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

XXX. Relation between fertilization and the number of engorged pollen grains among spikelets cooled at different pollen developmental stages

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Abstract : Cooling treatments at different stages of pollen development lowered the percentage of fertilized spikelets through decreasing the number of engorged pollen grains per anther at anthesis. Cooling during the period from anther differentiation to the tetrad phase decreased the number of engorged pollen grains mainly by decreasing the number of differentiated microspores. Cooling in the period from the early microspore phase to the late microspore phase, however, decreased the number of engorged pollen grains primarily by increasing the number of aborted microspores.

Cooling at the young microspore stage, which consists of the two phases of the tetrad and the early microspore phase, caused the largest decrease in the number of engorged pollen grains, resulting in the largest decrease in the percentage of fertilization. Cooling at the tetrad phase caused the largest decrease in the number of differentiated microspores, resulting in the largest decrease in the number of engorged pollen grains. On the other hand, cooling at the early microspore phase caused the largest increase in the number of aborted microspores, resulting in the largest decline in the number of engorged pollen grains.

These results indicate that the highest susceptibility to coolness of anthers at the young microspore stage, which has been estimated as the percentage of fertilized spikelets, is caused by the high susceptibility to coolness of the differentiation and development of microspores.
Key words : Anther, Cool injury, Cool temperature, Microspore, Pollen, Rice, Sterility.

イネの小胞子初期冷温処理による雄性不稔　第30報　花粉の発育時期別に冷温処理された穎花における受精率と葯当り充実花粉数との関係：佐竹徹夫（農林水産省北海道農業試験場）

要　旨：出穂前25～2日の期間に時期別に冷温処理を行い，穎花の受精率と小胞子および花粉の発育を調べた．受精率と充実花粉数との間に高い正の相関関係が認められたことから，処理時期による受精率の差異は充実花粉数の差異に基づくものと考えられる．冷温処理による充実花粉数の減少は，葯分化期～4分子期の期間においては主として分化小胞子数の減少によるものであり，小胞子前期～小胞子後期の期間においては主として退化小胞子数の増加によるものであった．

冷温による分化小胞子数の減少は4分子期に最も大きく，また冷温による退化小胞子数の増加は小胞子前期に最も大きかった．この結果，冷温による充実花粉数の減少とそれに基づく受精率の低下は小胞子初期(4分子期と小胞子前期を合わせた時期）の処理において最も大きかった．以上の結果より，これまで受精率によって評価されてきた小胞子初期の高い冷温感受性は，小胞子の分化と発育の両方の冷温感受性がこの時期に最も高いことに起因している．
キーワード：イネ，花粉，小胞子，葯，冷温，冷害．

In previous papers[8,10], we reported that the most sensitive stage regarding spikelet sterility due to cool temperature was the young microspore stage, which includes the phases of tetrad and early microspore. The sterility was caused by the failure of fertilization resulting from the immaturation of pollen grains in anthers[1,2]. The present paper reports that the percentage of fertilization is strongly affected by the number of engorged pollen grains per anther and the highest cooling susceptibility of anthers at the young microspore stage is caused by the high susceptbility to coolness of the differentiation and development of microspores.

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Materials and Methods

Experiment-1.

Two varieties differing in the degree of cool tolerance, Hayayuki (highly tolerant) and Norin-20 (susceptible), were used. Twenty seeds were directly sown in a circular pattern in each 4-liter plastic pot[6] and grown in a naturally lit room with a day/night temperature regime of 24/19°C. Each pot was provided with 0.9 g each of N, P and K. In order to obtain uniform main culms, tillers were removed as they appeared. The spikelets taken from the 3rd to 5th locations on the uppermost 3 primary branches on the panicles of main culms (specified spikelets : 9 spikelets per panicle) were used for experimnets.

Sixteen cooling treatments, in each of which 2 pots were cooled at 12°C for 3 days in a naturally lit room, were conducted successively at different stages during the period from 21 to 2 days before heading. After each cooling treatment, the pots were transferred back to the growth room and kept there until maturity. Panicles were labeled with the date of heading, and the stages at which the specified spikelets were subjected to cool temperatures were expressed by the number of days to heading from the beginning of cooling treatment.

The specified spikelets from 3 panicles were fixed with 50% ethanol at heading time to examine the development of anther and pollen. Anthers were excised from 20 spikelets of the fixed materials. The length of anther was determined as an average of 40 anthers taken randomly from the excised anthers. The number of engorged pollen grains stainable with iodin-potassium iodide solution per anther was determined as an average of 15～20 anthers sampled from the excised ones. After the ripening of seeds, the specified spikelets from 25 panicles were examined for the percentage of fertilization.

Experiment-2

Norin-20 was used. Twenty seeds were sown in a pot as in the experiment-1 and grown in a naturally lit room with a day/night temperature regime of 26/19°C. Twenty-four cooling treatments, in each of which 3 pots were cooled at 12°C for 4 days in a naturally lit room, were conducted successively at different stages during the period from 25 to 2 days before heading. After each cooling treatment, the pots were transferred back to the growth room.

The specified spikelets from 5 panicles were fixed with 50% ethanol at the beginning of the middle microspore phase to examine the number of differentiated microspores. Anthers were excised from all of the fixed spikelets and the number of microspores per anther was determined as an average of 15～20 anthers. The number of microspores at the beginning of middle microspore phase is considered to be nearly equal to the number of differentiated microspores, because only a few number of microspores degenerate during a short period to the beginning of middle microspore phase after tetrad[8].

The specified apikelets from 5 panicles were fixed with 50% ethanol at heading time to examine the number of engorged pollen grains. After the excision of anthers from all of the fixed spikelets, the number of engorged pollen grains per anther was determined as an average of 15～20 anthers. The number of aborted microspores was calculated by subtracting the number of engorged pollen grains from the number of differentiated microspores. The shape of 100 anthers was observed under the dissect microscope after staining them with iodin-potassium iodide solution and the percentage of abnormal loculi with little or no engorged pollen grains was measured. After the ripening of seeds, the specified spikelets from 30 panicles were examined for the percentage of fertilization.

Results

Fig. 1 shows the effect of cooling on the percentage of fertilization, anther length and the number of engorged pollen grains per anther at anthesis, when rice plants were cooled at different stages during pollen development. The decrease in the percentage of fertilization by cooling was the greatest at 11～12 days before heading and became smaller when the cooling stages became earlier or later. It was smaller in Hayayuki than in Norin-20, though the variation pattern was almost the same in these two varieties.

Cooling treatments at different stages induced the similar variations in the three characters. The percentage of fertilization significantly correlated with anther length (r=

0.95***) and with the number of engorged pollen grains per anther (r=0.93***). The number of engorged pollen grains per anther closely correlated with anther length (r=0.97***). These high correlations show that the percentage of fertilization was strongly affected by the development of anther and pollen.

Also in the experiment-2, the similar variation as in the experiment-1 was observed in the percentage of fertilization and the number of engorged pollen grains among cooling treatments. High positive correlation was observed between these two values (Fig. 2).

The number of differentiated microspores was estimated as the number of microspores at the beginning of middle microspore phase. The number of aborted microspores was calculated by subtracting the number of engorged pollen grains from the number of differentiated microspores.

As shown in Fig. 2, the numbers of differentiated and aborted microspores per anther were 800 and 150, respectively, in the control anthers. The number of differentiated microspores began to decrease from the cooling treatment at 22 days before heading (corresponding to the initiation stage of anther differentiation) and reached a minimum in the treatment at 11 days before heading (the tetrad phase). On the other hand, the number of aborted microspores did not show much difference among the treatments during the period from 22 to 11 days before heading. Consequently, the number of engorged pollen grains decreased with the proceeding of treatment stages, and reached a minimum in the treatment at 11 days before heading.

The number of differentiated microspores which was estimated for the control anthers was applied to those treatments later than 9 days before heading (the middle microspore phase, which is well after the end of microspore differentiation). The number of differentiated microspores in the treatment at 10 days before heading (the early microspore phase) was little less than that in the control. Abortion of microspores was greatest in the treatment at 10 days before heading and gradually decreased as the treatment was carried out at a later stage, however, it was hardly affected in the treatments later than 5 days before heading (the initiation phase of pollen engorgement). Consequently, the number of engorged pollen grains was the smallest in the treatment at the early microspore phase. Fig. 3 shows that the percentage of fertilization was closely correlated with the number of engorged pollen grains per anther through the whole data of the experiment-1 and 2(r=0.97***).

Fig. 4 shows the shapes of damaged anthers.

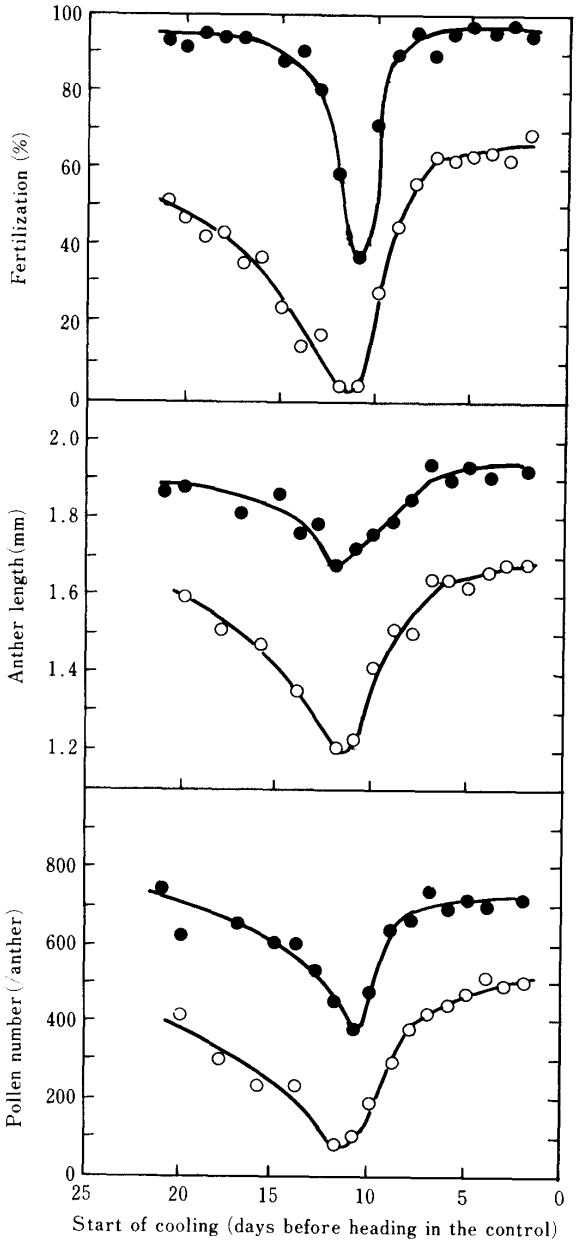

Fig. 1. Effect of cooling treatment on fertilization, anther length and the number of engorged pollen grains per anther at anthesis (experiment-1).
Cooling treatment : At 12°C for 3 days
Variety : ● : Hayayuki (cool tolerant),
○ : Norin-20 (cool susceptible).

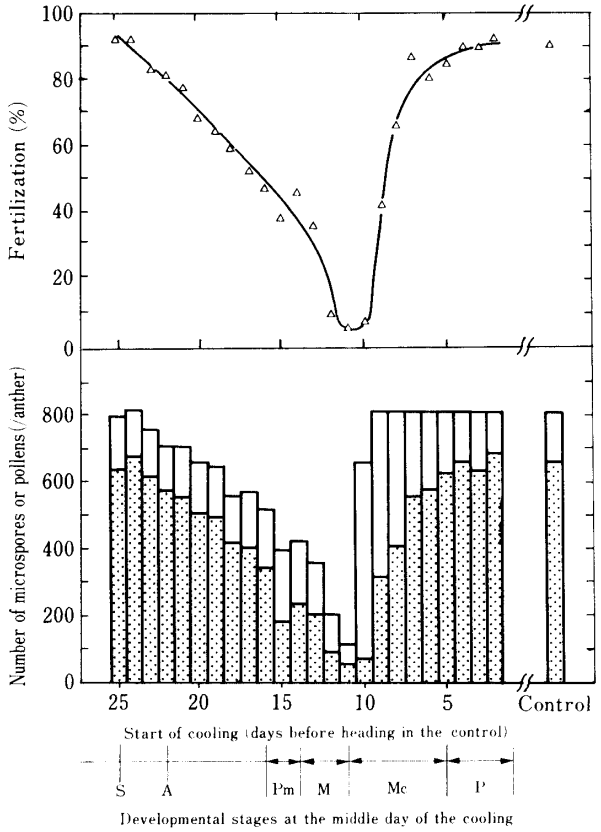

Fig. 2. Effect of cooling treatment on fertilization, the numbers of differentiated and aborted microspores and the number of engorged pollen grains per anther at anthesis (experiment-2).
Cooling treatment : At 12°C for 4 days
Variety : △ ; Norin-20 (cool susceptible)

Aborted microspores | Differentiated microspores
Engorged pollen grains |

Developmental stages at the middle day of the cooling :
S : Spikelet differentiation stage,
A : Anther differentiation stage,
Pm : Pollen mother cell formation stage,
M : Meiotic stage,
Mc : Microspore stage,
P : Pollen engorgement stage.

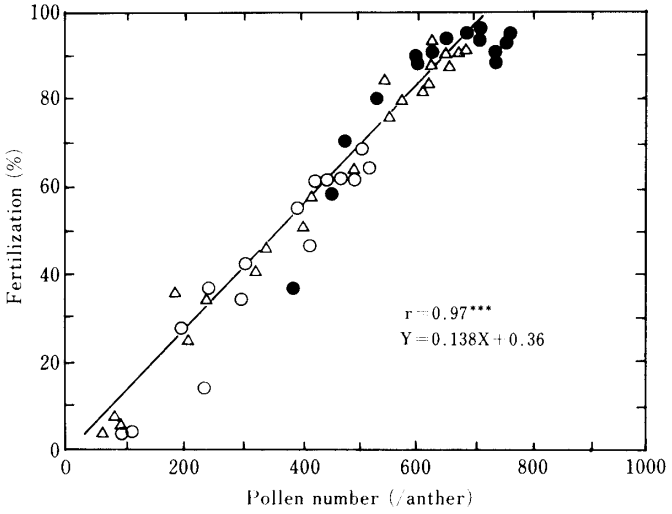

Fig. 3. Relation between fertilization and the number of engorged pollen grains per anther.
Varieties : ● : Hayayuki in experiment-1,
○ : Norin-20 in experiment-1,
△ : Norin-20 in experiment-2.

An anther is composed of four loculi : two large and two small. In normal anthers, both large and small loculi are fully developed and contain many engorged pollen grains. In the cooled anthers, the length of loculi shortened and the number of engorged pollen grains decreased. In extreme cases, loculi became flat and contain no pollen grains. The percentage of damaged loculi was distinctly larger in small loculi than in large loculi through all stages (Fig. 5). The number of engorged pollen grains per anther correlated significantly with the percentage of normal loculi.

Discussion

Nishiyama and Satake[5] demonstrated that spikelets on the upper part of a panicle are more susceptible to coolness for sterility induction at the booting stage than those on the lower part. Further, Nishiyama[4] showed that the different susceptibility to coolness among spikelets on a panicle was closely correlated with anther length and engorged pollen number per anther. Satake[9] found that enhancement in cool tolerance by raising water temperature prior to the critical stage was caused by increasing anther length and pollen number per anther. In addition to these reports, the present paper clarified that the decreased percentage of fertilization by cooling treatments at different stages of pollen development was also highly correlated with the decreased number of engorged pollen grains per anther (Fig. 3).

Calculating from the regression equation of Fig. 3, the number of engorged pollen grains per anther is 650 for obtaining 90% fertilization and 360 for 50% fertilization. These values were very close to those estimated by Nishiyama[4] and in the previous paper[9]. These results indicate that the number of

Fig. 4. Shapes of normal anther (a) and damaged anthers (b~f).
Anthers were stained with iodin-potassium iodide solution.
In b and c, only two of four loculi contain sufficient engorged pollen grains to dehisce and pollinate. No engorged pollen grains are seen in e and f.

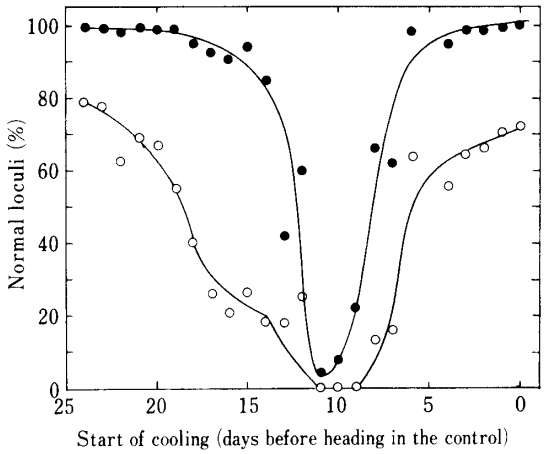

Fig. 5. Effect of cooling treatment on the development of large and small loculi.
●: Large loculus,
○: Small loculus.

engorged pollen grains per anther at anthesis is closely related to the success or failure of fertilization.

The decrease in the number of pollen grains per anther was often accompanied by the occurrence of abnormal loculi with little or no engorged pollen grains. The frequency of abnormal loculi is distinctly higher in small loculi than in large loculi (Fig. 3 and 4). Satake[7] reported that cytological abnormalities in microspores and tapeta were observed more often in small loculi than in large loculi. Nishiyama[3] also reported that small loculi were more sensitive to coolness than large loculi. The difference in cooling susceptibility between large and small loculi is a notable problem in relation to the mechanism of cooling tolerance.

Cooling treatments at different stages of pollen development lowered the percentage of fertilization through decresing the number of engorged pollen grains per anther at anthesis. As shown in Fig. 2, cooling in the period from anther differentiation to the tetrad phase decreased the number of engorged pollen grains mainly by decreasing the number of differentiated microspores, while cooling in the period from the early microspore phase to the late microspore phase decreased the number of pollen grains primarily by increasing the number of aborted microspores. These results coincide with the effects of water temperature before and during the critical stage on the differentiation and development of microspores, reported in the previous paper[9].

Cooling at the young microspore stage, which consists of the two phases of the tetrad phase and early microspore phase, caused the largest decrease in the number of engorged pollen grains, resulting in the largest decrease in the percentage of fertilized spikelets. Cooling at the tetrad phase caused the largest decrease in the number of differentiated microspores, resulting in the largest decrease in the number of engorged pollen grains. On the other hand, cooling at the early microspore phase caused the largest increase in the number of aborted microspores, resulting in the largest decrease in the number of engorged pollen grains, These results indicate that the highest susceptibility to coolness of anthers at the young microspore stage, which has been estimated as the percentage of fertilization, is caused by the high susceptibility to coolness of the differentiation and development of microspores.

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* In Japanese.

** In Japanese, the title was tentatively translated by the present author.