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Male sterile mutant from somatic cell culture of rice*

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Summary. Using MS medium supplemented with 6% sucrose and hormones, plantlets were regenerated from the explants of mature seeds and young panicles of IR_8 and IR_{54}. Out of 157 regenerated plants (R_1), three were found to be male sterile (ms): one from IR_8 and two from IR_{54}, including a fertile and sterile chimaera. In the second generations (R_2) of IR_{24} and IR_{54}, one line from each segregated into male sterile and fertile plants. These ms plants could be divided into two types with pollen failure: pollen free (without pollen) and pollen abortive. IR_{24} was a semi-restorer for ms-plants of the pollen free type derived from the second generation of IR_{54} somaclones. The segregation ratio of fertile : sterile in both R_2 of line 91 and the F_2 of ms-plant/IR_{24} fitted the formula 15/16 : 1/16 quite well, showing that the male-sterile was controlled by two independent nuclear genes. Until now, as we know, male-sterile could be produced by hybridization or mutagenesis: sometimes it could be found in nature by spontaneous mutation. Recently the cytoplasmic male-sterile of tobacco was produced by protoplast fusion. This is the first paper to report male-steriles in regenerated plants and their offspring obtained from somatic cell culture.

Key words: Male-sterile – Tissue culture – Somaclonal variation – Indica rice

Introduction

Male-sterility, which is found universally in different crops is a very important character and was widely used in utilization of heterosis for producing hybrid seed easily and on a large scale. The male-sterile could be found in nature from spontaneous mutations, such as the original pollen-free plant of rice (Anonymous 1973). It could be produced by hybridization (Shinjyo 1969). The famous Chinese male-sterile line of wild abortive type (WA) was believed to be derived from the natural crossing of cultivar rice with wild rice. It could also be obtained by mutagenesis using physical and chemical mutagens (Sarma 1985). So far there have been no reports of producing male-sterile not using one of the three methods mentioned above. This paper describes male-sterile plants found in the regenerated plant (the first generation, R_1) and segregated in the second generation (R_2) from somatic cell culture of Indica rice.

Materials and methods

Materials

The Indica varieties Qi Er-ai, Gui-zhao, IR_8, IR_{24}, IR_{36}, IR_{50}, IR_{52} and IR_{54} were used in this experiment. Mature dehulled seeds and young panicles of these varieties were inoculated as explants.

Tissue culture

The mature seeds were dehulled and the leaves and sheaths of the boots of young panicles were peeled until the last sheath and node emerged. For surface sterilization, the explants were soaked in 70% alcohol for 3 min, rinsed 3 times in sterilized water, followed by 0.1% mercuric chloride treatment for 10–15 min and then washed 3 times in sterilized water. The dehulled mature seeds were again dipped in the saturated solution of chlorid lime for 30–40 min and then washed 3 times in sterilized water. The young panicles were peeled out from the last sheath and inoculated on to the medium.

MS medium was used as the basal medium for inducing callus, supplemented with 6% sucrose, 2,4-D and kinetin 1 mg/l each; for subculture supplemented with 3% sucrose, 2,4-D 0.5 mg/l, ABA 0.1 mg/l and yeast extract 1,360 mg/l; for regenerating culture supplemented with 3% sucrose and NAA and kinetin 2 mg/l each. Callus induction was in the dark and

* Some of the tissue culture and plant regeneration work in this study was conducted at IRRI, Manila

plant regeneration in light. The culture temperature was 28 °C ± 2 °C.

Identification of fertile regenerated plants and their offspring

The regenerated plants were transplanted into pots or seeding beds and then transplanted to the field. The seed fertility of each regenerated plant (R_1) was checked and recorded. Meiotic observation and hand pollination were made at the same time on the sterile regenerated plants in order to identify the nature of the sterility (male, female or chromosomal).

In the 2nd generation (R_2), the seedlings were transplanted in individual plant progeny plots, each of which contained 60–70 plants, with one plant per hill. The numbers of lines of fertility segregation were recorded in each season. Test crossing with different varieties to the sterile plant were made.

The ms-plant surviving winter and aseptical sowing of hybrid seed

In order to maintain ms-plants for long research, especially to survive during winter, they were cultured in a test tube using the direct aseptic budding method for young panicles (Ling et al. 1983). The valuable hybrid seeds produced by cutting glume castration were usually contaminated by fungus and failed to germinate. Therefore, the hybrid seeds were sown aseptically on the medium with 1/2 inorganic salt concentration MS or liquid culture solution for rice solidified with 8% agar (Yoshida et al. 1972).

Histology and cytology

The young panicles of sterile R_1 plants were fixed in a 3 : 1 alcohol and acetic acid solution overnight for meiosis analysis and to examine embryo-sac structure. A 1% I-KI solution was used for examining the pollen fertility. The aceto-carmine smear method (Smith 1947) and paraffin cutting were used for meiosis and pistil structure observations, respectively. After dehydration through an alcohol series, the samples were embedded in paraffin, cut at 8–10 μ and stained with Ehrlich's Hematoxylin (Zheng 1979).

Results

Distribution of male sterile mutants in R_1 and R_2 regeneration

The distribution of male sterile mutants in somaclones of R_1 and R_2 generations of some Indica rice cultivars during 1984–1986 is shown in Table 1. Among 8 varieties in this experiment, male sterile mutants were only found in IR_8, IR_{24} and IR_{54}. IR_8 and IR_{24} both yielded a mutant in R_1 and R_2, respectively. In IR_{54}, two male sterile plants were found in the R_1 generation (spring 1984 and 1986) and one was segregated from the R_2 generation in spring 1985.

The ms plant and its expression in R_1 generation

Three ms plants (one from IR_8 and two from IR_{54}, Table 1) were found in the R_1 generation during 1984–1986. The fertility of the pollen and seed setting is shown in Table 2. The seed setting frequency in cross pollination was rather low in 826s, the ms plant in IR_8. Further studies should examine whether the female organ is also abnormal. (The chromosome pairing at meiosis of 826s is normal.) The other two ms plants in IR_{54} could set seeds after controlled hand pollination; the highest frequency of seed setting was 40%, showing that the female was normal and fertile.

One ms plant was found in each of the R_1 generations of IR_{54} in 1984 and 1986. One was a chimaera, which consisted of two fertile panicles (CH-F) and more than 10 sterile panicles (CH-S) (Fig. 1). The seed setting frequency of the CH-F part of the chimaera was more than 88%. The meiosis of the sterile part (CH-S) of the chimaera was normal, showing 12II at metaphase I. Under controlled hand pollination with 3 male parents, all three panicles set seeds (Table 2). This clearly indicated that the sterility of CH-S was not caused by chromosomal variation; therefore it was male sterile.

Chimaera expression in R_2 and F_1 generations

In all, 99 plants from two panicle lines in the R_2 generation of CH-F were developed: 12 of these were male sterile and the other 87 were normal fertile.

Two hybrid F_1 out of the three combinations crossed with CH-S as mother parent survived and grew to ma-

Table 1. The distribution of male sterile mutants in somaclones of the R_1 and R_2 generation[a]

Varieties	Generations	Normal fertile	Male sterile	Other[b] sterile	Total	Years
IR_8	R_1	15	1	0	16	1984 spr.
	R_2	11	0	0	11	
IR_{24}	R_1	110	0	8	118	1986 spr.
	R_2	44	1	0	45	
IR_{54}	R_1	126	2	13	141	1984, 1986 spr.
	R_2	124	1	3	128	1985 spr.
Total	R_1	251	3	21	275	
	R_2	179	2	3	184	

[a] The data on other varieties without male-sterile mutant are not listed
[b] Including chromosomal semi and female sterile

Table 2. The fertility of pollen and seed of ms plants derived from tissue culture

Materials		Stained frequency by I-IK (%)	Open pollination (%)	Male parent and seed setting frequency[a] (%)				
From R_1 generation	CH-S	0.4	0	/IR_{56} 40.0	/IR_{54} 12.0	/541 10.0		
	S_5-54	0	0	/Er-chiu-aiB 8.4	/IR_{36} 8.9	/IR_{54} 4.2	/Bowe-zha 10.3	
	826-S	0.8	0.2	/Er-chiu-aiB 6.7	/IR_{36} 0	/IR_{24} 0	/IR_{54} 0	
From R_2 generation	92-12	No pollen	0	/IR_{54} 32.0	/IR_{36} 15.0	/IR_{24} 11.1	/ID_{18} 18.8	/ID_{14} 6.25
	24257	0.3	0.25	/Gui-zhao 17.0	/Er-chiu-aiB 20.6	/Zheng shan 97B 29.5	/IR_{36} 17.9	

[a] Controlled hand pollination

Table 3. χ^2 test of ms-gene segregation in F_2 and R_2 of line 91. F: number of fertile plants; S: number of sterile plants

Generation	R_2			(91-12×IR_{24}) F_2			(91-20×IR_{24}) F_2		
Fertility	F	S	Total	F	S	Total	F	S	Total
No. observed	33	2	35	176	18	194	164	18	182
No. of exceptions	32.81	2.19	35	181.86	12.14	190	170.62	11.38	182
χ^2			0.0176			3.0174			3.3984
P			0.995			0.995			0.995

turity. In one combination (CH-S/IR_{56}) F_1, all of 8 F_1 plants were fertile. In the other combination (CH-S/$_{541}$) F_1 only two plants matured; one was fertile and the other sterile. The fertility of the (CH-S/$_{541}$) F_1 was segregated because, possibly, the male parent 541 was a regenerated plant from a tissue culture of IR_{54}. Some test and back-crosses are being made to study the male sterile.

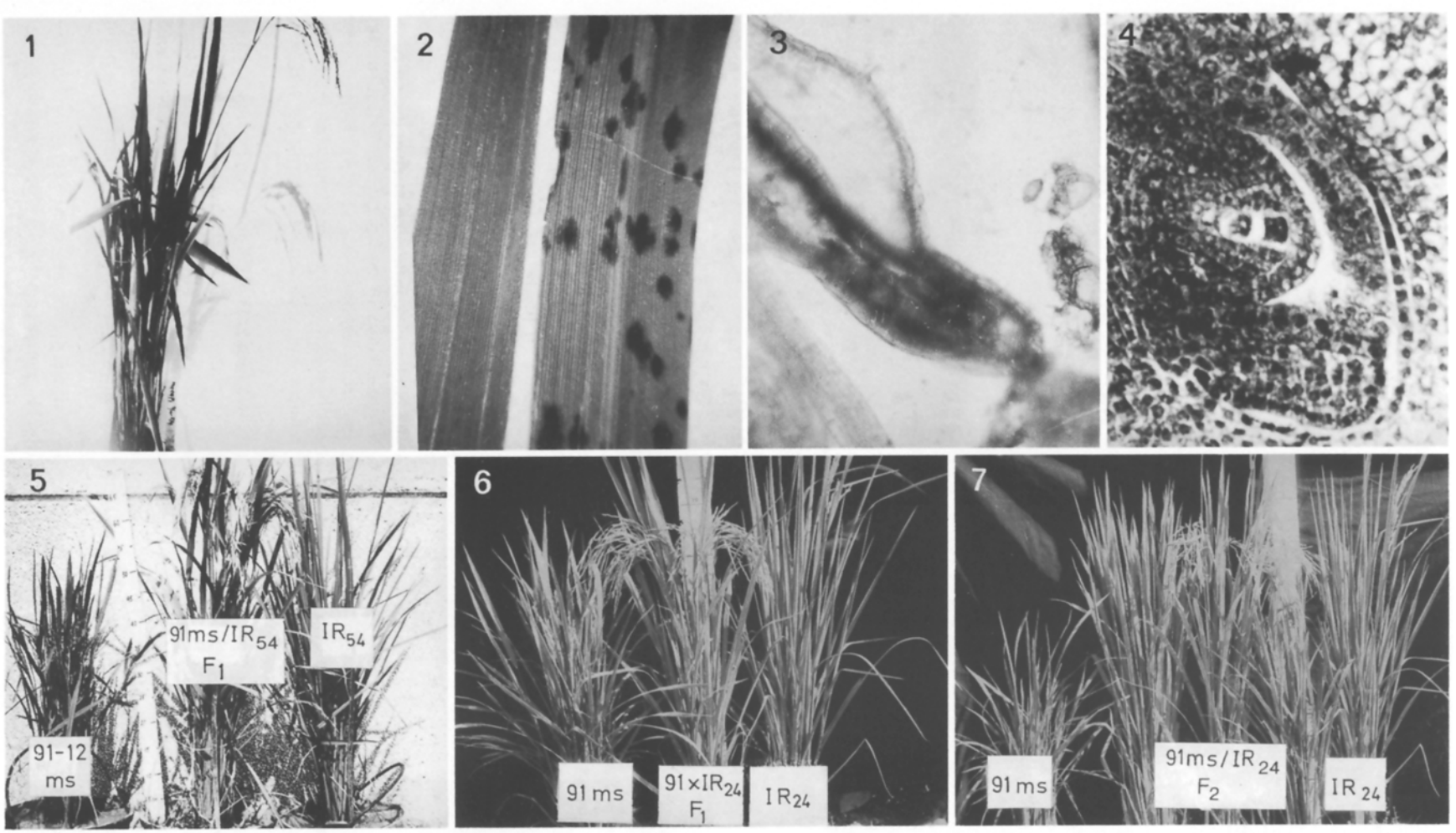
The expression of ms plants segregated from R_2 generation

Male sterile plants were segregated from each somaclonal line out of 128 of IR_{54} in 1985 and 45 of IR_{24} in 1986 during the R_2 generation (Table 1). Two ms plants out of 35 plants segregated in the segregating line of IR_{54} (line 91) and 10 ms plants out of 164 plants in the segregating line of IR_{24} (line 24257). The plants in the R_1 generation of both 91 and 24257 were fertile and had seed setting frequencies of more than 80%. Both original plants were regenerated from young panicle culture and subjected to subculture once. The ms plants of 24257 had abortive pollen which were not stained by I-KI. The frequency of seed setting in open pollination was 0.26% and in controlled hand pollination 17%–29% (Table 2). The hybrid seeds were set in all of panicles by crossing.

The ms plants segregated from line 91 were quite different from IR_{54}, the parent variety, in plant type, morphological and physiological characters. Ms plants were conspicuously shorter in height (55.8 cm) than IR_{54} (98 cm) (Fig. 5). Leaf colour was darker and a lot of brown spots were spread on the leaves (Fig. 2). The flowering date was 43 days earlier in the ms plant than in IR_{54} (Fig. 5). The anthers of the ms plant were almost empty, with very little pollen inside (Fig. 3). No pollen mother cells with meiosis were observed in the different stages of the young panicles, showing that microspore failed to develop before meiosis of pollen mother cells. The females had normal embryo-sacs (Fig. 4). Hybrid seed could be set if pollen from other plants was used. The highest frequency of seed-setting in crossing was 30% (Table 2).

Four hybrid F_1 plants of (91 ms/IR_{24}) were grown to maturity. The plant height of the hybrid F_1 was higher than that in the ms parent (Figs. 5 and 6). There were no spots on the leaves (Fig. 2). The frequency of seed setting in the F_1 hybrid was 44.7%–67.5%, demonstrating that only a half sterile gene of the 91 ms plant was restored by IR_{24}.

Fertile and sterile plants were segregated in the (91 ms/IR_{24}) F_2 generation (Fig. 7). The χ^2 test for segregation of the R_2 generation of line 91 and (91 ms/IR_{24})



Figs. 1–7. The ms plants derived from somatic cell culture of IR_{54} and its expression

Fig. 1. The male sterile and fertile chimaera plant regenerated from IR_{54}

Figs. 2–7. The ms plants segregated from line 91 in the R_2 generation of IR_{54} and its expression. **2.** Brown spots on the leaves of the ms plant (*right*) and the leaf of (91 ms/IR_{24}) F_1 (*left*). **3.** The empty (containing no pollen) anther of the ms plant (91-12). **4.** Section of embryo-sac of ms plant (91-12) showed the normal structure of embryo-sac (×450). **5.** The ms plant, 91-12 (*left*), the parent variety IR_{54}, (*right*), and their hybrid F_1 (*middle*). **6.** 91-12 (*left*), IR_{24} (*right*) and their hybrid F_1 (*middle*). **7.** 91-12 (*left*), IR_{24} (*right*) and their hybrid F2 (*middle*)

F_2 was expressed in the ratio of 15/16 (fertile) to 1/16 (sterile) which exactly fits the expected value of Mendel's law of 9:3:3:1 (Table 3). The result demonstrates that the sterility of the ms plant from line 91 was controlled by two pairs of independent genes. Only the individual plant with the genotype of homozygous recessive, $rf_1rf_1rf_2rf_2$ was phenotypically male sterile. All of the other genotypes such as Rf_1-Rf_2-, Rf_1-rf_2rf_2 and $rf_1rf_1Rf_2$- were not male sterile in phenotype.

Discussion

The heritable variation caused by tissue culture in different crops had been extensively studied since Larkin and Scowcroft (1981) advanced the concept of the somaclonal variation. Variation was chromosomal (Krikorian et al. 1983; Ling et al. 1987), physiological (Larkin et al. 1984), morphological (Meins 1983; Sun et al. 1983), biochemical (Schaeffer et al. 1984), disease resistance and tolerance to stress (Ling et al. 1985) and so on. Not many tissue culturists have related their work on male sterility to heterosis breeding. Ling et al. (1978) reported anther culture of hybrid rice and pointed out the possibility of obtaining a stable line from hybrid rice by anther culture. Kubo and Kumashiro (1984) and Kumashiro and Kubo (1984) produced a cytoplasmic male sterile line of tobacco by protoplast fusion. This paper is the first report of a male sterile mutant from somatic cell culture in rice.

The factors which might be involved in inducing mutants in vitro culture might be the effects of chemicals in the medium (various salts, hormones, organic substances and so on) and culture conditions (temperature, light and culture duration and so on). Mutagenic factor(s) causing ms mutants in tissue culture may be related to one factor or a group of factors. This needs to be studied further.

Gengenbach and Connelly (1981) and Brettell and Thomas (1980) obtained fertile mutants from a male sterile line of corn in tissue culture. They proved that mitochondrial DNA in cytoplasm of a male sterile line undertook mutation which caused the male sterile cytoplasm to become normal (fertile). These results showed that not only nuclear genes controlled male sterile but also cytoplasmic genes could mutate in tissue culture. Thus male sterile cytoplasm might be induced from normal fertile cytoplasm by tissue culture. This subject is very much worth studying in further detail. If male sterile cytoplasm mutants were obtained from normal cytoplasm, the nucleic-cytoplasmic intercooperative male sterile line could be established from ms plants controlled by a nuclear gene which could not be used in heterosis for lack of maintainer. For rice heterosis utilization in China, this success would break through the limitation of developing a maintainer line from WA cytoplasm and make the three lines varietal. This would play a huge role in hybrid rice production.

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