Studies on the Utility of Artificial Mutations in Plant Breeding.

XII. Induction and Gene Analysis of Male-sterile Mutants in Rice

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Seeds of three kinds of rice varieties were treated with EI (0.5%, v/v) and X-rays (25kR), then male-sterile mutants induced were screened in the $M_2 \sim M_4$ generations. In the present study, the plants that showed complete sterility in self pollination without abnormality of female organ were defined as "male-sterile mutants". Anthers of those plants were indehiscent, containing few or no good pollen grains. The EI treatments yielded the male-sterile mutants all over the three varieties with the frequencies of 0.30~0.38%, totaling eight mutants, while the X-irradiation was ineffective in inducing the mutants. F_1 hybrids from the crosses between the mutants and their original varieties exhibited a normal seed fertility under self- and open pollinations. The segregation ratio in F_2 showed that the mutants, except one, were controlled each by a single recessive gene. Allelism test with those recessive genes revealed that at least six different loci were responsible for the different male sterilities. These results may indicate that EI treatment is highly effective in inducing male-sterile mutants and that a considerable number of loci are concerned with male sterilities in rice.

Introduction

It is commonly known that cytoplasmic male sterility has so far extensively been applied to practical production of hybrid seeds. Recently, on the other hand, the utilization of genic male sterility also has become important from the standpoint of crop improvement. For instance, GLIMORE (1964) suggested that genic male sterility could be successfully used in recurrent selection, and FUJIMAKI (1975) also pointed out that the male sterility was available for backcross breeding. These ideas will be noticed from the practical significance, if some desirable type of the male sterility can be readily obtained.

Genic male sterilities of rice have so far been observed in no small numbers. However, most of them owed their origin to spontaneous mutations (ISHIKAWA 1929, TAKEZAKI 1932, HARA 1964, TREES and RUTGER 1978), and those which originated from artificially induced mutations are only seen in few recent reports (PAVITHRAN and MOHANDAS 1976, FUJIMAKI *et al.* 1977). The authors have endeavored to induce male-sterile mutations in rice, and found that the genic male sterility can be induced by mutagenic treatments comparatively with ease. The present paper describes a part of the results and discusses on induction procedure and genetics of the mutants.

In the induction and gene analysis of male sterilities, it seems necessary to give an accurate definition to the male sterility, because the use of the term is not consistent among different research workers. In this study, the plants that showed complete sterility in self pollination without the abnormality of female organ were defined as "male-sterile mutants" from the practical standpoint.

Materials and Methods

1. Induction of male sterility

Three rice varieties, Koshihikari, Etsunan No.77 and Toyonishiki were used in experiments I, II and III, respectively, for induction of male sterility. Through all the three experiments, air-dried seeds were treated with 25 kR of X-rays (113 R/min.) or with 0.5% v/v aqueous solution of ethyleneimine (EI) and the M_1 plants were grown in paddy field. After maturity, one panicle was randomly chosen from each M_1 plant and examined for seed fertility. The seeds from these panicles were gathered in bulk for the test in M_2 generation.

Seed-sterile plants, which can also involve those originating from female sterility, were selected in the M_2 generation by bagging panicles or observing anthers, and pollinated with sound pollen of the original variety to examine the function of female organ. The male-sterile mutants were thus screened out of those sterile plants, and their pollen abortion was checked by staining with cotton blue. For reference, frequencies of chlorophyll mutations at seedling stage were also examined in M_2 generation.

Experiment I. Seeds of Koshihikari were exposed to the X-rays or were soaked in the solution of EI for 2.5 hours at 21°C. One hundred M_1 plants were grown in each treatment.

The two M_2 populations from X- and EI treatments comprised 939 and 864 plants, respectively. Before the anthesis, one panicle from each M_2 plant was covered with a paraffin bag to examine the seed sterility in self pollination. As a result, in the EI treatment, there were observed four plants which showed no seed-setting in the selfing but obtained some seeds under open pollination.

M_3 and M_4 progenies were raised from the open-pollinated seeds of the four plants, and seed-sterile plants which segregated in M_4 generation were pollinated with pollen of the original variety, Koshihikari, to examine the function of female organ.

Experiment II. Seeds of Etsunan No.77 were treated with the EI solution for 3 hours at 21°C. The M_1 generation consisted of 200 plants. In the next generation, 531 M_2 plants were examined by means of the same method as applied in Experiment I and seven plants showing seed sterility in selfing were selected. These plants were transplanted to pots at the harvest time and continued to grow in a growth chamber. In the next summer, the regenerated plants were examined for the function of female organ by pollinating with the pollen of Koshihikari, which was used as a substitute for the original variety, Etsunan No.77.

Experiment III. Seeds of Toyonishiki were treated with the EI solution for 2.5 hours at 20°C. The number of plants grown in the M_1 and M_2 generations were 250 and 1000, respectively. In M_2 generation, visual observation of anther dehiscence was conducted for all the plants, and four plants carrying indehiscent anthers were selected. These plants were bagged without delay to confirm the seed sterility and to examine the function of female organ by pollinating with pollen of the original variety, Toyonishiki.

2. Gene analysis of male sterility

The eight male-sterile mutants obtained from Experiments I~III were crossed with their

original varieties, (Koshihikari was used instead of Etsunan No. 77 in Experiment II). In each cross combination, five F_1 plants were grown and observed for seed and pollen fertilities, and selfed seeds were harvested from three plants. The F_2 progeny consisting of 150 plants was examined for the segregation of male sterility.

Based on the results obtained, the allelic relation among male-sterile mutants were investigated for seven mutants which were proved to be governed each by a single recessive gene. Each of those mutants was crossed with the F_1 plant between the specific mutant and its original variety, and the segregation for male sterility was analysed in the subsequent generation, which contained 20 to 25 plants.

Results

1. Induction of male sterility

Through the three experiments, 15 seed-sterile plants were selected in the M_2 generation following EI treatments. In the manual pollination test, however, seven of them showed some abnormality of female organ, and the remaining eight were identified as "male-sterile mutants". These eight mutants consisted of 3 from the treatment of Koshihikari (Exp. I), 2 from Etsunan No. 77 (Exp. II) and 3 from Toyonishiki (Exp. III). Excepting a dwarf plant from Koshihikari, the male-sterile mutants were about the same as the original varieties in all the characters other than male-sterile character. The experimental results regarding these mutants are discussed below.

Table 1 shows the frequencies of chlorophyll-deficient and male-sterile mutants in the M_2 generation along with the seed fertility in the M_1 generation. It is not obvious what has caused that the M_1 seed-fertility of EI treatment is much lower in Exp. I than in Exp. II and III. In Experiment I, in any case, the two different treatments gave rise to nearly the same degree of the reduction of M_1 seed-fertility. However, the EI treatment induced chlorophyll mutations with the frequency of almost 3 times as high as the X-irradiation, and male-sterile mutants were induced only by the EI treatments. Besides, as clearly seen in Table 1, male-sterile mutants were induced in all EI treatments and at the same level of frequency, ranging from 0.30 to 0.38%.

Table 1. Frequency of mutations in the M_2 generation following X-ray and EI treatments

Treatment	M_1 seed-fertility (%)	Chlorophyll mutants in percent*	Male sterility		
			No. plants tested	No. mutants	Percentage
Exp. I :					
Control	87.2	0	117	0	0
X-rays 25 kR	30.5	2.0	988	0	0
EI 0.5%	30.7	5.6	864	3	0.35
Exp. II :					
Control	92.6	0	122	0	0
EI 0.5%	58.2	3.5	531	2	0.38
Exp. III :					
Control	95.5	0	100	0	0
EI 0.5%	64.3	3.4	1000	3	0.30

* Number of observed plants were much larger than those tested for male sterility.

Table 2. Seed and pollen fertilities of male-sterile mutants*

Original variety and mutant**	Seed fertility (%)			Pollen fertility (%)
	Self pollination	Open pollination	Manual pollination with normal pollen	
Exp. I:				
Koshihikari (K)	94.8	—	80.0	99.8
K 1	0	—	82.4	No pollen
K 2	0	—	80.0	0
K 3	0	—	86.4	0
Exp. II:				
Etsunan No. 77 (E)	84.0	79.9	74.1	98.5
E 1	0	8.5	73.2	No pollen
E 2	0	7.5	78.3	7.8
Exp. III:				
Toyonishiki (T)	95.0	97.1	76.7	99.1
T 1	0	13.5	81.4	No pollen
T 2	0	2.5	86.4	No pollen
T 3	0	9.0	75.0	14.3

* The fertilities shown were observed in the M_4 generation in Exp. I, and in the M_2 generation in Exp. II and Exp. III.

** K 1~3, E 1~2 and T 1~3 mean the mutants derived from Koshihikari, Etsunan No. 77 and Toyonishiki, respectively.

Table 2 shows the seed and pollen fertilities of male-sterile mutants. As shown in the table, seed fertilities of all the mutants were zero in self-pollination, while normal fertility (73.2~86.4%) was obtained when manually pollinated with normal pollen. Extremely low fertilities (2.5~13.5%) were shown under open pollination. Anthers of four mutants (K 1, E 1, T 1 and T 2) were devoid of pollen grains, two (K 2 and K 3) had only shrivelled and abortive pollen, and the other two (E 2 and T 3) contained some sound pollen grains though the percentages were very low.

Anthers of the mutants were unchanged in number per floret and the outward looks of unripe anthers were normal in size and color, irrespective of the indehiscence of anthers and the situation of pollen grains. Thus, it was impossible to detect the mutants before anthesis.

2. Gene analysis on the induced male sterility

In the crosses between male-sterile mutants and the original varieties, seed fertility of F_1 hybrids was as high as that of the original varieties in both of self- and open pollinations as shown in Table 3. Pollen fertility was also very high except a cross, E 2×Koshihikari. These facts suggest the possibility that the male sterility is controlled by a recessive gene or genes.

In the F_2 generation, a segregation ratio of 3 to 1 for male-fertile to male-sterile plants was observed in seven of the eight cross combinations as shown in Table 4. This indicates that the male sterility is controlled by a single recessive gene. The exceptional ratio in the cross of mutant E 2×Koshihikari fits to 15 : 1 ($\chi^2=0.300$, $p>0.5$), suggesting that the male sterility is controlled by duplicate genes.

The allelism test with the seven mutants gave the results as shown in Table 5. Suppose that the identical recessive gene is responsible for two mutants, e. g., A and B, the progeny of the cross $B \times (A \times \text{its original variety})$ should segregate into dominant and recessive phenotypes in the ratio of 1:1. Therefore, the segregation ratio 1:1 observed in the cross $K3 \times (K2 \times \text{Koshihikari})$ means

that both the mutants K2 and K3 are controlled by the gene of the identical locus. Excepting the above cross, segregation was not observed in any other crosses. This means that K2 or K3 and other five mutants possess different respective genes for male

Table 3. Seed and pollen fertilities of F_1 hybrids between male-sterile mutant and the original variety

Cross combination	Seed fertility (%)		Pollen fertility (%)
	Self pollination	Open pollination	
K1 $\times$ Koshihikari	89.9	94.5	99.8
K2 $\times$ Koshihikari	88.2	97.0	99.6
K3 $\times$ Koshihikari	86.0	94.7	99.6
E1 $\times$ Koshihikari*	88.1	86.2	94.3
E2 $\times$ Koshihikari*	85.1	91.3	64.3
T1 $\times$ Toyonishiki	93.8	96.6	98.3
T2 $\times$ Toyonishiki	96.6	96.6	99.3
T3 $\times$ Toyonishiki	89.6	91.8	99.8

* Koshihikari were used as a substitute for Etsunan No. 77.

Table 4. Segregation of male sterility in the F_2 generation from the cross of male-sterile mutant and the original variety

Cross combination	Number of plants observed		χ^2 -value 3:1	Probability
	Male-fertile	Male-sterile		
K1 $\times$ Koshihikari	118	30	1.766	$P > 0.1$
K2 $\times$ Koshihikari	113	36	0.056	$P > 0.8$
K3 $\times$ Koshihikari	119	31	1.502	$P > 0.2$
E1 $\times$ Koshihikari*	103	39	0.759	$P > 0.3$
E2 $\times$ Koshihikari*	139	11	24.969	$P < 0.01^{**}$
T1 $\times$ Toyonishiki	119	31	1.502	$P > 0.2$
T2 $\times$ Toyonishiki	110	37	0.002	$P > 0.9$
T3 $\times$ Toyonishiki	104	41	0.830	$P > 0.3$

* See Table 3

** Supposing that the segregation ratio is 15:1, χ^2 -value=0.300 and $P > 0.5$.

Table 5. Confirmation of allelic relation by the cross of mutant-A $\times$ (mutant-B $\times$ the original variety) : Segregation ratio of male-fertile versus male-sterile plants in the F_1 generation

Mutant (φ)	F_1 (σ)						
	K1 $\times$ K	K2 $\times$ K	K3 $\times$ K	E1 $\times$ K*	T1 $\times$ T	T2 $\times$ T	T3 $\times$ T
K1							
K2	20:0						
K3	23:0	12:13					
E1	20:0	20:0	21:0				
T1	21:0	20:0	22:0	22:0			
T2	20:0	20:0	24:0	20:0	23:0		
T3	20:0	20:0	21:0	20:0	20:0	20:0	

K: Koshihikari, K*: See Table 3, T: Toyonishiki.

sterility. Thus, it may be concluded that at least six kinds of recessive genes are responsible for the male sterilities induced.

Discussion

Induction of male sterility by means of mutagenic treatment has been reported in *Arabidopsis* (McKELVIE 1963, VEEN and WIRTZ 1968) and several crops, such as tomato (LESLEY, M. and LESLEY, J. 1958, YAMAKAWA 1968), jute (RAKSHIT 1967), pepper (DASKALOFF 1968), barley (YAMASHITA and UKAI 1979) and rice (PAVITHRAN and MOHANDAS 1976, FUJIMAKI *et al.* 1977). However, few reports have paid special attention to the frequency of the induced male-sterile mutations. In the present study, male-sterile mutants were obtained at fairly high frequencies of 0.30 to 0.38 percent in the M_2 generation following the EI treatments, but the X-irradiation was ineffective for the induction. This is consistent with the recent report of YAMASHITA and UKAI (1979) in which male-sterile plants of barley were also induced more frequently by EI than by gamma rays. Thus, it may safely be concluded that EI is much more effective for induction of male-sterile mutants than such radiations as X- and gamma rays, and that the mutation frequency of 0.3 percent or more is expected in the M_2 generation when rice seeds were treated with adequate dosage of EI.

In Experiment I, three generations from M_2 to M_4 were needed for determining the male-sterile mutants, because selection of seed sterility and checking the function of female organ had to be made in different generations. In Experiments II and I III, on the other hand, all the steps for determining the male-sterile mutants were conducted in M_2 generation by the joint checkings of male- and female organs. In Experiment III, detection of male sterility by visual observation of anthers was applied. Although there may be some possibility in this method of missing the male-sterile plants having dehiscent anthers, the experimental results showed that all the male-sterile mutants possessed indehiscent anthers alone. Thus, visual observation of anthers is considered highly reliable and, hence, is recommended for detecting male sterility from the viewpoint of saving time and labor.

The induced male-sterile mutants exhibited much lower seed fertility (2.5~13.5%) than the original varieties under open pollination, in spite of their normal function of female organ (Table 2). FUJIMAKI *et al.* (1977) also observed the same phenomenon in their male-sterile mutants. According to our observation, a large part of flowers of the mutants opened late in daytime and lost the chance of pollination with normal pollen from other plants. In addition, the M_2 population showed a reduced amount of available pollen due to the lack of uniformity in heading date and the inclusion of partially or completely sterile plants. These matters seem to have caused the low fertility of the male-sterile mutants. Actually, for instance, male-sterile mutants surrounded by the normal-type plants showed an enhanced seed fertility more than twice, 10~30% (unpublished). This level of seed fertility may be enough to obtain the seeds necessary for practical breeding works.

According to HOCKETT (1979), thirty-two nonallelic recessive genes have already been identified for male sterility in barley. As compared with barley, quite a few male-sterile mutants have been found also in rice since the first discovery by ISHIKAWA (1929). How-

ever, the allelic relationships among the genes have remained unknown up to date. The present finding of six different loci for male sterility is accordingly the first in this field, and this finding suggests that a considerable number of nonallelic genes take part in male-sterile characters in rice as well as in barley. The fact that the male-sterile mutants can be efficiently induced by EI may reflect that there exist many different loci responsible for male sterility and, at the same time, may indicate that the use of induced male-sterility is of practical value in breeding programmes.

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人為突然変異の利用に関する育種学的研究

XII. 水稻における雄性不稔突然変異の誘起ならびに遺伝子分析

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水稻を用いて雄性不稔突然変異の誘起を試み、得られた突然変異について遺伝子分析を行った。なお、本実験では実用上の見地より、袋かけによって完全不稔となりしかも雌性器官の機能には異常のみられないものを

雄性不稔突然変異体とした。

コシヒカリ，越南 77 号およびトヨニシキの 3 品種を供試し，それらの気乾種子にエチレンイミン (EI) 処理 (0.5% v/v, 2.5~3.0 時間) および X 線照射 (25 kR, コシヒカリのみ) を行った結果，EI 処理の次代に，各品種より 0.30~0.38% の頻度で合計 8 個体の雄性不稔突然変異体を得た。これらの突然変異体はいずれも葯が裂開せず，また大部分は花粉を全く形成しないかまたは異常花粉のみを有していたが，2 個体には僅かながら正常花粉も観察された。

ついで，得られた雄性不稔突然変異体と原品種との交雑を行い， F_1 の稔性および F_2 における分離比を調べた結果，1 つの突然変異体では雄性不稔性に重複遺伝子が関与していると推察されたが，他の 7 種の突然変異体では雄性不稔性が単一の劣性遺伝子に支配されていることが明らかになった。さらに，後者の 7 種の突然変異に関与する遺伝子相互の対立関係を調べた結果，座位を異にする 6 個の雄性不稔遺伝子が検出された。これらの結果から，水稻においても大麦と同様，雄性不稔性に関与する遺伝子座がかなり多いこと，および雄性不稔性は多くの場合単遺伝子劣性突然変異によって生ずることがわかった。