelet fertility as shown in Table 17. The plants tested could be easily classified into two classes, sterile (0-5%) and fertile (70-100%). Segretion of the sterile and fertile classes in each disomic family was tested to fit the 1 : 1 ratio.

Table 17. Segregation of fertile and sterile classes in test families from three way cross., (*ms-boro*) *rf* – Taichung 65 × F_1 disomic plants of Aikoku or Norin 15 trisomics × *Rf* – Taichung 65

Test Family	Number of plants			χ^2-value
	fertile	sterile	total	1 : 1
D – A	62	52	114	0.877
D – B	53	45	98	0.653
D – C	60	49	109	1.110
D – D	43	50	93	0.526
D – E	55	60	115	0.213
D – F	50	42	92	0.695
D – G	48	55	103	0.457
D – H	68	56	124	1.161
D – I	52	65	117	1.444
D – J	54	59	113	0.221
D – K	50	51	101	0.009
D – L	45	52	97	0.502

Since each of 12 disomic families gave the 1 : 1 ratio, and Taichung 65, Aikoku and Norin 15 with the male sterility-inducing cytoplasm derived from Chinsurah Boro II were completely sterile under the present experimental condition, it is concluded that the dominant fertility-restoring gene *Rf* of the strain *(ms-boro)Rf*-Taichung 65 is responsible for the fertility restoration.

Out of twelve trisomic families derived from the three way cross, eleven satisfied the 1 : 1 ratio. This fact indicates that neither of above eleven different extra chromosomes in these families carries the fertility-restoring gene. The highly significant deviation from the 1 : 1 ratio, with much less fertile and excessive sterile plants, was observed in the family concerned trisomic C line (Table 18). T-C in Table 18 is to be a critical trisomic family, whose extra chromosome carries the fertility-restoring gene. Pollen fertility of the critical trisomic family is shown in Table 19. The disomic and trisomic F_1 plants with the normal cytoplasm derived from original trisomic line C x *(ms-boro)Rf*-Taichung 65 revealed more than 95 percent pollen fertility. Out of 32 partially pollen fertile plants from the three way cross, twenty were disomic and the remaining twelve were trisomic. Different degrees of pollen fertility between the disomic and trisomic plants in the family were observed as given in Table 19.

Table 18. Segregation of fertile and sterile classes in test family from three way cross, *(ms-boro) rf* – Taichung 65 × F_1 trisomic plants derived from Aikoku or Norin 15 trisomics × *Rf* – Taichung 65

Family	Fertile		Sterile		Number of plants	χ^2-value
	2n	2n + 1	2n	2n + 1		1 : 1
T – A	111	2	110	0	223	0.040
T – B	65	0	82	4	151	2.920
T – C	20	12	161	3	196	88.898**
T – D	91	9	86	3	189	0.640
T – E	110	0	121	0	231	0.523
T – F	74	1	72	1	148	0.027
T – G	83	0	106	1	190	3.031
T – H	77	8	62	18	165	0.151
T – I	117	4	135	6	262	1.526
T – J	92	1	82	0	175	0.691
T – K	75	4	73	4	156	0.025
T – L	40	0	55	0	95	2.368

** : Significant at the 1% level.

Table 19. Dgree of pollen fertility in critical trisomic family, T – C

Family	2n or 2n+1	Pollen fertility (%)														
		0	5	10	——	30	35	40	45	50	55	60	——	90	95	100
F_1	2n														1	1
	2n+1														2	
Family from three way cross :																
	2n	161							3	17						
	2n+1	3				3	7	2								

(2) Linkage test for *Rf* gene

The results of the linkage tests among the three genes (*Rf*, *fl* and *pgl*) are shown in Table 20. The recombination value between *fl* and *Rf* was about 0.4 percent, *pgl* and *Rf* was about 12 percent, and *fl* and *pgl* was about 20 percent. The arrengement order and their distance of the three genes tested are shown in Figure 2.

Table 20. Linkage test among three genes, *fl*, *pgl* and *Rf*, in the first backcross or F_2 generation.

Gene ration	Cross combination	AB	Ab	aB	ab	Number of plants	Linkage phase	Recombination value (%)
B_1F_1	(*ms-boro*) *Fl Rf/fl rf* × *fl rf*	265	2	0	244	511	C	0.4±0.28
B_1F_1	(*ms-boro*) *Pgl Rf/pgl rf* × *pgl rf*	266	30	39	244	579	C	11.9±1.35
F_2	(*n-boro*) *Fl pgl* × *fl pgl*	2920	1370	1460		5750	R	20.4±5.30

Note : *fl* ..... faded leaves
Rf ..... fertility restoring gene.
pgl ..... pale green leaves.
(*ms-boro*)··· male sterility-inducing cytoplasm derived from Chinsurah Boro II.
(*n-boro*)····· normal cytoplasm.

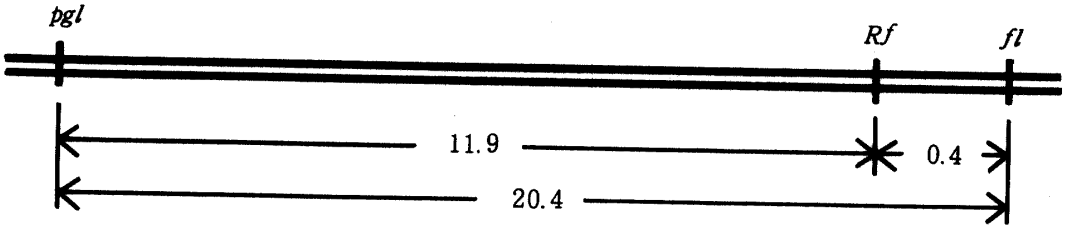

Fig 2. Linkage map of three genes

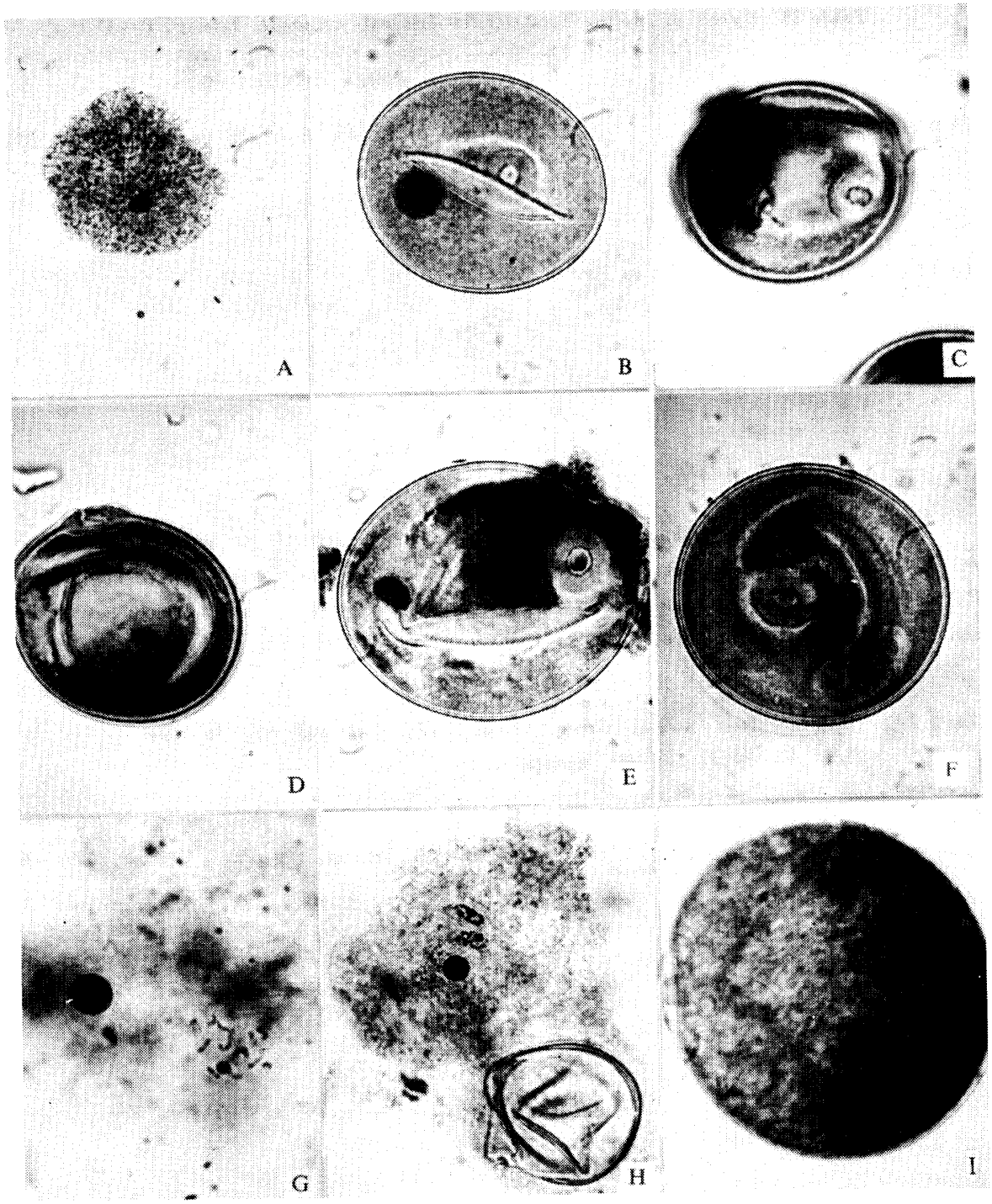

Fig 3. Microspores in plants of Taichung 65

A. Tetrad stage.
B. One-nucleate stage
C. prophase of first pollen mitosis.
D. Anaphase of first pollen mitosis.
E. Two-nucleate stage.
F. Prophase of second pollen mitosis.
G. Metaphase of second pollen mitosis.
H. Three-nucleate stage.
I. Mature pollen.

D. Cyto-histological investigation of pollen degeneration in anthers of male sterile plants, (*ms–boro*) *rf*–Taichung 65

In the plants of Taichung 65, young microspores liberated the tetrabs developed and became mature pollen grains with three nuclei after passing through one and two nucleate stages as given in Fig. 3-A to -H. The proceeding of microspore development in Taichung 65 was the same as that of observed by Kihara and Hirayoshi (48) in *Oryza sativa.* In cytoplasmically male sterile plants, (*ms-boro*)*rf*-Taichung 65, the pollen mather cells were however normally produced and meiosis in the PMC's also proceeded normally as given in Table 21. But after liberation from tetrabs the microspores failed to develop and disintegrate by the time. At the one-nucleate stage, about 25 percent nuclei of 1024 male sterile microspores seemed to be smaller than those of Taichung 65 as shown in Table 21 and Fig. 4-A. Furthermore, almost of all the microspores at two-nucleate stage contained only a single nucleus (Table 21 and Fig. 4-B). The pollen grains at the flowering stage of the male sterile plants were globular but smaller in size and were slightly stained by iodide solution (Fig. 4-C).

The anthers of the male-sterile plants and Taichung 65 were similar in the structure of tapetal tissue as shown in Fig. 5.

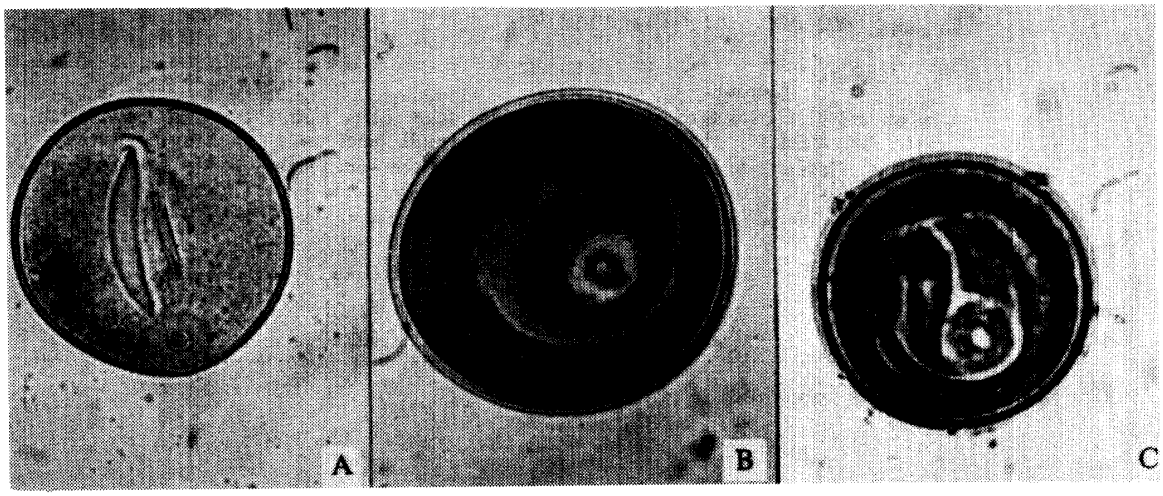

Fig 4. Abnormal microspores in cytoplasmic male-sterile plants

A. One-nucleate stage microspore with small nucleus.
B. Microspore at two-nucleate stage having one nucleus only.
C. Sterile pollen at flowering stage.

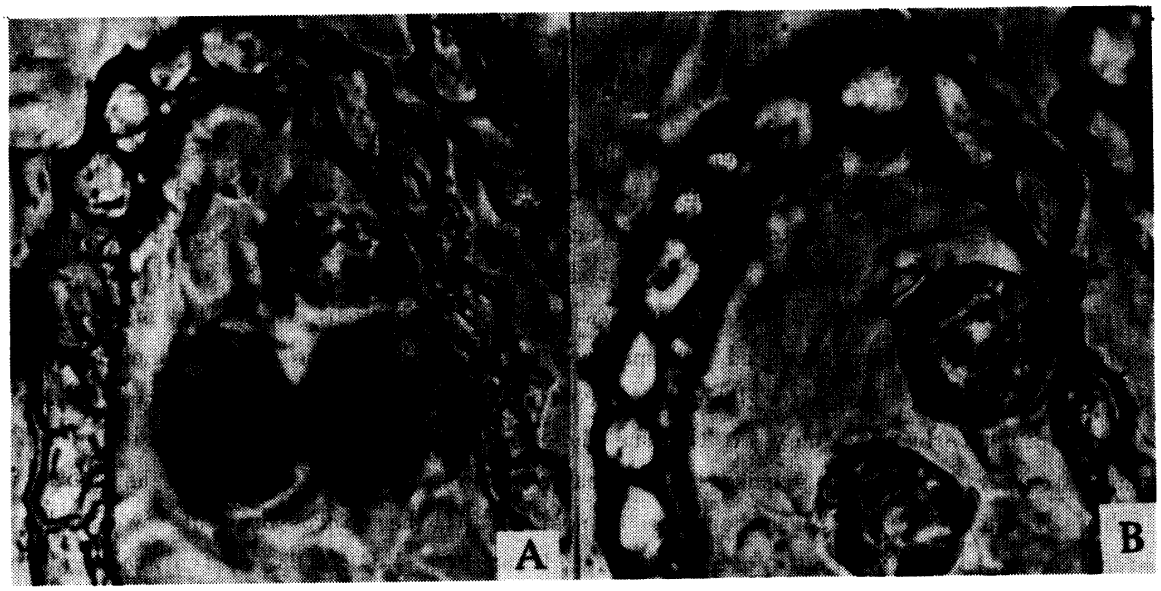

Fig 5. Tapetal tissue of Taichung 65 and its cytoplasmic male-sterile plants

A. Taichung 65.

B. Male sterile plant.

Table 21. Cytological observation of microspore development in Taichung 65 and (*ms-boro*) *rf*-Taichung 65.

Line	Meiosis		Mitosis	
	% of cells with univalent at the first metaphase	% of cells with chromosome bridges at the first anaphase	% of cells with small nucleus at one-nucleate stage	% of cells with single nucleate at two-nucleate stage
Taichung 65	0.5 (566) *	0.3 (590)	0.2 (1010)	2.3 (1108)
(*ms-boro*) *rf*-Taichung 65	1.9 (521)	0.2 (573)	24.2 (1024)	81.9 (1096)

* : Number of PMC's observed.

E. Distributions of male sterility-inducing cytoplasm and fertility-restoring genes

(1) Commercial lowland-rice cultivated in Japan

In the cytoplasm testcross, all of F_1 progenies revealed completely male fertility as given in Table 22 and spikelet fertility was also high (ca. 94 percent). It was therefore indicated that each cytoplasm of all varieties tested was normal.

In the nuclear gene test for fertility restoration, out of 150 F_1 progenies, 131 were completely male-sterile and the remaining 19 gave partially male fertility which fell in

a range from 25.3 to 53.3 percent as shown in Table 23. However, spikelet fertility of these 19 progenies were far lower ranged (from 1.7 to 11.5 percent) than their pollen fertility (Table 23). From the results of the nuclear gene test, these 19 varieties were found to possess weak fertility-restoring gene or genes.

Table 22. Pollen fertility of 150 F_1 hybrids raised from cytoplasm and nuclear gene tests.

Testcross	Pollen fertility (%)											Number of testcrosses
	0	10	20	30	40	50	60	70	80	90	100	
Cytoplasm											150	150
Nuclear gene	131			3	8	8						150

Table 23. A list of the male parents showing partially male fertility in nuclear gene test, and pollen and spikelet fertilities of the test progenies.

Male parent	Fertility (%) of test progenies	
	Pollen	Spikelet
Aikawa 44	40.2	8.1
Benkei	50.8	6.0
Etsunan 18	49.7	7.1
Hyuga-mochi	34.4	6.6
Isenishiki 722	51.6	4.6
Iwai-mochi	37.9	3.6
Kairyo Hattanryu	40.1	4.6
Kyonohana 1	34.9	3.3
NaKate-asahi 1	44.4	11.5
Nojobo	50.5	6.2
Norin 31	50.5	10.3
Oita-omachi 50	48.6	1.7
Okayama-mochi	51.0	10.0
Shigahabutae-mochi	25.3	5.1
Shinhabutae-mochi	44.3	6.6
Shinriki 798	47.6	7.0
Shinson-mochi	43.8	4.5
Tarobee-mochi	40.4	5.5
Yamada-nishiki	53.3	6.1

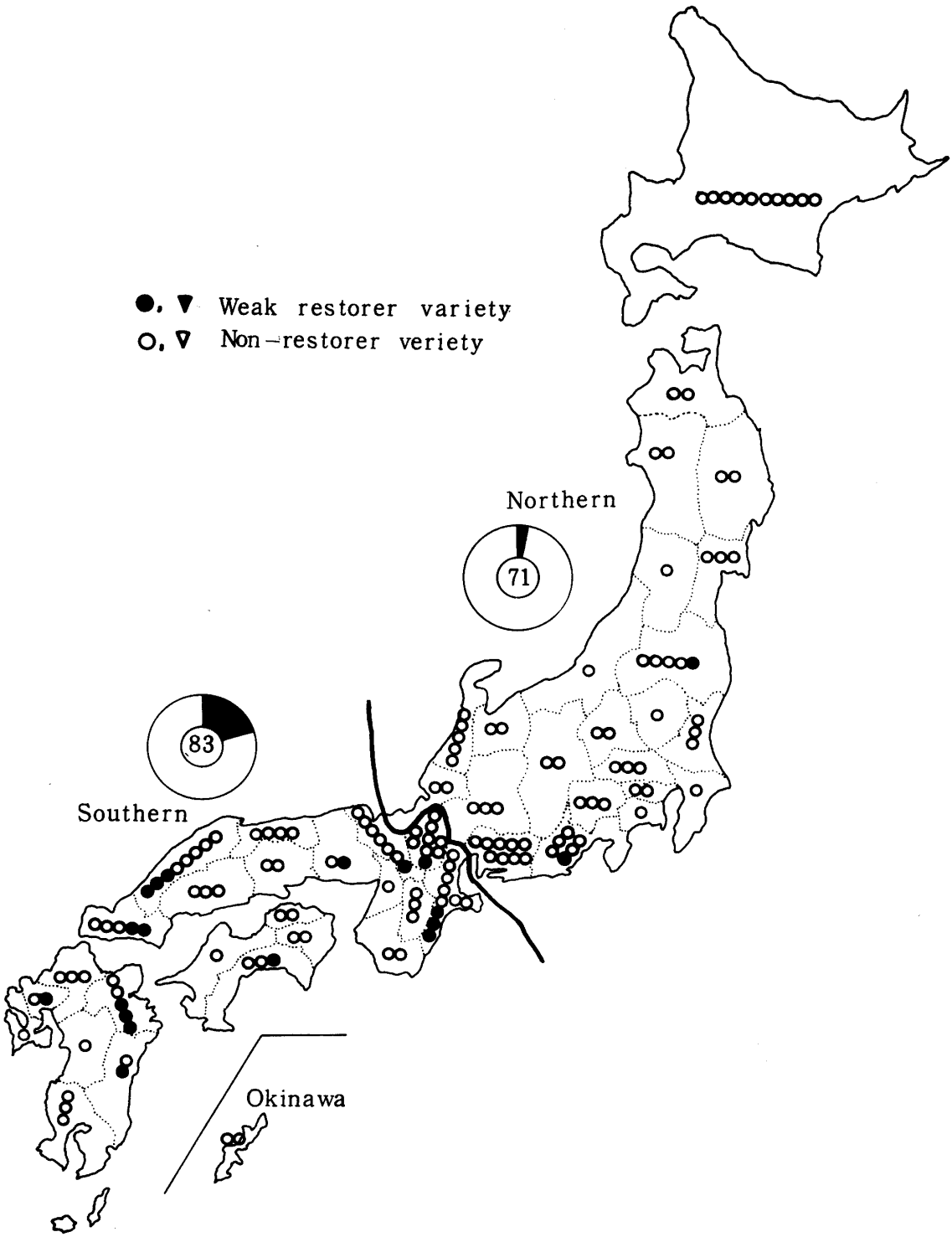

Fig 6. Geographical distribution of weak restorer and non-restorer varieties in Japanese lowland-rice tested

The male sterility-inducing cytoplasm and fertility-restoring gene of Chinsurah Boro II, an Indica variety, were transferred into Taichung 65, a Japonica variety, as previously mentioned in this paper. Accordingly, F_1 hybrid carrying (*ms-boro*) *Rf rf* genotype revealed a partially male fertility (ca. 50 percent), although a high spikelet fertility (ca. 93 percent) was expected. The fertility-restoring gene *Rf* from Chinsurah Boro II and the weak fertility-restoring gene (s) in here were quite different in the percentage of spikelet fertility.

The geographical distribution of 135 non-restorer varieties including four cytoplasm testers and 19 weak restorer ones is shown in Fig. 6. Allmost all the weak restorer varieties were distributed in southern region and only two were found in northern region. The southern region is represented by Kyushu including Okinawa, Chugoku, Shikoku and Kinki districts, and the northern region includes Chubu, Kanto, Tohoku and Hokkaido districts.

(2) Foreign rice varieties

Cytoplasm test: Out of 146 test progenies, 142 revealed the 80 percent or still higher pollen fertility and the remaining four showed about 40 percent pollen fertility as given in Table 24. The spikelet fertility of the latter four test progenies was about 80 percent or much higher as were the former 142. These four paternal parent varieties, which revealed partially pollen fertility, were Assam, Bhutmuri, Chinsurah Boro I, and Tepa. When these four F_1 test progenies were crossed again as the maternal parent with their each cytoplasm tester, the progenies were segregated into completely pollen sterile and partially pollen fertile classes in a ratio of 1 : 1 as given in Table 25. These facts indicated that each of these four varieties contained an effective restoring gene and the male sterility-inducing cytoplasm as same as variety Chinsurah Boro II. Above male sterility-inducing cytoplasm was detected in the varieties belonging to the boro rice obtained from India and East Pakistan. On the other hand, male sterility-inducing cytoplasm was not detected among the varieties belonging to aman and aus rice groups grown in the same region. The normal cytoplasm was exclusively distributed in all other countries.

Table 24. Frequency of the varieties giving rise to F_1 hybrid with different grades of fertility, when crossed to cytoplasm tester.

Fertility	Fertility grade (%) of F_1 hybrid									Total number of varieties tested
	20	30	40	50	60	70	80	90	100	
Pollen		1	3				16	98	28	146
Spikelet						2	36	92	16	146

Table 25. Segregation of pollen fertility in four B_1 F_1 populations used in the cytoplasm test, (boro rice × cytoplasm tester) × cytoplasm tester.

Boro rice variety	Pollen fertility (%)							Number of plants	χ^2-value
	0	10	20	30	40	50	60		1 : 1
Assam	80			1	50	24		155	0.161
Bhutmuri	74			2	70	6	1	153	0.163
Chinsurah Boro I	96			1	20	69		186	0.194
Tepa	62			1	21	32	1	117	0.419

Nuclear gene test for fertility restoration: Out of 153 tested progenies, 54 revealed partially pollen fertility (ca. 40 percent), though their spikelet fertility was a 70 percent or still higher, 28 showed far lower spikelet fertility as compared with their pollen fertility, and the remaining 71 revealed complete sterility in both spikelet and pollen as given in Table 26. Each of F_1 progenies, which revealed far lower spikelet fertility than their pollen fertility, was crossed with cytoplasm testers, respectively. The spikelet fertility of their 28 prgenies thus raised did not restore higher fertility than their F_1 plants. It may be considered than each of these 28 varieties possessed a weak restoring gene or genes. Fifty-four varieties, which revealed 70 percent or much higher spikelet fertility in the test crosses, were considered to carry an effective restoring gene or genes.

Table 26. Frequency of the varieties giving rise to F_1 hybrid with different grades of fertility, when crossed to nuclear gene tester.

Fertility	Fertility grade (%) of F_1 hybrid											Total number of varieties tested
	0	10	20	30	40	50	60	70	80	90	100	
Pollen	69	1	2	9	53	17	2					153
Spikelet	74	19	6					1	22	25	6	153

The distribution of effective fertility restorer varieties, weak restorer ones and non-restorer ones is shown in Table 27. There were found no effective restorers in Korea, the northern part of China and Australia. Also effective restorers could not be found in Japanese commercially lowland-rice varieties (Table 22). Every native rice from Taiwan possessed an effective restoring gene or genes, while certain commercially lowland-rice varieties so-called "ponglai" did not contain any effective restoring gene. Three ecotypic varieties, namely, aman, aus, and boro, have been introduced from India and East Pakistan.

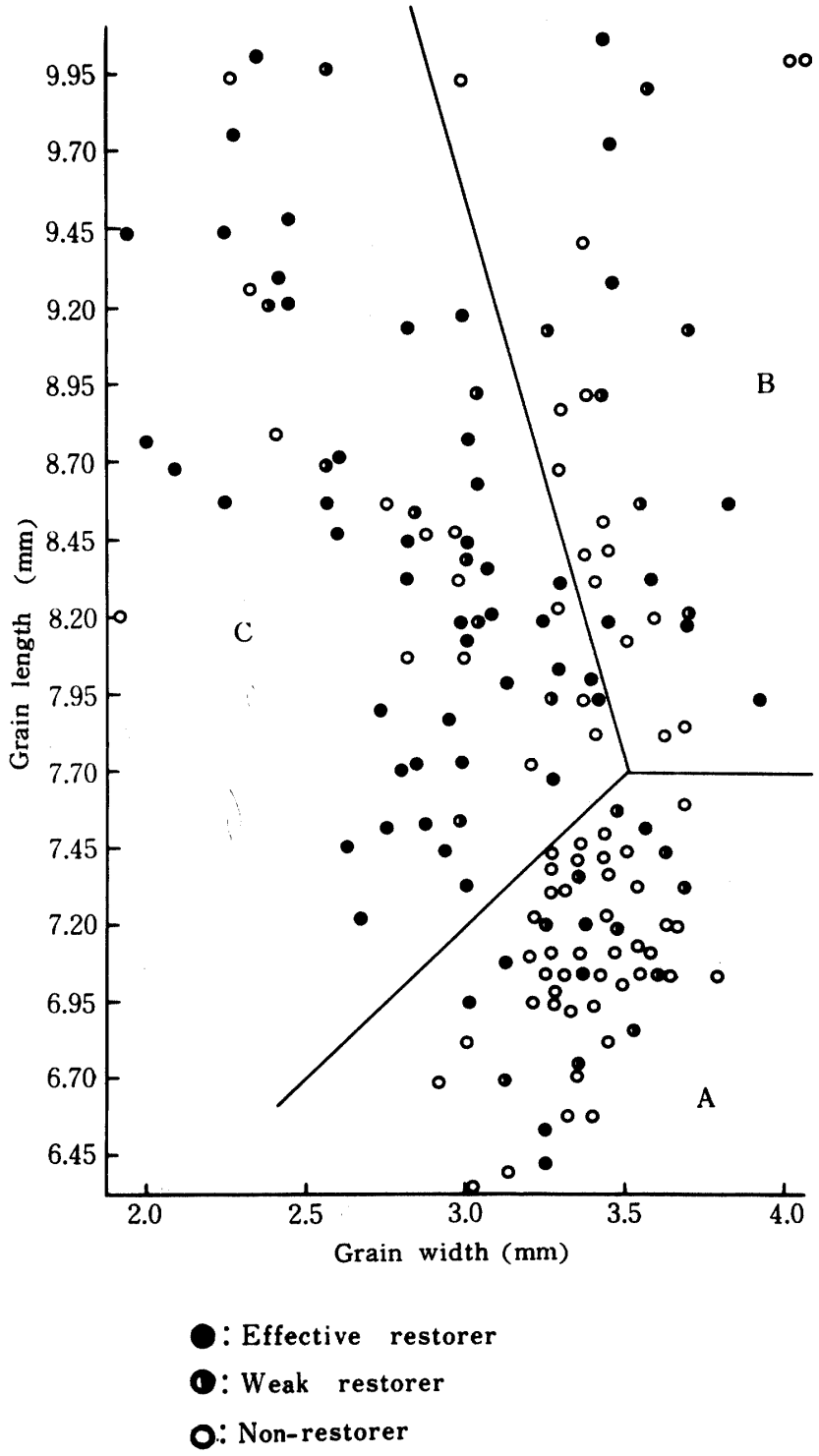

Fig 7. Relationship between grain type by Matsuo and restoring gene in 153 foreign rice varieties tested

Table 27. Geographical distribution and frequency of the fertility-restoring gene among the varieties tested.

Native place of varieties	Number of restorer varieties			Total number of varieties tested
	Effective	Weak	Non	
Korea		1 (1)*	6 (5)	13
China				
North		1	2	3
South and middle	12	2	14	28
Taiwan	(7)	2	4	13
Philippines	5	1		6
Tailand	1	1	1	3
India and East Pakistan				
Aus		2	3	5
Boro	4			4
Aman	5			5
Celebes			1	1
Java island				
Bulu		1	6	7
Tjereh	5			5
Egypt	3	1	3	7
Portugal	1	1	1	3
Italy	5	2	7	14
France	2	3	5	10
USSR	1	2	3	6
USA	3	6	8	17
Australia		1	2	3
Total	54	28	71	153

* : The number in parentheses indicates that of the native varieties.

All of the aman and boro varieties studied belonged to Indica rice and possessed exclusively certain effective restoring genes. On the contrary, the aus group varieties called as Javanica or intermediate type by Jennings (36) and Morinaga *et al.* (59), respectively, contained the weak or non-restoring genes. There are two different ecotypic varieties, bulu and tjereh, in Java island. The bulu varieties belonging to Javanica rice contained the weak or non-resorer genes, but the tjereh ones belonging to Indica rice involved the effective restoring genes. It indicates that the detection of the effective restoring gene in Javanica rice varieties including the aus and bulu groups is more or less difficult

because the presence of the effective gene is rare in the rice groups. There are found no effective restorer varieties in California, U.S.A., where Japonica rice is exclusively grown. On the other hand, three effective ones were detected in Louisiana and Texas where Indica is mainly distributed. All the effective, weak and non-restorer varieties are distributed in the European countries.

Cultivated varieties of rice, *Oryza sativa*, in the world were divided into three grain types, A, B, and C, by Matsuo (53). He reported that A type varieties were mainly distributed in Japan, B in Java island, and C in India and the southern part of China. The relationship between the grain types and the restoring genes is shown in Fig. 7. Frequency of the effective restorer varieties in the three grain types, A, B, and C, were 8.5, 27.6 and 62.9 percent, respectively.

V DISCUSSION

A. Inheritance of cytoplasmic male sterility

When the male sterile line was pollinated by five other lines, A, B, X, Y, and Z lines, which were completely male-fertile or partially male-fertile (ca. 50 percent), F_1 hybrids revealed monogenic segregation ratio, respectively. The selfings of these six lines including male sterile line also proved that fertility-restoring gene should be present in the nucleus. Moreover, the same results were also obtained two additional experiments (Table 12 and 13).

From the results so far obtained the genotpe of line B may be regarded as *(ms-boro) Rf rf* but the F_1 hybrid of line C x B was only partially male-fertile as that of line C x A (Table 8). The selfed progeny of line B segregated into completely male-fertile and partially male-fertile classes in the ratio of 1 : 1 (Table 9). It was thus assumed that in the line B, *(ms-boro) Rf rf*, the pollens having recessive gene *rf* degenerated at a certain stage of development due to its interaction with the male sterility-inducing cytoplasm and did not participate in fertilization. For the same reason the pollen fertility of line B may be partially male-fertile (50 percent). The normal function of female gametes was proved by selfing and in the segregation into partailly male-fertile and completely male-sterile classes in the 1 : 1 ratio in the F_1 hybrid plants of line B x Taichung 65 (Table 7 and 9). Therefore, the effect of the present fertility-restoring gene in the male sterility-inducing cytoplasm can be considered as gametophytic. Such examples as hereby found were also observed by Buchert (7) in the USDA type of cytoplasmic sterile corn (19) and by Shinjyo *et al.* (79) in Lead Rice cytoplasm detected by Watanabe *et al.* (87). Jones *et al.* (37, 38) and Maunder and Picktt (54) reported that the mode of inheritance of cytoplasmic male-sterility in onion and in sorghum, respectively. In onion and sorghum, both *Rf Rf* and *Rf rf* genotype plants with male sterility-inducing cytoplasm revealed completely male fertility, while a *rf rf* genotype plant with that cytoplasm was completely male-sterile. Accordingly, male fertile and sterile classes were

observed in a ratio of 1 : 1 in an F_2 generation. The effect of the fertility-restoring gene in the male sterility-inducing cytoplasm in onion and sorghum is of sporophytic type, and the sporophytic type gene or geges is detected in many other crops.

The fertility-restoring gene of variety Tadukan which was used by Kitamura (50, 51) was introduced into Japonica rice variety in the present investigation. The hybrid population observed in the B_4F_1 generation segregated into partially male-fertile and completely male-sterile classes in the ratio of 1 : 1. In Kitamura's reports, any abortion was not seen in both male and female gametes, but due to interruption of anther dehiscence the flowers became sterility. The results of Kitamura differed completely from the present case.

Two fertility-restoring genes for the male sterility-inducing cytoplasm of variety Lead Rice detected by Watanabe *et al.* (87) was discovered in a Japonica variety, Fukuyama, and in the strain *(ms-boro)Rf*-Taichung 65 by Shinjyo *et al.* (79). The effects of *Rfx* gene named temporarily from Fukuyama and *Rf* from *(ms-boro)Rf*-Tachung 65 were the gametophytic in the Lead Rice cytoplasm. Accordingly, both kinds of heterozygous plants having either gene in that cytoplasm revealed partially male fertility (ca. 50 percent), while the spikelet fertility in both plants was a 80 percent or still higher. Although, *Rfx* gene was acted as weak restoring one for cytoplasm of Chinsurah Boro II. *Rf* and *Rfx* genes did not restored fertility in *Oryza sativa* f. *spontanea* cytoplasm detected by Katsuo and Mizushima (46). The author could not find any restorer variety having both effective and weak restoring genes for cytoplasm of Chinsurah Boro II in the study on distributions of male sterility-inducing cytoplasm and fertility-restoring genes. These facts suggest that weak and effective genes probably being *Rfx* and *Rf* are allelic in their relation. It also suggest that the male sterility-inducing cytoplasm of Chinsurah Boro II is more or less resembled to that of Lead Rice comparing with above cytoplasm of *Oryza sativa* f. *spontanea*.

Katsuo and Mizushima (46), Erickson (23), and Athwal and Virmani (3) reported cytoplasmic male sterile lines of which the cytoplasmic sources were derived from different rice varieties or wild rice, respectively. Although, the mode of inheritance of the male sterility in the present investigation can not compare with above ones, because any effective restoring gene or genes for their cytoplasms except for cytoplasm of Lead Rice have not been reported unfortunately.

Intervarietal hybrid sterility in rice, *Oryza sativa*, have been studied genetically and reported by Kato (43), Terao and Mizushima (84), Oka (62, 63, 64, 65), Morinaga and Kuriyama (58, 59), and many other workers as reviewed by Chang (11). They could not detect cytoplasmic male sterility in their intervarietal hybrid progenies, and moreover Oka (64, 65), and Katsuo and Mizushima (46) considered that the intervarietal hybrid sterility did not contain cytoplasmic male sterility. But recently four cytoplasmically male-sterile lines have been obtained from the intervarietal hybrid progenies by Shinjyo and Omura (75), Watanabe *et al.* (87), Erickson (23), and Athwal and Virmani (3). Consequently, these facts may suggest that the cytoplasmic male sterility is also one of fac-

tors for the intervarietal hybrid sterility.

B. Application of genetic results to developing program of three lines needed hydrid rice breeding

(1) Development of male sterile line from *rf rf* genotype variety with normal cytoplasm

To develop the pure male-sterile line which has fully identical characteristics to a given variety except for male sterility, a male sterile line of *(ms-boro)rf rf* genotype and the variety of *(n-boro)rf rf* one must be carried along as described by Jones and Clarke (38), and Jones and Davis (39) in a male sterile onion. The methods of developing and maintaining the male sterile line through the seed are illustrated in Fig. 8. As the male sterile line can not be selfed, the seed is secured by continually backcrossing to the variety. After a few backcrossings the male sterile line should be practically identical with the recurrent paternal variety except for the male strility, but the male sterile line should be secured by continually backcrossing to the variety. This backcross seed make it possible to perpetuate the male sterile line as well as produce the male-sterile maternal parent used in the production of hybrid seed.

(2) Development of both male-sterile and restorer lines from *rf rf* genotype variety with normal cytoplasm

A *(ms-boro)Rf Rf* line or variety should be chosen as non-recurrent maternal parent and it is crossed with a given variety, *(n-boro)rf rf* genotype, used as recurrent pollinator as shown in Fig. 9. It may be expected that the hybrid plants in B_1F_1 generation segregate into partially male-fertile and completely male-sterile classes in a ratio of 1 : 1. Then the partially male-fertile plant in each backcross generation is selected by observation of pollen fertility and is crossed as maternal parent to the recurrent pollinator. After being backcrossed five or six times to the recurrent pollinator, the hybrid plants are almost identical with their pollinator except for the pollen fertility. In that generation, completely male sterile plants must be selected and must be perpetuated by the method of Fig. 8, while the restorer line showing completely male fertility must be selected in the next generation derived from the selfings of partially male-fertile plant and the line is perpetuated by selfing.

(3) Development of maintainer and male-sterile lines from *(ms-boro)Rf Rf* or *(n-boro)Rf Rf* genotype variety

Firstly, a maintainer line, *(n-boro)rf rf*, which is fully identical with a given variety except for the gene constitution concerning fertility restoration or cytoplasmic type must be developed. To obtain the maintainer line from the *(ms-boro)Rf Rf* or *(n-boro)Rf Rf* genotype variety, both varieties should be crossed as the recurrent pollinator to a *(n-boro)rf rf* variety or line which is non-recurrent maternal parent.

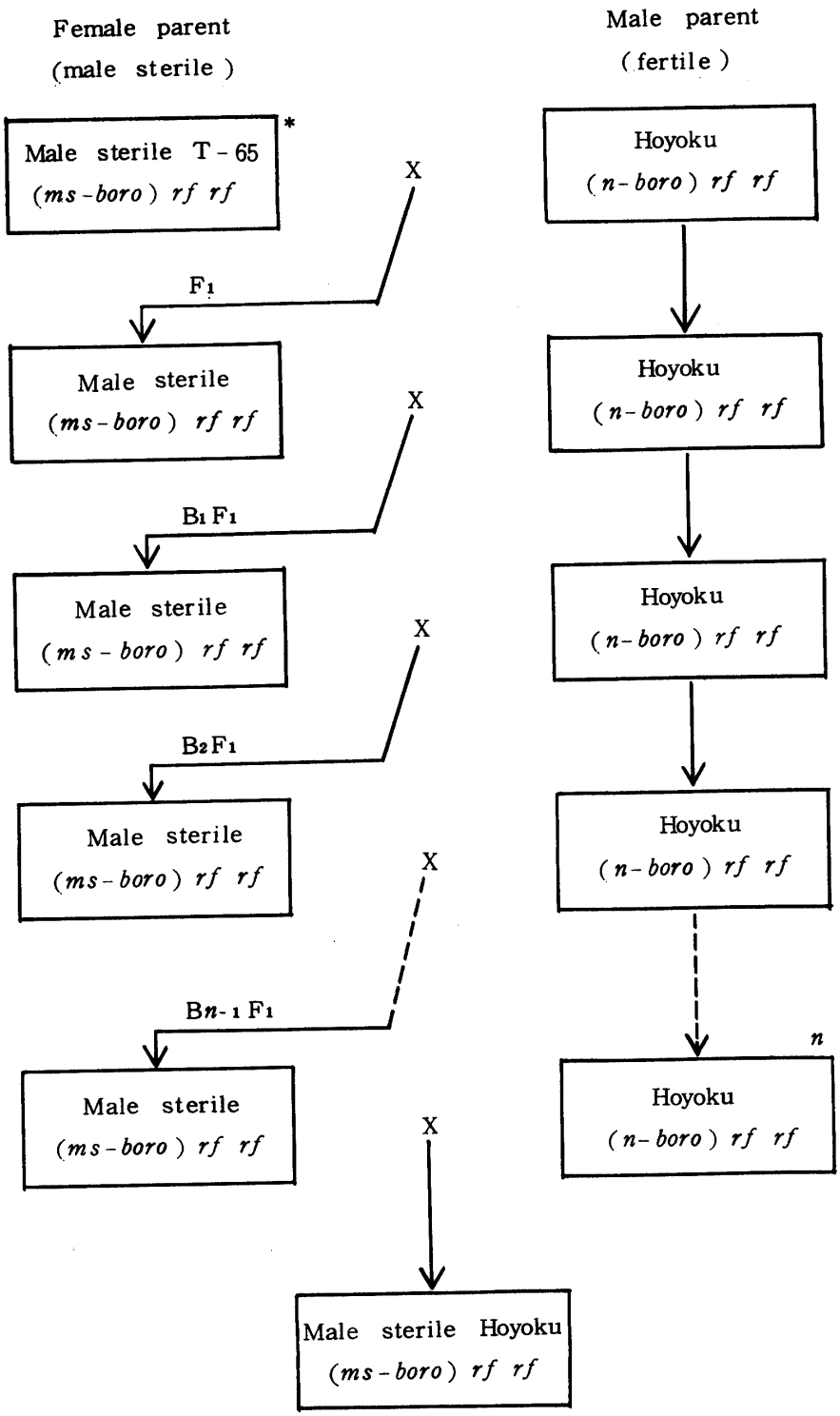

Fig 8. Method of developing male sterile line Hoyoku

* : Male sterile Taichung 65 was used as female parent in the original cross in this case.

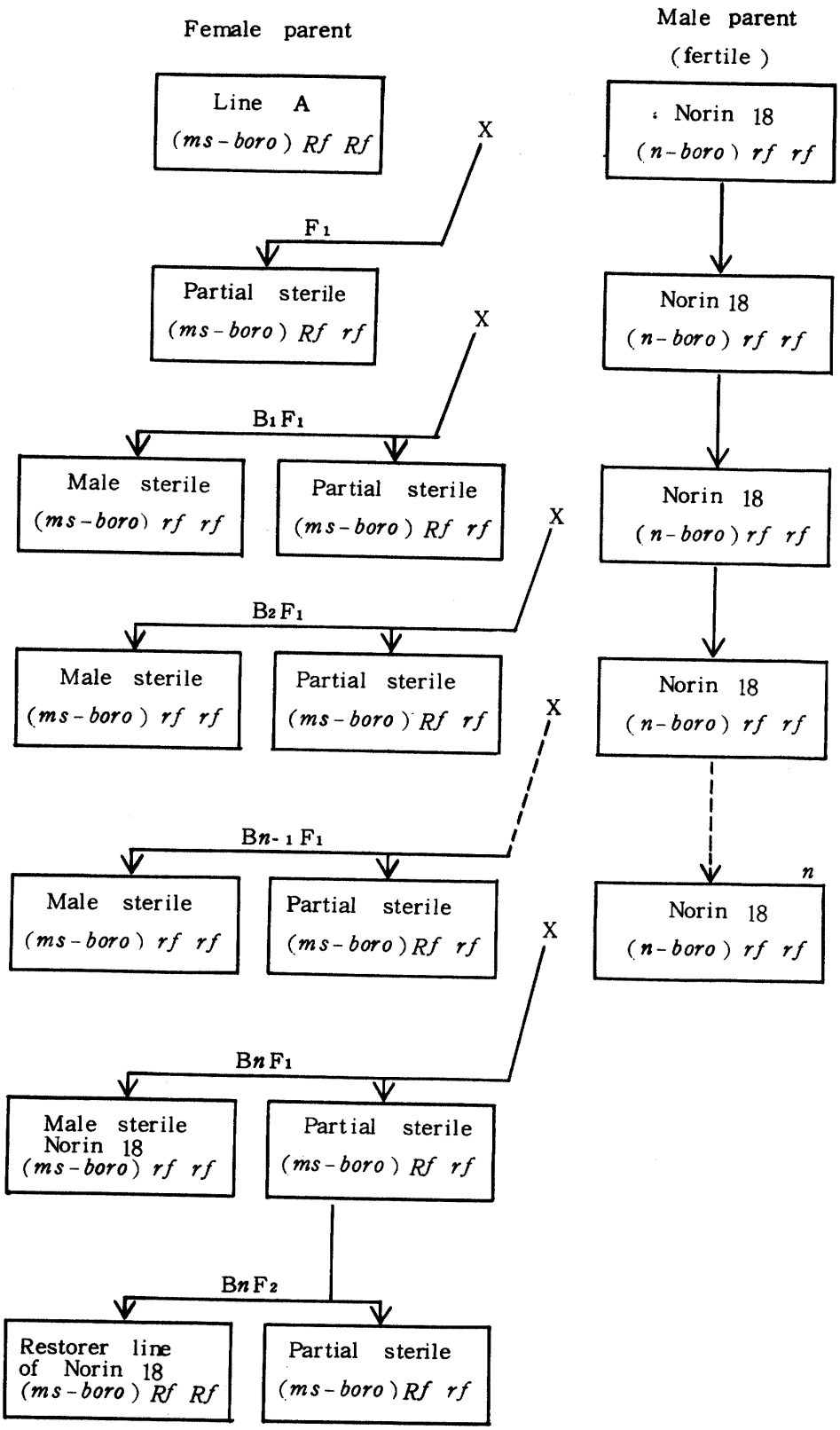

Fig 9. Method of developing both male sterile and restorer line of Norin 18

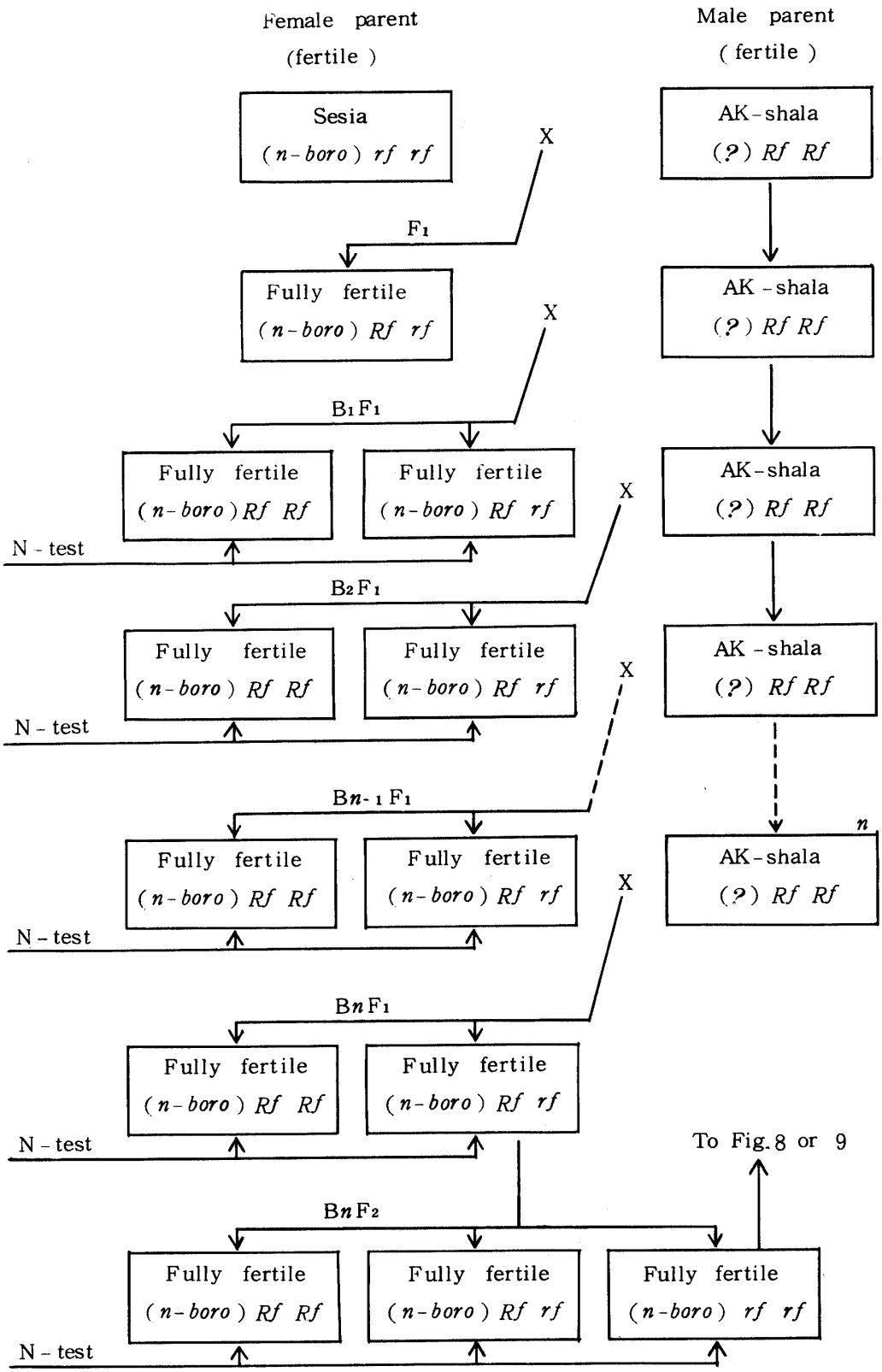

Fig 10. Method of developing maintainer line, (*n*-boro) *rf rf*, of (*ms*-boro) *Rf Rf* or (*n*-boro) *Rf Rf* genotype veriety

Two different F_1 progenies may be expected to be completely male and spikelet fertile. First backcrossing progenies in two different combinations will be also expected to be completely male fertile, although they segregate into *(n-boro)Rf Rf* and *(n-boro)Rf rf* genotype plants in a ratio of 1 : 1. Therefore, a heterozygous plant in each backcrossing generation must be selected by the nuclear gene test concerning fertility restoring gene constitution as aleady shown in Table 5, and the selected plant must be repeatedly backcrossed with the recurrent pollinator. When the abvanced backcrossed plants are expected to be almost identical with the pollinator, the heterozygous plant concerning fertility restoring gene constitution is self-pollinated, and the maintainer line having *(n-boro)rf rf* genotype must be selected as given in Fig. 10.

In the second procedure, the selected maintainer line is used as the recurrent pollinator and is crossed to a male sterile line or restorer line with male sterility-inducing cytoplasm which is non-recurrent maternal parent (Fig. 8 or 9). The male sterile line is produced by the method of Fig. 8, and the male sterile and restorer lines are produced by the method of Fig. 9.

(4) Developments of three lines from heterozygous plants

If *(n-boro)Rf rf* genotype plant is obtained in a variety, the plant will be self-pollinated. The selfings segregate genotypically into *(n-boro)Rf Rf*, *(n-boro)Rf rf* and *(n-boro)rf rf* in a 1 : 2 : 1 ratio, although they are completely male fertile. From the selfings, the maintainer and restorer lines are selected by the nuclear gene test. The male sterile line will be developed by using maintainer line as the recurrent pollinator as given in Fig. 8.

If *(ms-boro)Rf rf* plant is obtained in a variety, the plant will be self-pollinated, and completely male fertile plant will be selected from the selfed progeny. The maintainer and male-sterile lines are produced by the method of Fig. 8 and 10.

C. Effects of cytoplasm and nuclear genotype for fertility restoration on main quantitative characters

Grogan and Sarvella (29) studied inbred lines of maize and reported that Texas-type male sterility-inducing cytoplasm was effective in reducing the stalk length above ear, the length of internode above ear and the length of tassel culm, while no effect was detected on the internode number in a stalk, the length of stalk below ear, leaf sheath length and heading date. According to the report of Hori and Tsunewaki (31), *Aegilops ovata* cytoplasm delayed heading date about fifteen days and reduced plant height about eleven cm.

In this investigation, the shorter culm and three shorter upper-level internodes were appeared alone on the male sterile line as those of corn. They are highly significant differences for those of five other lines and are mainly attributed to interaction of nuclear genotype for fertility-restoring genes and the male sterility inducing cytoplasm. The heading date, ear number per plant and leaf number (or node number) of the male

sterile line are not significant for those of the other lines. These facts indicate that the perpetuation of male sterile line through seed and production of hybrid seed are easy comparatively. Because shorter culm with no restrictions in other important characters on the male sterile line as well as corn is most desirable for pollinating system in the field.

D. Chromosomal location of *Rf* gene

Nagao *et al.* (61) reported tentatively twelve linkage groups in Japonica rice. About 70 genetic marker strains involved about 50 different genes determined already their loci on chromosomes were introduced from the Hokkaido University and the Kyushu University into the University of the Ryukyus, and were crossed as paternal parent to a restorer line with the male sterility-inducing cytoplasm from Chinsurah Boro II. The F_2 segregations for the genetic marker genes and *Rf* were observed. All of the F_2 progenies gave only a independent ratio between *Rf* and the genetic marker genes. A complete set and several lines of trisomics in rice have been reported by Iwata *et al.* (34) and Watanabe (unpublished), respectively. The trisomic lines were introduced from above workers and used as genetic testers in this investigation. The tested family related the trisomic C line is expected to be a critical one, whose extra chromosome carries the fertility-restoring gene. According to the report by Iwata *et al.* (35), only two genes of *fl* (faded leaves) and *pgl* (pale green leaves) have been carried by the extra chromosome of the trisomic C line, and have been named temporarily as *fl-pgl* linkage group. It is clear that the fertility-restoring gene belongs to the *fl-pgl* likage group from the results of trisomic and the marker gene analyses.

E. Cyto-histological investigation of pollen degeneration in anthers of male-sterile plant, (*ms–boro*) *rf*– Taichung 65

In the cytoplasmic male sterile plants of various crops, the meiosis in PMC's has been reported to be normal (2) in *Beta vulgaris*, in *Allium Cepa* (83), etc. Fukasawa (25) also concluded in *Aegilops* that the degeneration of pollen grains began at the stage of the first division of pollen nuclei. Katsuo (45) and Watanabe *et al.* (87) reported that the cytoplasmic male-sterile line had been obtained from the successive backcrosses with Japonica variety to a wild rice (*Oryza sativa* f. *spontanea*) and a Burmese variety, Lead Rice, respectively. These two differently cytoplasmic male-sterile lines produced the pollen grains which could be stained by iodide solution as same as those of the parent varieties. Any abortion could not be found during the microspore development. Furthermore, Katsuo (45) indicated that no particle of polysaccharide was found in his cytoplasmic male sterile line. In his case, some microspores carried out nuclear divisions producing pollen grains with two or three nuclei, but were abortive judging from the occurrence of micro-chromatin bodies.

In the present study, microspores with a small nucleus were always found at one-nucleate stage and contained a single nucleus even after two-nucleate stage, showing that

almost all of the microspores loss the ability to carry out nucleus division at all.

In cytoplasmically male-sterile sugar beet, pollen abotion was associated with tapetum hypertrophy or plasmodium (2, 32). Similar phenomena were reported also by Sakai (72) in rice plants damaged by low temperatures and Shibuya (74) in semi-sterile rice plants. In the present materials, no histological abnormality was found in the tapetum. This indicates that mechanisms of the cytoplasmic male sterility in rice is similar to that in wheat.

F. Distributions of male sterility-inducing cytoplasms and fertility-restoring genes

(1) Commercial lowland-rice cultivated in Japan

Any male sterility-inducing cytoplasm and effective fertility-restoring gene did not detected in the commercial lowland-rice cultivated in Japan. About 87 percent of the varieties tested were considered to have *rf rf* genotype with the normal cytoplasm, because their F_1 test progenies were completely male-sterile in the nuclear gene test and were completely male-fertile in the cytoplasm test. It indicates that the male-sterile and the restorer lines needed in hybrid rice breeding are easily developed in Japanese rice varieties, although a few varieties contain the weak restoring gene or genes which are considered to be not useful as the restorer for the male sterile line.

Table 28. Frequency of varieties possessing weak fertility-restoring gene (s) and non-fertility-restoring one in different classes of their origin (bred by hybridization or by pureline selection) and endosperm character (waxy or non-waxy)

Region	Endosperm	Breeding by hybridization		Pure-line selection		Number of varieties
		Non-restorer	Weak-restorer	Non-restorer	Weak restorer	
North	Waxy	8 (100) *		0	0	8 (100)
	Non waxy	59 (93.6)	1 (1.6)	2 (3.2)	1 (1.6)	63 (100)
South	Waxy	6 (46.2)	0	0	7 (53.8)	13 (100)
	Non waxy	50 (71.4)	3 (4.3)	10 (14.3)	7 (10.0)	70 (100)

* : The number in parentheses indicates percentage.

According to the breeding history of the varieties tested by two publications (17, 41), almost all the varieties tested from the northern region of Japan were developed by hybridization methods and only three by pure-line selection from their native varieties as given in Table 28. Almost all the weak restorer varieties from the southern region were developed by selection from their natives. This fact may indicate that the weak restoring gene or genes is easily found in other varieties developed by the pure-line selection or in many native varieties. The gene(s) will give an obstacle when the male-sterile and the restorer lines are developed from the weak restorer varieties.

(2) Foreign rice varieties

The male sterility-inducing cytoplasm as same as variety Chinsurah Boro II was only detected in the varieties belonging to the boro rice group obtained from the Indian Center including India and East Pakistan where is considered to be birth place of cultivated rice, *Oryza sativa*, according to the report by Vavilov (86). Considering the results up to this point, the normal cytoplasm would be detected throughout almost all the varieties cultivated in the world, while the male sterility-inducing cytoplasm would be found only in a few varieties cultivated in India, East Pakistan, and their neighboring countries.

In the nuclear gene test, each of 54 varieties which revealed 70 percent or much higher spikelet fertility is considered to carry an effective restoring gene(s) and the effect of the effective gene(s) is gametophytic. Such effective gene(s) found in these varieties must be tested critically to know whether their restoring gene(s) is the same as the *Rf* gene of Chinsurah Boro II in its action. Each of these 28 varieties showing far lower spikelet fertility than pollen one may possess a weak restoring gene or genes. Such a example as this was also observed in certain commercial lowland-rice cultivated in Japan. Each of 71 varieties revealed completely male sterility in the nuclear gene test may have non-restorer gene.

Every native variety from Taiwan possessed an effective restoring gene(s), while certain commercial lowland-rice varieties so-called "Ponglai" did not contain any effective restoring gene. It may be considered in this connection that no native Taiwan variety was used as the parent when Ponglai varieties were developed by hybridization methods as stated Huang (33).

The results obtained in the present investigation (Table 27 and Fig. 6) and in Japanese rice (Table 22 and Fig. 5) showed that the non-restorer varieties were mainly distributed in the temperate countries. On the other hand, the effective restorer ones were mainly grown in the tropics, while the weak restorers were found everywhere and in all three grain types by Matsuo (53). As a rule, the male sterile and restorer lines can be developed rather easily in the temperate countries where Japonica rice is cultivated, comparing to the tropics where Indica rice is generally grown. The author could not find any restorer variety having both effective and weak restoring genes. It may suggest that the weak and effective restoring genes are allelic in their relation.

VI SUMMARY

This research work was carried out to elucidate five genetical problems on the cytoplasmic male sterility and fertility restoration, and to probe the possibility of future hybrid rice breeding in the sophisticated way by making use of the cytoplasmic male sterility in this important cereal crop. The conclusions arrived at are summarized in the following paragraphs.

A. Inheritance of male sterility

A case of cytoplasmically male sterility controlled by a fertility-restoring gene *Rf* was found by the present author in a cultivated rice, *Oryza sativa* L. The male sterility-inducing cytoplasm symbolized by (*ms-boro*) and fertility-restoring gene *Rf* were derived from an Indica variety, Chinsurah Boro II, and the experiments were made by the isogenic lines having the same genetic background of a Japonica variety, Taichung 65.

When a plant with male sterility-inducing cytoplasm (*ms-boro*) had nuclear genotype *Rf Rf*, it was completely male-fertile; when it had *Rf rf*, partially male-fertile (ca. 50 percent); and when it had *rf rf*, completely male-sterile with no resticition in ovule fertility. A plant with normal cytoplasm (*n-boro*) would be completely male-fertile regardless of the nuclear genotype for fertility-restoring genes. The F_1 plants of (*ms-boro*) *rf rf* x (*n-boro*)*Rf rf* segregated into partially male-fertile and completely male-sterile classes in a ratio of 1 : 1, while the F_1 plants of (*ms-boro*)*rf rf* x (*ms-boro*)*Rf rf* did not segregate, and all of the plants revealed only partially male fertility. The selfed progeny of (*ms-boro*)*Rf rf* line segregated into completely male-fertile and partially male-fertile classes in a ratio of 1 : 1. The effect of fertility-restoring gene is thus found to be gametophytic type in the male sterility-inducing cytoplasm. The F_1 plants of (*ms-boro*)*rf rf* x *Rf Rf* have revealed a 90 percent or higher spikelet fertility, though they are partially male-fertile. The sources of cytoplasm and fertility-restoring gene may be used for breeding "hybrid rice".

It is clear that the developments of three lines (male-sterile, maintainer and restorer lines) needing for hybrid rice breeding are possible by using methods of Figs. 8, 9 or 10.

B. Effects of cytoplasm and nuclear genotype for fertility-restoration on main quantitative characters

Seven quantitative characters, namely, heading date, ear number, leaf number of main culm, culm length, and each of three internodes (first, second and third) counted from the uppermost internode to lower, of six isogenic lines were studied.

About 7 cm shorter culm and its related three shorter internodes were appeared alone on the male sterile line, but were not related in those of five other isogenic lines. Nevertheless, the male sterile line had the shorter culm including three shorter internodes, the leaf number (or node number), heading date, and ear number per plant of the male sterile line, were the same degree as those of the other lines. These are the most

desirable factors for cross pollinated system in the production of hybrid seed in commercial scale.

C. Linkage analysis for fertility-restoring gene *Rf*

Two analyzing methods were employed. Firstly, each of 12 F_1 trisomic plants derived from 12 trisomic lines x strain (*ms-boro*)*Rf*-Taichung 65 was selected cytologically, and the selected F_1 trisomic plants were crossed again as paternal parent to (*ms-boro*)*rf*-Taichung 65 (male sterile line). From the results of spikelet fertility of the F_1 progenies derived from above three way cross, it is clear that the fertility restoring gene is carried by the extra chromosome of trisomic C line which is to be the seventh chromosome assumed by Iwata and Omura (35). In the second, the recombination values among three genes, *fl*, *pgl*, and *Rf*, which were carried by the same chromosome were calculated by conventional methods used first backcross or F_2 progenies. The values between *fl* and *Rf* is about 0.4 percent, *pgl* and *Rf* is about 12 percent, and *fl* and *pgl* is about 20 percent. The arrangement order of the genes is expected to be *pgl-Rf-fl*.

D. Cyto-histological investigation of pollen degeneration in anthers of male sterile plants

The development of pollen grains in male sterile plants with male sterility-inducing cytoplasm derived from Chisurah Boro II were observed. Their anthers are slender and contain globular but small pollen grains which are not deeply stained by iodine. Meiosis is normal and pollen tetrads are normally formed, but the microspores do not develop beyond the one-nucleate stage. The tapetum of the male sterile plants is normal.

E. Distributions of male sterility-inducing cytoplasm and fertility-restoring genes

(1) Commercial lowland-rice cultivated in Japan

One hundred and fifty lowland-rice varieties recommended by forty-seven prefectural authorities of Japan in 1962 were crossed with two different testers in order to screen male sterility-inducing cytoplasm and fertility-restoring genes.

Nineteen varieties (12.7 percent) carrying weak fertility-restoring gene(s) and 131 non-carriers (87.3 percent) were found. Almost all the varieties carrying the weak restoring gene(s) were concentrated in the southern Japan (Kyushu, Shikoku, Chugoku and Kinki Districts) and were developed by pure-line selection from their native varieties.

As to cytoplasm type, male sterility-inducing cytoplasm such as that found in Chinsurah Boro II was not found. Namely, all varieties tested possessed a normal cytoplasm.

(2) Foreign rice varieties

One hundred and fifty-three rice varieties of the species, *Oryza sativa*, which were

introduced from sixteen different countries, were crossed to both the cytoplasm and the nuclear gene testers using them as the maternal and paternal parents, respectively, in order to make clear the distributions of the male sterility-inducing cytoplasm and various fertility-restoring genes.

Out of 146 varieties tested, only four possessed the same male sterility-inducing cytoplasm as the variety Chinsurah Boro II, while the remaining 142 contained the normal cytoplasm. The former four varieties were introduced from India and East Pakistan, *i. e.* the Indian Center as called by Vavilov, and all of them belonged to boro rice group.

As to the nuclear genes for fertility restoration, 54 out of 153 varieties tested were found to be effective restorer, 28 to be weak restorer, and the remaining 71 to be non-restorer. As a rule, non-restorer varieties were mostly concentrated in the temperate countries where Japonica rice was mainly grown. On the other hand, the effective restorer varieties were mainly distributed in the tropics where Indica rice was exclusively grown. The weak restorer varieties were distributed everywhere and were found in all the three grain types by Matsuo.

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Ⅷ LITERATURE CITED

1. Allard, R. W. 1956. Formulas and table to facilitate the calculation of recombination values in heredity. Hilgardia **24** : 235～278.
2. Artschwager, E. 1947. Pollen degeneration in male sterile sugar beet with special reference to the tapetal plasmodium. J. Agr. Res. **75** : 191～197.

3. Athwal, D. S. and S. S. Virmani 1972. Cytoplasmic male sterility and hybrid breeding in rice. Rice Breeding pp. 615~620. Intern. Rice Res. Inst., Los Banos, Laguna, Philippines.
4. Bateson, W. and A. E. Gairdner 1921. Male sterility in Flax, subject to two types of segregation. J. Genetics **11**: 269~275.
5. Blackeslee, A. F. 1922. Variation in *Datura* due to changes in chromosome number. Am. Naturalist **56**: 16~31.
6. Blackeslee, A. F. and J. Belling 1924. Chromosomal mutation in the jimson weed, *Datura stramonium*. J. Hered. **15**: 194~210.
7. Buchert, J. G. 1961. The stage of the genome-plasmon interaction in the restoration of fertility to cytoplasmically pollen-sterile maize. Proc. Natl. Acad. Sci. **47**: 1436~1440.
8. Burnham, C. R. 1962. Discussion in Cytogenetics. pp. 139~167. Burgess Publishing Co., Minnesota, U.S.A.
9. Carnahan, H. L., J. R. Erickson, S. T. Teseng, and J. N. Rutger 1972. Outlook for hybrid rice in the U.S.A. Rice Breeding pp. 603~607. Intern. Rice Res. Inst., Los Banos, Laguna, Philippines.
10. Caspari, E. 1948. Cytoplasmic inheritance. Advances in Genetics **2**: 1~66. Academic Press, New York.
11. Chang, T. T. 1964. Present knowledge of rice genetics and cytogenetics. Intern. Rice Res. Inst. Techn. No. **1**: 1~96.
12. Chaplin, J. F. and Z. T. Ford 1965. Agronomic and chemical characteristics of male sterile ful-curd tobacco as influenced by cytoplasm of different *Nicotiana* species. Crop Sci. **5**: 436~438.
13. Chittenden, R. J. and C. Pellew 1927. A suggested interpretation of certain cases of anisogeny. Nature **119**: 10~12.
14. Clayton, E. E. 1950. Male sterile tobacco. J. Hered. **41**: 171~175.
15. Correns, C. 1904. Experimentelle Untersuchungen über die Gynodioecie. Ber. Deut. Bot. Ges. **22**: 506~517.
16. Correns, C. 1908. Die Rolle der männlichen Keimzellen bei der Geschlechtsbestimmung der gynodioecischen Pflanzen. Ber. Deut. Bot. Ges. **26A**: 686~707.
17. Depertment of Promotion, Ministry of Agriculture and Forestry, Govenment of Japan 1962. Sui-rikuto shoreihinshu no tokuseihyo (A list of characteristics of recommended lowland and upland rice varieties) p. 95 (In Japanese).
18. Duvick, D. N. 1959. The use of cytoplasmic male-sterility in hybrid seed production. Econ. Bot. **13**: 167~196.
19. Duvick, D. N. 1965. Cytoplasmic pollen sterility in corn. Advances in genetics **13**: 1~56. Academic Press, New York.
20. East, E. M. 1932. Studies on self sterility. IX. The behaviour of cross between self sterile and self fertile plants. Genetics **17**: 175~202
21. Edwadson, J. R. 1956. Cytoplasmic male-sterility. Bot. Rev. **22**: 696~738.

22. Edwadson, J. R. 1962. Cytoplasmic differences in T-type cytoplasmic male-sterile corn and its maintainer. Am. J. Bot. **49**: 184～187.
23. Erickson, J. R. 1969. Cytoplasmic male sterility in rice (*Oryza sativa* L.) Agron. Absts. p. 6. (Abst.).
24. Fukasawa, H. 1953. Studies on restoration and substitution of nucleus in Aegilotricum, I. Appearance of male sterile durum in substitution crosses. Cytologia **18**: 167～175.
25. Fukasawa, H. 1956. Studies on restoration and substitution of nucleus (genome) in Aegiloticum, III. Cyto-histological investigation of pollen degeneration in anthers of male-sterile plants. Cytologia **21**: 97～106.
26. Fukasawa, H. 1959. Nucleus substitution and restoration by means of successive backcrosses in wheat and related genus *Aegilops*. Japan. J. Bot. **17**: 55～91.
27. Gairdner, A. E. 1929. Male-sterility in flax. II. A case of reciprocal crosses differing F_2. J. Genetics **21**: 117～124.
28. Goodspeed, T. H. and P. Avery 1939. Trisomic and other types in *Nicotiana sylvestris*. J. Genetics **38**: 381～458.
29. Grogan, C. O. and P. Sarvella 1964. Morhological variations in normal, cytoplasmic male and restored counterparts in maize, *Zea mays* L. Crop Sci. **4**: 567～570.
30. Hermsen, J. G. Th. 1970. Basic information for the use of primary trisomics in genetic and breeding research. Euphytica **19**: 125～140.
31. Hori, T. and K. Tsunewaki 1969. Basic studies on hybrid wheat breeding. II. Effects of three alien cytoplasms on main quantitative characters of hexaploid wheats. Japan. J. Breed. **19**: 425～430.
32. Hosokawa, S., T. Takeda, Y. Otani, and M. Ikehata 1954. Cyto-histological studies on male sterility of sugar beet, with special reference to pollen degeneration and tapetal plasmodium. Japan. J. Breed. **4**: 196～202. (In Japanese).
33. Huang, C. H. 1969. Rice varietal improvement in Taiwan. Rept. JCRR, Taiwan pp. 16. (Type-writting).
34. Iwata, N., T. Omura, and M. Nakagahra 1970. Studies on the trisomics in rice plants (*Oryza sativa* L.). I. Morphological classification of trisomics. Japan. J. Breed. **20**: 230～236.
35. Iwata, N. and T. Omura 1972. Linkage analysis by use of trisomics in rice (*Oryza sativa* L.). II. Japan. J. Breed. **22** (Supl. 1) : 49～50. (In Japanese).
36. Jennings, P. R. 1966. Evaluation of partial sterilily in Indica x Japonica rice hybrids. Intern. Rice Res. Inst. Techn. Bull. No. **5**: 1～63.
37. Jones, H. A. and S. L. Emsweller 1937. A male-sterile onion. Proc. Am. Soc. Hort. Sci. **34**: 582～ 585.
38. Jones, H. A. and A. E. Clarke 1943. Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Am. Soc. Hort. Sci. **43**: 189～194.
39. Jones, H. A. and G. N. Davis 1944. Inbreeding and heterosis and their relation to the development of new varieties of onion. USDA. Techn. Bull. **874**: 1～28.

40. Joppa, L. R., F. H. McNeal and J. R. Welsh 1966. Pollen and anther development in male sterile wheat (*Triticum aestivum* L.). Crop Sci. **6** : 296～297.
41. Kagoshima Agricultural Experimental Station 1967. Suito shoreihinshu no Kaisetsu (Explanation on characteristics of recommended lowland-rice varieties). p. 225. (In Japanese).
42. Katayama, T. 1950. Ine mugi no bunketsu kenkyu. Third Ed., Yokendo, Tokyo. (In Japanese).
43. Kato, S. 1930. On the affinity of the cultivated varieties of rice plant, *Oryza sativa* L. J. Agr. Kyushu Univ. **2** (9) : 241～276.
44. Katsuo, K. 1959. Studies on the cytoplasmic difference among rice varieties, *Oryza sativa* L. II. On the sterility of Fujisaka 5-type plants with the cytoplasm of Chinese wild variety, *O. sativa* L. f. *spontanea*. Japan. J. Breed. **9** : 257. (In Japanese).
45. Katsuo, K. 1960. On the abortive pollen of Fujisaka No. 5-type plants with the cytoplasm of Chinese wild variety, *Oryza sativa* L. f. *spontanea*. Japan. J. Breed. **10**: 262 (In Japanese).
46. Katsuo, K. and U. Mizushima 1958. Studies on the cytoplasmic difference among rice varieties, *Oryza sativa* L. I. On the fertility of hybrids obtained reciprocally between cultivated and wild varieties. Japan. J. Breed. **8** : 1～5. (In Japanese).
47. Kihara, H. 1951. Substitution of nucleus and its effect on genome manifestations. Cytologia **16**: 177～193.
48. Kihara, H. and I. Hirayoshi 1942. Entwichklung des pollenkorns bei *Oryza sativa* L. Nogyo oyobi Engei (Agriculture and Horticulture) **17**: 685～689. (In Japanese).
49. Kihara, H. and K. Tsunewaki 1967. Genetic principles applied to the breeding of crop plants. Heritage from Mendel p. 403～418. Univ. Wisc. Press.
50. Kitamura, E. 1962. Studies on cytoplasmic sterility of hybrids in distantly related varieties of rice, *Oryza sativa* L. I. Fertility of F_1 hybrids between strains derived from a certain Philippine x Japanese variety crosses and Japanese varieties. Japan. J. Breed. **12**: 81～84.
51. Kitamura, E 1962. Studies on cytoplasmic sterility of hybrids in distantly related varieties of rice, *Oryza sativa* L. II. Analysis of nuclear genes in Japanese varieties controlling cytoplasmic sterility. Japan. J. Breed. **12**: 166～168.
52. Lesley, J. W. 1932. Trisomic types of the tomato and their relations to the genes. Genetics **17**: 545～559.
53. Matsuo, T. 1952. Genecological studies on cultivated rice. Bull. Natl. Inst. Agr. Sci. (Japan) Ser. D, No. **3** : 1～111. (In Japanese).
54. Maunder, A. B. and R. C. Pickett 1959. The genetic inheritance of cytoplasmic-genetic male sterility in grain sorghum. Agron. J. **51**: 47～49.
55. McClintock, B. 1929. A cytological and genetical study of triploid maize. Genetics **14**: 180～222.
56. Michaelis, P. 1933. Entwicklungeschichtlich-genetische untersucchungen an *Epilobium*. II. Die bedeutung des *Epilobium-leteum-hirsutum*-Bastardes. Z. Indukt Abstammu.

Vererblehre **65**: 1～71, 353～411.

57. Michaelis, P. 1954. Cytoplasmic inheritance in *Epilobium* and its theoretical significance. Advances in Genetics **6**: 287～401.
58. Morinaga, T. and H. Kuriyama 1955. Japonica type rice in the subcontinent of India and Java. Japan. J. Breed. **5**: 149～153.
59. Morinaga, T. and H. Kuriyama 1958. Intermediate type of rice in the subcontinent of India and Java. Japan. J. Breed. **7**: 253～259.
60. Nagamatsu, T. and C. Shinjyo 1960. Studies on the classification of Okinawan local rice varieties. II. The classification by sexual affinity. Sci. Bull. Coll. Agr. Univ. Ryukus No. **7**: 162～180.
61. Nagao, S., M. Takahashi, and T. Kinoshita 1964. Genetic studies on rice plants. XXIX. Present status of rice linkage studies and some intriguing association problems. J. Fac. Agr. Hokkaido Univ. **54**: 29～41.
62. Oka, H. I. 1953. Phylogenetic differentiation of the cultivated rice plant. I. Variation of various characters and character combinations among rice varieties. Japan. J. Breed. **3** (2): 33～43. (In Japanese)
63. Oka, H. I. 1953. Phylogenetic differentiation of the cultivated rice plant. IX. Influence of intervarietal hybrid sterility on segregation ratios in rice. Japan. J. Breed. **3** (1): 31～39. (In Japanese).
64. Oka, H. I. 1954. Phylogenetic differentiation of the cultivated rice plant. II. Classification of rice varieties by intervarietal hybrid sterility. Japan. J. Breed. **3** (3, 4): 1～6. (In Japanese).
65. Oka, H. I. 1963. Consideration on the genetic basis of intervarietal sterility in *Oryza sativa*. Genetics and Cytogenetics, p. 158～174. Elsevier Publishing, New York.
66. Owen, F. V. 1942. Male sterility in sugar beets produced by complementary effects of cytoplasmic and Mendelian inheritance. Am. J. Bot. **29**: 692.
67. Owen, F. V. 1945. Cytoplasmically inherited male-sterility in sugar beets. J. Agr. Res. **71**: 423～440.
68. Owen, F. V. 1950. The sugar beet breeder's problem of establishing male sterile populations for hybridization purposes. Proc. Am. Soc. Sugar Beet Tech. **6**: 191～194.
69. Peterson, P. A. 1958. Cytoplasmically inherited male sterility in *Capsicum*. Am. Naturalist **92**: 111～119.
70. Rhodes, M. M. 1931. Cytoplasmic inheritance of male-sterility in *Zea mays*. Science **73**: 340～341.
71. Rhodes, M. M. 1933. The cytoplasmic inheritance of male sterility in *Zea mays*. J. Genetics **27**: 71～93.
72. Sakai, K. 1949. Cyto-histological and thermatological studies on northern parts of Japan with special reference to abnormal hypertrophy of tapetal cells due to low temperature. Hokkaido Agr. Exp. Stat. **43**: 1～46. (In Japanese).

73. Sasahara, T. and K. Katsuo 1965. Studies on the cytoplasmic difference among rice varieties, *Oryza sativa* L. III. On the abortive pollen of Fujisaka No. 5-type plants with the cytoplasm of Chinese wild variety, *Oryza sativa* L. f. *spontanea*. Japan. J. Breed. **15**: 191~196. (In Japanese).

74. Shibuya, T. 1966. Studies on partial male sterility in rice plants. Japan. J. Breed. **16**: 174~178. (In Japanese).

75. Shinjyo, C. and Omura 1966. Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. I. Fertilities of F_1, F_2 and offsprings obtained from their mutual reciprocal backcrosses ; and segregation of completely male sterile plants. Japan. J. Breed. **16** (Supl. 1) : 179~180. (In Japanese).

76. Shinjyo, C. 1969. Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. II. The inheritance of male sterility. Japan. J. Genetics **44**: 149~156.

77. Shinjyo, C. 1972. Distributions of male sterility-inducing cytoplasms and fertility-restoring genes in rice. I. Commercial lowland-rice cultivated in Japan. Japan. J. Genetics **47**: 237~243.

78. Shinjyo, C. 1972. Distributions of male sterility-inducing cytoplasms and fertility-restoring genes in rice. II. Varieties introduced from sixteen countries. Japan. J. Breed. **22**: 329~333.

79. Shinjyo, C., R. Nishime, and Y. Watanabe 1974. Inheritance of fertility restoring gene *Rfx* and *Rf* in male sterile cytoplasm derived from variety Lead Rice (preliminary report). Japan. J. Breed. **24** (Supl. 1) : 130~131. (In Japanese).

80. Sirks, M. J. 1938. Plasmatic inheritance. Bot. Rev. **4** : 113~131.

81. Stephens, J. O. and R. F. Holland 1954. Cytogenetic male-sterility for hybrid sorghum seed production. Agron. J. **46**: 20~23.

82. Tahir, C. M. 1970. Distribution of fertility-restoring genes in hexaploid wheat for *Ae. ovata* and *T. timopheevi* cytoplasms. I. Japan. J. Breed. **20**: 1~6.

83. Tatebe, T. 1952. Cytological studies on pollen degeneration on male sterile onion. J. Hort. Sci. Japan. **21**: 73~75. (In Japanese).

84. Terao, H. and U. Mizushima 1939. Some consideration on the classification of *Oryza sativa* L. into subspecies, so-called Japonica and Indica. Japan. J. Bot. **10**: 213~258.

85. Tsuchiya, T. 1962. The establishment of a trisomic series in a two-rowed cultivated variety of barley. Can. J. Genet. Cytol. **9** : 667~682.

86. Vavilov, N. I. 1951. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica **13**: 13~54.

87. Watanabe, Y., S. Sakaguchi, and M. Kudo 1968. On the male-sterile rice plant possessing the cytoplasm of Burmese variety, "Lead Rice". Japan. J. Breed. **18** (Supl. 2) : 77~78. (In Japanese).

88. Welch, J. E. and E. L. Grimball 1947. Male sterility in the carrot. Science **106**: 594.

89. Wilson, J. A. and W. M. Ross 1961. Cross breeding in wheat, *Triticum aestivum*. I. Frequency of the pollen restoring character in hybrid wheat having *Aegilops ovata*

cytoplasm. Crop Sci. 1 : 191～193.

90. Wilson, J. A. and W. M. Ross 1962. Cross breeding in wheat, *Triticum aestivum* L. II. Hybrid seed set on a cytoplasmic male-sterile winter wheat composite subjected to cross-pollination. Crop Sci. 2 : 415～417.

イネにおける細胞質雄性不稔と稔性回復の遺伝学的研究

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摘 要

筆者はインド型イネChinsurah Boro Ⅱ品種が雄性不稔細胞質と稔性回復遺伝子をもつことを発見し，連続戻交雑法を用いて両因子を日本型イネの台中65号へ導入した。育成された上記 isogenic 系統を主材料にして，雄性不稔の遺伝，他形質に対する細胞質と稔性回復遺伝子の効果，稔性回復遺伝子の座位，雄性不稔イネにおける花粉退化の時期，雄性不稔細胞質と稔性回復遺伝子の地理的分布，および雑種イネ育種に必要な三系統の育成法を研究した。結論の要約は下記のとおりである。

A 雄性不稔の遺伝

インド型イネChinsurah BoroⅡに由来する雄性不稔細胞質を（*ms-boro*），その稔性回復遺伝子を*Rf*と命名した。（*ms-boro*）*Rf Rf*の遺伝子型をもつ系統は完全雄性稔，（*ms-boro*）*Rf rf*は部分雄性稔（花粉稔性約50％）で，（*ms-boro*）*rf rf*は完全雄性不稔であった。一方上記3系統の雌性配偶子は健全であった。正常細胞質（*n-boro*）をもつ個体は核内遺伝子型に関係なく，すべて完全雄性稔になった。

（*ms-boro*）*rf rf* × （*n-boro*）*Rf rf*のF_1世代においては，部分雄性稔と完全雄性不稔個体が1：1の比に分離したが，（*ms-boro*）*rf rf* ×（*ms-boro*）*Rf rf*のF_1では稔性の分離は観察されず，すべての個体が部分雄性稔（花粉稔性約50％）になった。（*ms-boro*）*Rf rf*系統の自殖次代には完全雄性稔および部分雄性稔個体が1：1の比に分離した。したがって（*ms-boro*）*Rf rf*個体においては，花粉形成期のある時期に*rf*遺伝子をもつ花粉は雄性不稔細胞質との相互作用で死滅し，*Rf*花粉のみが正常に発育するといういわゆる雄性配偶体不稔性と結論した。

（*ms-boro*）*rf rf* × *Rf Rf*のF_1個体の花分稔性は50％を示すが，種子稔性は90％以上になる。したがって本雄性不稔細胞質と稔性回復遺伝子は雑種イネの育成に利用できるものと考えられる。

なお，雑種イネ育成に必要な3系統，すなわち雄性不稔系統，雄性不稔維持系統および稔性回復系統の育成方法についても理論的に示した。

B 量的形質に対する細胞質と核内遺伝子の効果

作出可能な6 isogenic 系統を育成し，5反覆の乱魂法を用いて，1970年の第1期作と第2期作で栽培し，出穂日，穂数，主稈葉数，稈長，第1節間長，第2節間長および第3節間長（節間は最上位から数えた。）を測定し，系統間の比較を行った。

雄性不稔系統の稈長は他の5系統に比較して約7㎝短く，1％水準で有意であった。雄性不稔系統の短稈性は，おもに第1～第3節間長の短縮に起因する。他の5系統の稈長間には有意差はなかった。雄性不稔系統の示す出穂日，穂数，主稈葉数は他の系統と同程度であった。雄性不稔系統のこのような特性は交雑圃における受粉体制に好影響をもたらすものと考えられる。

C *Rf* 遺伝子の座位

まず三染色体系統を用いて，*Rf* 遺伝子の座乗染色体を確定し，つぎに既2標識遺伝子系統との交雑を行ない座位を明らかにした。

三染色体分析では3系交雑法を適用した。まず Trisomics × (*ms−boro*) *Rf Rf* の交雑F_1から三染体個体を染色体数の観察によって迸抜し，つぎにF_1の三染色体植物を父本にして雄性不稔系統へ交雑し，次代植物の種子稔性を調査した。その結果*Rf*遺伝子はTrisomic C系統の過剰染色体，つまり岩田らの第7染色体に座上することが判明した。

Rf fl 間の組換価は約0.4%で，*pgl* と*Rf* 間のそれは約12%，*pgl* と *fl* 間は約20%であった。したがって第7染色体上における遺伝子の配列順序は *pgl−Rf−fl* である。

D 花粉退化の細胞組織学的研究

本雄性不稔系統における花粉の発育過程を観察した。減数分列は正常に進行し，花分四分子も正常に形成される。しかし花粉1核期でその発育を停止し，2核期以後は観察されない。タペート細胞は正常である。出穂期の不稔花粉はヨード・ヨードカリ液で染色されない。不稔花粉は球形で発芽孔を有するが，形は正常花粉よりも小さい。

E 雄性不稔細胞質と稔性回復遺伝子の分布

(1) 日本の水稲奨励品種について

細胞質と核内遺伝子型の検定法を考案し，その検定法に基づいて1962年度の日本水稲奨励品種150品種を検定した。

19品種（12.7%）の品種は弱稔性回復遺伝子をもち，他の131品種は非回復遺伝子をもっていた。弱回復遺伝子をもつ品種のほとんどは京都以南に集中的に分布した。これらの品種のほとんどは在来品種から分離育種法によって育成された品種であった。

供試日本稲品種には雄性不稔細胞質は発見されなかった。

(2) 外国品種について

本研究に用いたイネ品種は15国から蒐集した153品種でった。

細胞質検定親に遺伝子型（*n−boro*）*rf rf* の6系統を，核内遺伝子検定親には遺伝子型(*ms−boro*) *rf rf* の6系統を用いた。これらの検定親と153品種との交雑を行なった。主としてF_1の花粉および種子稔性から，それぞれの品種の細胞質型と核内遺伝子型を推定した。雑種不稔性の併発によって，これらの推定が困難であった組合せについては，B_1F_1か自家受粉による後代系統の稔性から推定した。

細胞質の検定を行なった146品種のうち，4品種が Chinsurah Boro Ⅱと同じ雄性不稔細胞質（*ms−boro*）をもち，他の142品種は正常細胞質をもっていた。雄性不稔細胞質をもつこれら4品種は，Vavilov がIndian Center と名づけたインドおよび東パキスタンから蒐集したboro型のものであった。

核内遺伝子型検定を行なった153品種のうち54品種は効果的な稔性回復遺伝子を，28品種は弱回復遺伝子を，残りの71品種は非回復遺伝子をもっていた。効果的な稔性回復遺伝子はインド型イネが主に栽培される熱帯地方に，一方非回復遺伝子は日本型が栽培される温帯地方に分布していることがわかった。弱回復遺伝子はいずれの地域にも見られる。