Oryza rufipogon由来の系統RT18Aにおける細胞質雄性不稔および稔性回復の遺伝

Inheritance of Cytoplasmic Male Sterility and Restoration of Fertility in Rice Line, RT18A, derived from *Oryza rufipogon*

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Key words : Gametophytic control, Nucleus substitution line, *Oryza rufipogon, O. sativa,* Restoring gene

Summary

The usage of limited cytoplasm of crpos is genetically vulnerable. It may cause outbreak of diseases and insects, which attack a particular cytoplasm preferentially. Therefore, it is necessary to develop other sources of cytoplasmic male sterility. In this paper, the inheritance of male sterility and fertility restoration induced by the interaction between cytoplasm and a nuclear gene in rice line will be reported.

To develop isogenic lines, K18, a line of *Oryza rufipogon*, was used as the initial female parent, followed by successive backcrosses to Taichung 65 as the recurrent male parent. In B C_8F_1 generation the male sterile line was developed and designated as RT18A. The line had sterile pollen so that spikelets were also revealed to be sterile. On the other hand, RT61C was also developed by the successive backcross method. It was found that the line carried both male sterile cytoplasm (cms_1) and restoring gene ($Rf_1{}^1$) derived from K61C. To clarify inheritance of cytoplasmic male sterility and identify cytoplasm in RT18A, crossing tests were carried out, and the following results on inheritance of cytoplasmic male sterility and restoration fertility were obtained.

Fertility was conditioned by the interaction between a pair of nuclear restoring genes, $Rf_1{}^1$ and *rf*, and the male sterile cytoplasm, msc_1. Pollen grains with the $Rf_1{}^1$ gene displayed normal development under the msc_1 cytoplasm, while pollen grains with the *rf* gene degenerated under this cytoplasm. Therefore, the degree of pollen and spikelet fertility in the msc_1 plants varied with the genotype. In pollen fertility, $(msc_1)Rf_1{}^1Rf_1{}^1$ plants showed complete fertility; $(msc_1)Rf_1{}^1rf$ plants 50% fertility - normal pollen grains and degenerated ones; and $(msc_1)rfrf$ plants complete sterility. In spikelet fertility, both $(msc_1)Rf_1{}^1Rf_1{}^1$ and $(msc_1)Rf_1{}^1rf$ plants showed a high degree of fertility, while the $(msc_1)rfrf$ plants showed complete sterility. In contrast, in the plants with the male fertile cytoplasm of Taichung 65, *mfc*, pollen grains developed normally and displayed an equal opportunity of fertilization regardless of their genotype. Pollen grains with the *rf* gene of the $(msc_1)Rf_1{}^1rf$ plants could not be used for fertilization and this type of pollen abortion was gametophytic.

This mode of inheritance was the same as that observed in RT61C. The abortion of sterile pollen grains also started at an earlier stage of pollen development as RT61A, i.e. the grains were smaller and less stained. It was considered that this stage of pollen degeneration was controlled by the characteristics of cytoplasm. Therefore, cytoplasm of RT18A must be the same as that of RT61A, i.e. msc_1. However, for positive identifiction the more detailed examinations are needed with the use of other restoring lines.

Introduction

The cytoplasmic male sterility and fertility restoration system is one of the effective means for facilitating F_1 hybrid seed production in rice. Differences of the fertility in reciprocal crosses between *indica* and *japonica* types of a cultivated rice variety (*O. sativa*) were observed by Sampath and Mohanty (1954) for the first time, and they suggested that sterility was caused by the interaction between *indica* cytoplasm and the *japonica* nucleus. Afterwards, similar results on the cytoplasm difference in rice were also reported (Kitamura, 1962; Katsuo and Mizushima, 1958). None of the researchers, however, clarified inheritance of sterility. Inheritance of male sterility and fertility restoration was originally reported by Shinjyo (1969) in the offspring of the cross between *indidca* variety, Chinsurah Boro II, and *japonica* variety, Taichung 65. The cytoplasm of Chinsurah Boro II induced male sterility with a recessive allele *rf*, and it restored fertility with a dominant allele $Rf_1{}^1$.

The first cytoplasmic male sterile line used for commercial F_1 rice hybrids was developed in China in 1973. The line originated from a wild rice (*O. sativa f. spontanea*) native to Hainan Island (Yuan, 1977). The cytoplasm was designated as WA. Most of the commercial rice hybrids in China and elsewhere are produced by the use of this WA cytoplasm (Zhou, 1994). Limited use of a particular cyto-

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plasm is very risky due to genetic vulnerability, as major losses through diseases or insects that attack the particular cytoplasm may occur. For avoiding this kind of risk, it is desirable to use some other kinds of cytoplasm.

The present authors analyzed inheritance of cytoplasmic male sterility in the three lines, RT61, RT98 and RT102, which were developed by successive backcrossing of Taichung 65, as the recurrent male parent, to lines of *O. rufipogon* (Motomura *et al.*, 1992, 2001, 2003). In any case, fertility was conditioned by the interaction between the nuclear restoring genes at a single locus and cytoplasm. However, characteristics of the cytoplasm and the restoring gene among the three lines differed from each other and they were labeled as follows: msc_1 and Rf_1^1 for RT61C; msc_2 and Rf_1^2 for RT98C; msc_3 and Rf_1^3 for RT102C.

Another developed line, RT18A, was also an isogenic line of Taichung 65. The line carried male sterile cytoplasm but not restoring genes. However, the characteristic of cytoplasm was very similar to those of RT61C (Motomura, 1992; Motomura *et al.*, 1994). Therefore, it was supposed that the mode of inheritance for male sterility and fertility restoration in RT18A was the same as that in RT61C, if the restoring gene of RT61C was introduced to RT18A. In the current study inheritance of cytoplasmic male sterility in RT18A was analyzed with use of the restoring gene of RT61C.

Materials and Methods

Development of the nucleus substitution lines, RT18A and RT61C

Two lines of *O. rufipogon,* W0125 and W0180, originating in India, were obtained from the National Institute of Genetics in Shizuoka, Japan. They were renamed K18 and K61 in our laboratory. In order to facilitate the analysis of the mode of inheritance of cytoplasmic male sterility, it is necessary to remove hybrid sterility. In the current experiment, nucleus substitution lines of Taichung 65 were developed by the following procedures.

K18 was used as the female parent crossed with the male parent Taichung 65. The F_1 with spikelet sterility was backcrossed as the female parent to Taichung 65. The resulting BC_1F_1 plants revealed spikelet sterility again. Although backcrossing was continued through BC_8F_1 generation, resulting progeny in each generation generated only spikelet sterility. A plant in the BC_8F_1 was selected and designated as RT18A. Its morphology looked very much like Taichung 65. This sterile line was maintained by crossing with Taichung 65.

On the other hand, RT61C was also developed by successive backcross methods. However, the line carried both male sterile cytoplasm (cms_1) and restoring gene (Rf_1^1) derived from K61C.

Cross combination for analysis of cytoplasmic male sterility and fertility restoration

To investigate the mode of inheritance the following crosses were carried out: (a) RT18A / RT61C, (b) RT18A / RT61C // RT61C, (c) RT18A / RT61C // Taichung 65, (d) F_2: RT18A / RT61C, (e) Taichung 65 // RT18A/RT61C, (f) F_2: Taichung 65 // RT18A/RT61C, (g) RT18A /// Taichung 65 // RT18A / RT61C.

Observation of pollen and spikelet fertility

For the observation of pollen fertility, several spikelets with mature anthers per plant were picked and fixed in a 75% ethyl alcohol solution. Pollen grains were stained with an I_2KI solution and observed under an optical microscope. Pollen fertility was judged by shape, size and stained color: fertile - grain with morphologically spherical and darkly stained, and sterile - grain with spherical but small and stained in light brown. For the observation of spikelet fertility, three panicles per plant were sampled, and the percentage of fertile spikelets was calculated.

Results and Discussion

Analysis of restoring gene in the male sterile cytoplasm

Almost all the pollen grains of Taichung 65 were spherical and darkly stained, showing a complete fertility (Fig.1). Similar pollen grains were also observed in RT61C (Fig.2). Since this line revealed high spikelet fertility, these pollen grains also must be fertile. On the other hand, RT18A carried only sterile pollen grains, that is, they were spherical but small and stained in light brown (Fig.3).

There were two types of pollen grains with the ratio of 1 to 1 in the F_1 plants obtained from the cross between RT18A and RT61C: spherical and darkly stained as those of Taichung 65 and RT61C, and spherical but small and stained in light brown as those of RT18A. Spikelet fertility of these F_1 plants was more than 80% (section a in Table 1). It proved that fertilization carried out normally. That

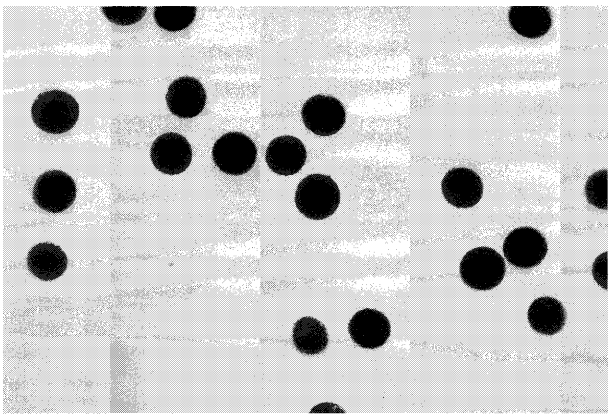

Fig. 1. Pollen grains of Taichung 65 (*mfc*)*rfrf*

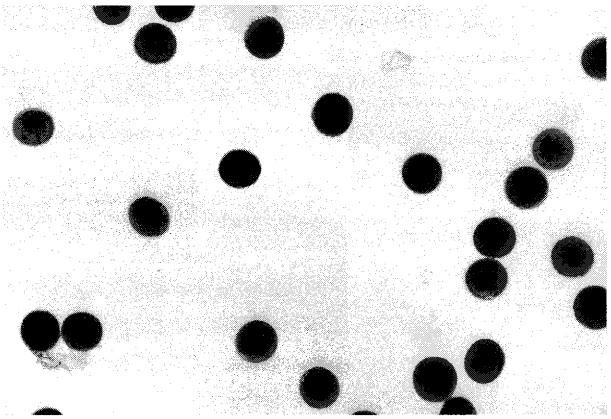

Fig. 2. Pollen grains of RT61C $(msc_1)Rf_1^1Rf_1^1$

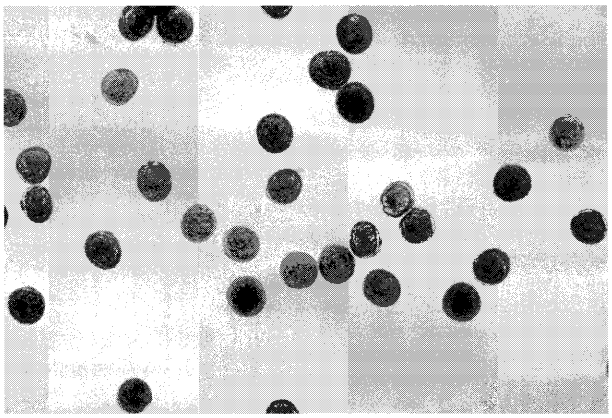

Fig. 3. Pollen grains of RT18A $(msc_1)rfrf$

is, the pollen grains which were spherical and darkly stained were viable. Genes controlling fertility were seemed to be monogenic because of being a 1 to 1 ratio for both pollen types. The viable pollen grains were considered to carry Rf_1^1 gene derived from RT61C, while the sterile ones carried *rf* gene under the male sterile cytoplasm of RT18A.

The F_1 was pollinated by RT61C and pollen and spikelet fertility of the resultant plants were researched. All of them revealed high spikelet fertility. However, they were divided into two groups by difference of pollen fertility, one with 100% and the other with 50%. The numbers of the fertile and sterile plants were 103 and 127, respectively, that is, the ratio was one to one (section b in Table 1). It showed monogenic segregation, being assumed that the plants with 100% pollen fertility carried $Rf_1^1Rf_1^1$ genotype and the plants with 50% pollen fertility Rf_1^1rf genotype.

On the other hand, in the cross between F_1 of RT18A and RT61C and Taichung 65 spikelet fertile plants with more than 60% and completely sterile plants were obtained in a 1:1 ratio (section c in Table 1). Like above, the ratio showed monogenic segregation. The genotypes of the plants were considered Rf_1^1rf and *rfrf*, respectively.

Segregation of restoring genes in F_2 population of the cross of RT18A and RT61C

All the plants revealed high spikelet fertility, more than 70%, in the F_2 population obtained from the cross between RT18A and RT61C. That is, plants not segregated

Table 1. Distribution of pollen and spikelet fertility in progenies obtained from the crosses among RT18A, RT61C and Taichung 65

	Cross combination	Organ	No. of plants in each fertility class							Total No. of plants	x^2 value for 1:1
			0 - 40	50	60	70	80	90	100 (%)		
(a)	RT18A/RT61C	Pollen		46						46	
		Spikelet					4	27	15	46	
(b)	RT18A/RT61C// RT61C	Pollen		103					127	230	2.504
		Spikelet				5	12	109	104	230	
(c)	RT18A/RT61C// T65	Pollen	152						125	277	2.632
		Spikelet	152		2	7	7	65	44	277	2.632
(d)	F_2:RT18A/RT61C	Pollen		117					133	250	1.024
		Spikelet				10	9	120	111	250	
(e)	T65//RT18A/ RT61C	Pollen							112	112	
		Spikelet					2	53	57	112	
(f)	F_2:T65//RT18A/ RT61C	Pollen							139	139	
		Spikelet					31	88	20	139	
(g)	RT18A///T65// RT18A/RT61C	Pollen	77	96						173	2.087
		Spikelet	77			4	4	37	51	173	

Table 2. Theexpression of pollen characteristics and spikelet fertility under different combination of cytoplasm type and genotype of restoring gene in the substituted line, RT18A

Genotype	Pollen characteristics		Spikelet fertility
	Size	Color	
$(msc_1)Rf_1^1Rf_1^1$	normal	dark purple	normal
$(msc_1)Rf_1^1rf$	normal	dark purple	normal
	small	light brown	
$(msc_1)rfrf$	small	light brown	sterile
$(mfc)Rf_1^1Rf_1^1$	normal	dark purple	normal
$(mfc)Rf_1^1rf$	normal	dark purple	normal
$(mfc)rfrf$	normal	dark purple	normal

in spikelet fertility. However, they segregated into two types in pollen fertility, 50% and 100%. The numbers of plants were 117 and 133, respectively, suited to a 1:1 ratio (section d in Table 1). It was considered that the former had Rf_1^1rf and the latter $Rf_1^1Rf_1^1$. Since the F_1 genotype was assumed to be Rf_1^1rf, two types of pollen grains (Rf_1^1 and rf) should be present with the same ratio. If the rf pollen was fertilized in the F_1 plants, some completely sterile plants should be generated in the F_2 generation. In such a case, sporophytic control of pollen fertility might be involved. In fact, no completely sterile plants appeared in the F_2 generation, indicating that the Rf_1^1rf plants produced Rf_1^1 viable and rf nonviable pollen grains. Therefore, it was considered that the male sterility was controlled by the pollen genotype but not by the plant genotype. This mode of gene action was gametophytic.

Analysis of restoring gene in the male fertile cytoplasm of Taichung 65

Taichung 65 used as the female was crossed with F_1 obtained from the cross between RT18A and RT61C. All offspring revealed high spikelet fertility and 100% pollen fertility (section e in Table 1). As they were inherited Rf_1^1 from male parent and rf from female parent respectively, the genotype must be Rf_1^1rf. Therefore, the result showed that the pollen grain with rf gene was also fertile and that the cytoplasm of Taichung 65 was normal for fertility. When the offspring was self-fertilized, all the plants in F_2 revealed pollen and spikelet fertility normally (section f in Table 1). There had to be three kinds of genotypes, $Rf_1^1Rf_1^1$, Rf_1^1rf and $rfrf$ with Taichung 65 cytoplasm in the F_2 population. On the other hand, RT18A was crossed with the F_1 produced by three way crosses above, i.e. the cross of maternal Taichung 65 with the F_1 between RT18A and RT61C. In the resultant offspring, two types of plants segregated: the plants with complete pollen and spikelet sterility, and the plants with 50% pollen but high spikelet fertility. The numbers were 77 and 96, respectively, fitted to a 1:1 (section g in Table 1). Although the genotype of the male parent had to be Rf_1^1rf, the results showed that both Rf_1^1 and rf pollen grains could be fertilized normally. That is, the cytoplasm of Taichung 65 was normal without causing male sterility.

The relationships between pollen and spikelet fertility and the combination of cytoplasm and genotype were put together in Table 2. Under the male sterile cytoplasm cms_1, Rf_1^1 pollen grain was developed normally, so that they were stained dark purple in the solution. While rf pollen grains were degenerated in an earlier stage, so that they were small and stained in light brown. As half of the fertile pollen grains being viable in $(msc_1)Rf_1^1rf$, spikelet fertility was normal in this genotype. Spikelets were sterile in only $(msc_1)rfrf$. Under male fertile cytoplasm mfc, all pollen grains were viable regardless of genotype.

Based on the results obtained, it was concluded that the inheritance of male sterility and fertility restoration was conditioned by a pair of alleles, dominant and recessiveness, at a single locus interacting with the male sterile cytoplasm, and that the gene action of male sterility was gametophytic.

This mode of inheritance was the same as that observed in RT61C. The abortion of sterile pollen grains also started at an earlier stage of pollen development as that in male sterile plant RT61A, i.e. the grains were smaller and less stained. It was considered that that stage of pollen degeneration was controlled by the characteristics of cytoplasm. Therefore, cytoplasm of RT18A must be the same as that of RT61A, i.e. msc_1. However, for positively identifying them more detailed examinations are needed with use of other restoring lines.

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Oryza rufipogon 由来の系統RT18A における細胞質雄性不稔および稔性回復の遺伝

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キーワード：*Oryza rufipogon*、*O.sativa*、核置換系統、稔性回復遺伝子、配偶体支配型

要　約

細胞質雄性不稔性を用いたイネのヘテロシス育種においては、中国の海南島で発見されたWA細胞質が主に用いられている。しかしこの様な特定の細胞質のみを用いた限定的な利用では遺伝的脆弱性が心配される。その解決のためには、多くの雄性不稔細胞質を発見･同定し、遺伝分析を行って優良素材を探索する必要がある。著者等はその点の研究を進めており、今回次のような研究を行った。

Oryza rufipogon のK18系統を1回親母本にし、栽培イネ品種、台中65号を反復親父本に用いて8回の連続戻交雑を行って、雄性不稔系統RT18Aを得た。同系統の花粉は球形であるが正常花粉に比べて小さく、また正常花粉が染色液で濃紫色になるのに対して同花粉は薄い褐色を示した。これらの花粉は活性を持たず、したがって種子稔性も完全不稔であった。この花粉退化の様式は既に報告したRT61C系統のそれと似ており、同系統の稔性回復遺伝子を用いてRT18Aの雄性不稔性の遺伝分析を行い、併せてRT18A系統の細胞質の同定を試みた。両系統および台中65号を材料に用いて交雑実験を行い、以下の結果を得た。

稔性は細胞質と核内の稔性回復遺伝子との相互作用により支配されていた。すなわち、正常細胞質（*mfc*）のもとでは稔性回復遺伝子（Rf_1^1-*rf*）の優劣に関係なく、花粉が正常に発育し、受精が行われるため種子稔性も正常であった。雄性不稔細胞質（msc_1）のもとでは、Rf_1^1 遺伝子を持つ花粉は正常であるが、*rf* 遺伝子をもつ花粉は途中で発育を停止し不稔となった。そのため雄性不稔細胞質のもとでは遺伝子型により花粉および種子稔性が異なった。(msc_1)Rf_1^1 Rf_1^1 では花粉は球形で濃染し正常であり、種子稔性も正常であった。(msc_1)Rf_1^1*rf* では Rf_1^1 花粉は正常であるが *rf* 花粉は不稔であった。種子稔性は、(msc_1)*rfrf* 個体以外は高かった。(msc_1)Rf_1^1*rf* 個体の自殖後代では、不稔個体が生ぜず、配偶体支配型の花粉不稔を示すことがわかった。

以上の結果はRT61Cに見られた雄性不稔および稔性回復の遺伝に酷似しており、RT18Aの細胞質はRT61Cのそれと同一の可能性が高かった。しかし、確実な同定のためには他の複数の稔性回復遺伝子との間で示される稔性反応を調べる必要がある。

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