COMPARISON OF THE MALE STERILITY INDUCED BY LOW TEMPERATURES WITH THE GENETICAL MALE STERILITY IN RICE. I. OBSERVATION ON INDEHISCENT ANTHERS AT ANTHESIS[1)]

Souhei SAWADA and Toshiro KINOSHITA

Plant Breeding Institute, Faculty of Agriculture,
Hokkaido University, Sapporo, Japan

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Introduction

Low temperature has little or no harmful effects on the pistil but has injurious effects on the stamen (HAYASE *et al.* 1969). Therefore, the sterility in so-called "destructive type cold injury" is physiological male sterility. On the other hand, various types of genetical male sterility has been found in rice (SHINJYO and OMURA 1966, SHIBUYA 1966, WATANABE *et al.* 1968, KO *et al.* 1976, FUJIMAKI *et al.* 1976).

Comparison of physiological and cytoplasmic-genetic male sterility has been made by the authors, in hoping that this line of examination may be effective for elucidating the mechanism of pollen sterility. In this paper, the results obtained from observations at anthesis is reported.

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Materials and Methods

The cytoplasmic male sterile rice used in this experiment is listed in Table 1. The strain Shiokari 'MS' is B_3F_1 generation which is derived from the successive backcrossings, (I–127 × Shiokari) × Shiokari. The strain A–58 MS is also obtained from the progeny of backcrossings by the similar method. 'H–103 F_1' is an F_1 population of the cross, I–127 × H–103. I–127

1) Contribution from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University and Laboratory of Forage Crops, Obihiro University of Veterinary Medicine, Obihiro, Hokkaido, Japan.

TABLE 1. The cytoplasmic-genetic male sterile strains

Shiokari MS	; B_3F_1 of (I-127×Shiokari)×Shiokari
A-58 MS	; B_3F_1 of (I-127×A-58)×A-58
H-103 F_1	; F_1 of I-127×H-103
I-127 (Taichung 65 MS)	genotype; [*cms-boro*] *rf rf*
	$B_{12}F_1$ of (Chinsurah Boro II×Taichung 65)×Taichung 65

is an isogenic strain of Taichung 65 which prossesses [*cms-boro*] cytoplasm together with the nucleus genotype of Taichung 65 and was produced from the successive backcrossings, (Chinsurah Boro II×Taichung 65)×Taichung 65 by SHINJYO (1970).

The seedlings were grown in the greenhouse of Faculty of Agriculture, Hokkaido University and transplanted into polyvinyl pots. After that, plants were shifted in the greenhouse of Obihiro University until maturity. The dehiscence of anthers were observed in the spikelets which were collected after flowering by using a magnifying glass. Anther dehiscence was designated even though one theca opened. Pollen grains were stained with I-KI solution. More than five hundreds of pollen grains were observed for the calculation of pollen fertility. Five panicles from an individual plant were investigated at maturity for seed fertility.

In physiological male sterility, two varieties, Hayayuki and Norin 20 were treated at 12 days before heading in a phytotron which was conditioned at 15°C during day and at 12°C in the night. The duration of low temperature treatments were 0, 3 and 6 days. The methods employed for determining the dehiscence of anthers and pollen fertility were essentially the same as those used in the genetical male sterility.

Results

Shiokari MS and A-58 MS were the cytoplsmic-genetic male sterility and fertility restoring genes were not contained as well as the original strains, Shiokari and A-58. Therefore, they were complete sterile. In contrast with this, 'H-103 F_1' indicated 9% of seed fertility and it is assumed that a fertility restoring gene or genes were contributed from H-103.

Four types of anthers arose in the sterility as shown in previous paper (SAWADA 1974). As the duration of low temperature treatment became longer, there was a tendency that the indehiscent anthers increase in the both varieties. In this experiment, a cold tolerant variety Hayayuki produced a small number of **b** and **c** type anthers, while in Norin 20 which is

susceptible, **b** and **c** type anthers increased under longer duration of the treatments.

Similar types of indehiscent anthers were also found in the cytoplasmic-genetic male sterile strains. The frequency of anther types differed among the two strains and the F_1 (Table 2). In Shiokari MS, the percentage of dehiscent anthers was only 1% and all the remainder belonged to **a** type. The dehiscent type and the three types of indehiscent anthers were observed in A-58 MS, where the percentage of the dehiscent anthers was 2%. Indehiscent types of **a** and **b** were 73% and 20% respectively. 'H-103 F_1' produced 76% of dehiscent anthers and 24% of type **a**. The dehiscent anthers of 'H-103 F_1' indicated an incomplete openning of theca in which only small pore appeared at the tip. It induced a high seed sterility owing to non-fertilization by insufficient pollen grains. The complete sterility of Shiokari MS and A-58 MS were also due to the non-pollination caused by the indehiscence of anthers. Thus, the phenomena of anther dehiscence resembled to the sterility in the low temperature treatment.

As to non-flowering spikelets, longer treatments of low temperature increased the rate. In 'H-103 F_1' and Shiokari MS, non-flowering spikelets were none or scarce, while A-58 MS showed relatively a high rate, 25%. In the non-flowering spikelets, most of anthers belonged to **b** and **c** types.

TABLE 2. Frequency of anther types, percentage of non-flowering spikelets and seed fertility

Strain and treatment	Frequency of anther types[1] (%)				% of non-flowering spikelets	No. of spikelets observed	Seed fertility
	d	**a**	**b**	**c**			
Shiokari MS	1	99			1	417	0
A-58 MS	2	73	20	4	25	527	0
H-103 F_1	76	24			0	429	9
Hayayuki 0[2]	96	2	2		0	307	97
" 3	73	22	5		1	309	56
" 6	31	56	11	2	5	276	18
Norin 20 0	83	9	7	1	0	368	89
" 3	12	60	25	3	7	381	11
" 6	2	27	44	27	9	363	2

1) **d**, **a**, **b** and **c** indicate dehiscent anthers and the three classes of indehiscent anthers, respectively. Anther length and number of pollen grains diminish from **a** to **c**.

2) Duration of low temperature treatment in the phytotron.

TABLE 3. Frequency of various types of spikelets

d	**a**	**b**	**c**	Shiokari MS	A-58 MS	H-103 F_1
6						253 (59)
5	1					33 (8)
4	2				1	36 (8)
3	3			2*	6 (1)	25 (6)
2	4			2	4 (1)	16 (4)
1	5			8 (2)**	27 (6)	21 (5)
1		5			1	
	6			405 (97)	266 (62)	44 (10)
	5	1			9 (2)	
	5		1		1	
	4	2			7 (2)	
	4	1	1		2	
	4		2		1	
	3	3			3 (1)	
	3	2	1		1	
	2	4			2	
	2	3	1		1	
	2	2	2		1	
	1	5			2	
	1	4	1		1	
	1	2	2		3 (1)	
		6			48 (11)	
		5	1		20 (5)	
		4	2		11 (3)	
		3	3		2	
		2	4		4 (1)	
			6		3 (1)	
Total				417 (100)	427 (100)	429 (100)

* Number of spikelets observed.
** Percent against total.

As shown in Table 3, Shiokari MS produced the combination of anther types, such as **d**3**a**3 (indicating 3 anthers of dehiscent type and 3 anthers of **a** type), **d**2**a**4, **d**1**a**5 and **a**6 in the observed spikelets. Among them, the frequency of **a**6 showed the highest rate, 97%. In A-58 MS, the frequency

TABLE 4. Pollen sterility of indehiscent anthers **a** and **b**

Strain	Anther type	Pollen sterility (%) 0~10~20~30~40~50~60~70~80~90~100	No. of anthers observed	Mean (%)
Shiokari MS	**a**	28 2	30	6.2
A-58 MS	**a**	9 15 5 1	30	14.1
" MS	**b**	6	6	98.5
H-103 F_1	**a**	12 14 4	30	13.5

of anther types varied in a wide range from **d4a2** to **c6**, where **a6** was the highest one. Among the 7 types of spikelets occurred in 'H-103 F_1', the types containing more than one dehiscent anther occupied about 90%.

Average percentages of pollen sterility in the **a** type anthers were 6.2%, 14.1% and 13.5% in Shiokari MS, A-58 MS and 'H-103 F_1' respectively. These anthers contained a considerable amount of pollens looking healthy in spite of the indehiscence of anthers. Pollen sterility of **b** type anthers in A-58 MS was 98.5% (Table 4).

Discussion

According to KINOSHITA *et al.* (1973), flowering duration of spikelets within a panicle of I-127 (Taichung 65 MS) became longer than that of the isogenic line, Taichung 65 and the time of flowering differed in some conditions between the both strains. The similar phenomena of flowering habits were observed in the sterile type injury (SAWADA 1975). In addition to these facts, the results obtained from this investigation indicate that the cause of both sterility is non-pollination due to anther indehiscence. Namely, the similar anther types were observed at anthesis in both physiological and genetical male sterility.

SAWADA (1974, 1976 a, b) detected that in destructive type cold weather injury, three types of indehiscent anthers occurred and these anthers derived from the incomplete development at different stages, and the frequency of indehiscent anthers varied with the degree of temperature and the time exposed to low temperature. From the frequency of anther types and pollen sterility of indehiscent anthers, it could be deduced that each anther of spikelets in Shiokari MS stopped development presumably at a later stage of pollen development and anthers of 'H-103 F_1' made a little progress towards those of the normal and that of A-58 MS stopped at earlier stages in the microspore development after the tetrad.

Further investigations on the stage at which microspores ceased the development may give informations of the mechanism of the character expression in both physiological and genetical male sterility.

Summary

The sterility induced by low temperature and the cytoplasmic-genetic male sterility were compared at anthesis.

Two male sterile strains, Shiokari MS and A–58 MS and an F_1 population 'H–103 F_1' were used in this experiment, together with the two normal strains, Hayayuki and Norin 20 which were treated in a phytotron at 12 days before heading for 0, 3 and 6 days.

The following results were obtained.

1. The direct cause of sterility in cytoplasmic-genetic male sterility was non-pollination due to anther indehiscence as well as those in low temperature injury.
2. Three indehiscent types of anthers were observed in both genetical and physiological male sterility, though the frequency of the indehiscent anthers differed among the strains, F_1 and the treatments (Table 2).
3. The combinations of dehiscent and indehiscent types in an individual spikelet also differed remarkably among the sterile strains and F_1 (Table 3). Pollen sterility of **a** type anthers was relatively low in spite of the indehiscence of anthers (Table 4).
4. In most of the cases in the low temperature treatments and A–58 MS, indehiscent anthers arose from various developmental stages of pollens while in Shiokari MS and 'H–103 F_1', indehiscent anthers were an outcome of the abnormality at a definite stage of microspore development. It is noted that the anther type of A–58 MS resembles closely to that in the 3 days treatment in Norin 20.

Thus it is suggested that the mechanism of the pollen sterility due to the both physiological and genetical causes stands for a common phenomenon.

Literature cited

FUJIMAKI, H., S. HIRAIWA, K. KUSHIBUCHI and S. TANAKA 1976. A male sterile gene induced by mutation and its use for new breeding system in rice. Japan J. Breed. 26 (Supl. 1): 66–67. (In Japanese)

HAYASE, H., T. SATAKE, I. NISHIYAMA and N. ITO 1969. Male sterility caused by cooling treatment at the meiotic stage in rice. II. The most sensitive stage to cooling and the fertilizing ability of pistils. Proc. Crop Sci. Soc. Japan

38: 706-711.

KINOSHITA, T., H. KIKUCHI and M. TAKAHASHI 1973. Flowering habits in male sterile rice. Rep. Hokkaido branch. Jap. Soc. Breed. 13: pp. 18 (In Japanese)

KO, T. and H. YAMAGATA 1976. Studies on the induction of male sterile strains in rice. 2. EI-induced mutations toward male sterility. Japan J. Breed. 26 (Supl. 1): 64-65. (In Japanese)

KITAMURA, E. 1962. Studies on cytoplasmic sterility of hybrids in distantly related varieties of rice, *Oryza sativa* L. I. Fertility of the F_1 hybrids between strains derived from a certain Philippines×Japanese variety crosses and Japanese varieties. Japan J. Breed. 12: 81-84. (In Japanese with English summary)

SAWADA, S. 1974. Anther indehiscence in rice plants as a cause of sterility when induced by low temperatures. Res. Bull. Obihiro Univ. 8: 629-636.

SAWADA, S. 1975. Effects of low temperature at the booting stage on flowering habit, pollination and fertilization in rice varieties. Res. Bull. Obihiro Univ. 9: 589-597.

SAWADA, S. 1976 a. A view on the sterility in the destructive type cool weather damaged rice. Japan J. Breed. 26 (Supl. 1): 140-141. (In Japanese)

SAWADA, S. 1976 b. Frequency of indehiscent anthers and sterility, induced by low temperatures at the booting stage in rice plants. Res. Bull. Obihiro Univ. 9. (In press).

SHIBUYA, T. 1966. Studies on partial male sterility in rice plants. Japan J. Breed. 16: 174-178. (In Japanese with English summary)

SHINJYO, C. and T. 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)

SHINJO, C. 1970. Cytoplasmic-genetic male sterility in cultivated rice. *Oryza sativa* L. I. The process of developing complete male-sterile and restorer lines, and their stability against natural environments. Bull. College Agr. Univ. Ryukyus 17: 261-272. (In Japanese with English summary)

WATANABE, Y., S. SAKAGUCHI and K. 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)