Male Sterility Caused by Cooling Treatment at the Meiotic Stage in Rice Plants

III. Male abnormalities at anthesis

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In the previous papers^{1,4)} the authors reported that the most severe damage in fertility induced by cooling treatments was found if the period of time between the heading stage and the middle dates of low temperature treatments was 10 to 11 days (the meiotic stage of pollen mother cells) regardless of the length of treatment. Pistils of low-temperature treated plants showed good receptivity when pollinated with sound pollens. Consequently, the failure at fertilization may have been caused by male abnormalities.

The authors⁵⁾ previously reported that sterility was mainly due to anther indehiscence when rice plants were artificially cooled at booting stage. The present study was carried out to confirm this suggestion by growing rice plants under controlled conditions and cooling them at the meiotic stage.

throughout the whole life. In a natural light room, seeds of Hayayuki were sown on June 20, 1968 and grown at 24°C day and 19°C night. Cooling treatments were done at 12°C for 2 to 4 days.

For each plot, panicles at the initial stage of the meiotic division of pollen mother cells were selected before cooling by measuring distances between the last two auricles³⁾. Uniform spikelet samples were taken from only the upper three primary branches omitting their two apical spikelets and secondary branches. At anthesis which had opened during the day were collected and observed on male injuries every evening. Pollen germination on pistils were observed after cotton blue staining and pollen ripeness was estimated with acetocarmine staining.

RESULTS

MATERIALS AND METHODS

Two early varieties of rice plants, Hayayuki and Norin 20, were used. In an artificial light room, seeds of Norin 20 were sown on February 21, 1968, grown at 23°C day and 18°C night

Table 1 showed the percentages of fertilized spikelets of the control and cooled plants. Heading dates delayed with the increase of cooling days and fertility decreased strikingly in the 4 day cooling treatments. Moreover, 4 day

Table 1. Effect of cooling treatment at the meiotic stage on the percentage of fertilized spikelets in rice plants.

Room	Variety	Treatment	Heading date	Fertility (%)
Natural light (N)	Hayayuki	Control (C)	Aug. 7.0	96
		2 day cooling (2T)	8.3	88
		3 day cooling (3T)	9.1	79
		4 day cooling (4T)	10.0	42
Artificial light (A)	Norin 20	Control (C)	May 2.1	91
		2 day cooling (2T)	3.7	90
		4 day cooling (4T)	5.3	31

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cooling treatments prolonged the period from heading to anthesis and the blooming period (fig. 1). At normal anthesis, filaments elongated and anthers emerged above or outside the open spikelets. And when spikelets closed, anthers were left outside them. Cooling treatment, however, increased the number of anthers remaining in spikelets (table 2).

The number of pollen which adhered to the stigma and those which germinated on it were shown in fig. 2 and 3. In the control spikelets, about 30 to 200 pollens were shed on each stigma and about 10 to 60 of them germinated. Cooling treatments decreased the rate of pollen germination as well as the number of pollens adhered on stigma. In the spikelets which had less than 19 pollens on a stigma or/and less than 9 germinated ones, anther dehiscence was prevented (fig. 4) and many pollens remained in the anthers (fig. 5). Most of these pollens were weakly or not stained with acetocarmine (fig. 6), indicating immature pollens.

Causes of the infertilization for damaged

Table 2. Number of anthers remaining in spikelets after anthesis.

Room (Var.)	Treat- ment	No. of anthers observed (a)	No. of remaining anthers (b)	Ratio (b/a ×100)
N (Hay)	C	642	11	1.7
	2T	678	16	2.4
	3T	684	19	2.8
	4T	654	64	9.8
A (N 20)	C	504	8	1.6
	2T	546	20	3.7
	4T	606	96	15.9

See table 1 for abbreviations.

spikelet were estimated from the results of observation (fig. 7). When severe male abnormalities occur in anther development, pollen ripening and anther dehiscence or pollen discharge, fertilization may be expected to fail in most cases. Even if they occur in incomplete pollination or in a little germination of pollens, there is a little possibility of fertilization. The greater cause of infertilization consists of incomplete anther development, pollen unripeness and anther

Fig. 1. Effect of cooling treatment at the meiotic stage on flowering in rice plants.

○: Control, ●: 2 day cooling (2T), △: 3 day cooling (3T),
×: 4 day cooling(4T)

See table 1 for abbreviations.

indehiscence in rice plants subjected to servere cooling for 4 days. Among these causes pollen unripeness is recognized to be the most influential factor.

Fig. 2. Number of pollens adhered on stigma.

- : More than 20 pollens.
- ▨ : 1 to 19 pollens.
- : No pollen.

See table 1 for abbreviations.

Fig. 4. Degree of anther dehiscence in damaged spikelets.

- : Sound spikelets.
 - : Completely dehisced anthers
 - ▨ : Partially dehisced anthers
 - : Indehisced anthers
- } Damaged spikelets.

See table 1 for abbreviations.

DISCUSSION

Our previous paper¹⁾ showed that the anther is the weak organ of rice plants when cooled at the meiotic stage. To clarify the cause of steri-

Fig. 3. Number of pollens germinated on stigma.

- : More than 10 pollens.
- ▨ : 1 to 9 pollens.
- : No pollen.

See table 1 for abbreviations.

Fig. 5. Amount of remained pollens in anther loculi of damaged spikelets.

- : Sound spikelets.
 - : A little or no pollens remained
 - ▨ : About half of pollens remained
 - ▨ : Most of pollens remained
 - : All of pollens remained
- } Damaged spikelets.

See table 1 for abbreviations.

Fig. 6. Amount of pollens stained densely with acetocarmine.

See table 1 for abbreviations.

lity, microscopic observations were carried out at anthesis.

Injuries observed were arranged in the developmental order from pollen differentiation to germination, as follows: (1) a cessation of anther development, (2) pollen unripeness, (3) anthers remaining in spikelets after anthesis, (4) partial or no dehiscence, (5) pollens remaining in anther loculi, (6) a little or no shedding of pollens on stigma, (7) failure of pollen to germinate on stigma.

As shown in fig. 7 the main cause of infertilization can be explained by the cessation of the developmental processes prior to anther dehiscence (item 1, 2 and 4). Pollen unripeness is considered to be the greatest factor which induces anther indehiscence and may be produced by the lack of nutrient supply from transitory and tapetal tissues²⁾.

SUMMARY

Spikelets were examined for male abnormalities at anthesis in rice plants which were cooled

Fig. 7. Sterility and main causes of infertilization in damaged spikelets.

Solid line indicates sterility.

See table 1 for abbreviations.

at the meiotic stage of pollen mother cells. The abnormalities were a cessation of anther development, pollen unripeness, anthers remaining in spikelets after anthesis, partial or no dehiscence, pollens remaining in anther loculi, a little or no shedding of pollens on stigma and failure of pollen germination on stigma. The main cause of sterility was anther indehiscence, resulting from pollen unripeness to a considerable degree.

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〔和 文 摘 要〕

水稻の減数分裂期低温処理による雄性不稔

第3報 開花期に観察されたおしべおよび花粉の異常

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ファイトトロンの自然光室および人工照明室で水稻を培養し、減数分裂期に低温処理をおこない、開花期に雄性の異常を調査した。葯の發育から受精にいたる各段階、すなわち、葯の發育、花粉の充実、花糸の伸長、葯の裂開、花粉の飛散、受粉、柱頭上における花粉の発芽のそれぞれに異常が観察された。不稔の主因は葯の不裂開および不完全裂開であり、これらの裂開不良葯の花粉は充実していない場合がおおかつた。