イネの小胞子初期冷温処理による雄性不稔 第5報

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

V. Estimations of pollen developmental stage and the most sensitive stage to coolness**

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We have clarified several facts on male sterility caused by cooling treatment at the so-called meiotic stage in rice plants. The cooling induced the immaturation of pollen grains and resulted in anther indehiscence[2]. This was the main cause of the sterility[1]. From the second contraction phase of microspores, the increasing rates of respiratory activity, and protein and free proline contents in the anthers became lower in the cooled plants than those in the control plants[5,6]. But the causal physiological changes from cooling to this phase are not yet clarified. For studying these changes, more precise studies should be made on estimations of pollen developmental stages and the most sensitive stage to coolness.

MATERIALS AND METHODS

Two varieties differing in cool hardiness were used. Hayayuki is very strong and Norin 20 weak. Every twenty seeds were directly sown in a circle to each of 1/5,000 a Wagner's pot and the plants were grown in natural light rooms of the phytotron through their life. The growing temperature conditions were 24°C day—19°C night for Hayayuki and 26°C day—19°C night for Norin 20. In rice plants grown under such favourable temperatures the percentage of fertilized spikelets was about 95% in these two varities.

In the experiments of estimating pollen developmental stage, cooling treatment was started at the meiotic stage when distance between the last two auricles (auricle distances) were —16 to—13cm in Hayayuki and—13.5 to—10.5cm in Norin 20. Coolings were made at 12°C for 4 days in Hayayuki and for 3 days in Norin 20. By these coolings, the sterility induced was 58% in Hayayuki and 67% in Norin 20. To follow the progress of pollen developmental stages in control and cooled plants, more than 6 panicles were sampled at 10 a.m. every day from the start of cooling treatment to anthesis. They were fixed by a mixture of formalin acetic acid-alcohol and stored in a refrigirated room. After 1 to 3 months' storage, panicles were washed with 50% alcohol. Spikelets examined were taken from the special location of panicles on the upper three branches excluding two apical spikelets and the secondary branches from each of them. After the measurement of palea length, pollen developmental stages were determined in preparations smeared with acetocarmine.

In the experiments of determining the most sensitive stage of spikelets to coolness, materials used were five apical spikelets on the upper three primary branches of panicles on main stems. To make cooling treatment at different stages of pollen development, panicles were selected by the auricle distances from—17 to 0 cm in these two varieties and were grouped by the auricle distances at 1cm interval. Cooling treatments were made at 12°C for 4 days in Hayayuki and for 3 days in Norin 20. For the es-

* The title of this series is revised from this report. The former title was "Male sterility caused by cooling treatment at the *meiottic stage* in rice plants".

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timation of progress of pollen developmental stage during the cooling, samplings were made for Norin 20 at the start and end of each cooling and for Hayayuki on the middle date of each cooling. Palea length and pollen developmental stages were examined at every location of spikelets on more than 30 panicles grouped by the auricle distances. The percentage of sterile spikelets was also estimated at every location of every panicle group.

RESULTS

1. Progress of pollen developmental stage.

Fig. 1 shows the progresses of auricle distances, palea and anther length, and pollen developmental stage in control and cooled rice plants. Growing rates of all these three characters were lowered by cooling treatments, but they recovered their normal rates as soon as the plants were moved back to the normal rooms. The full length of paleae was attained about the second recovering phase of micro-

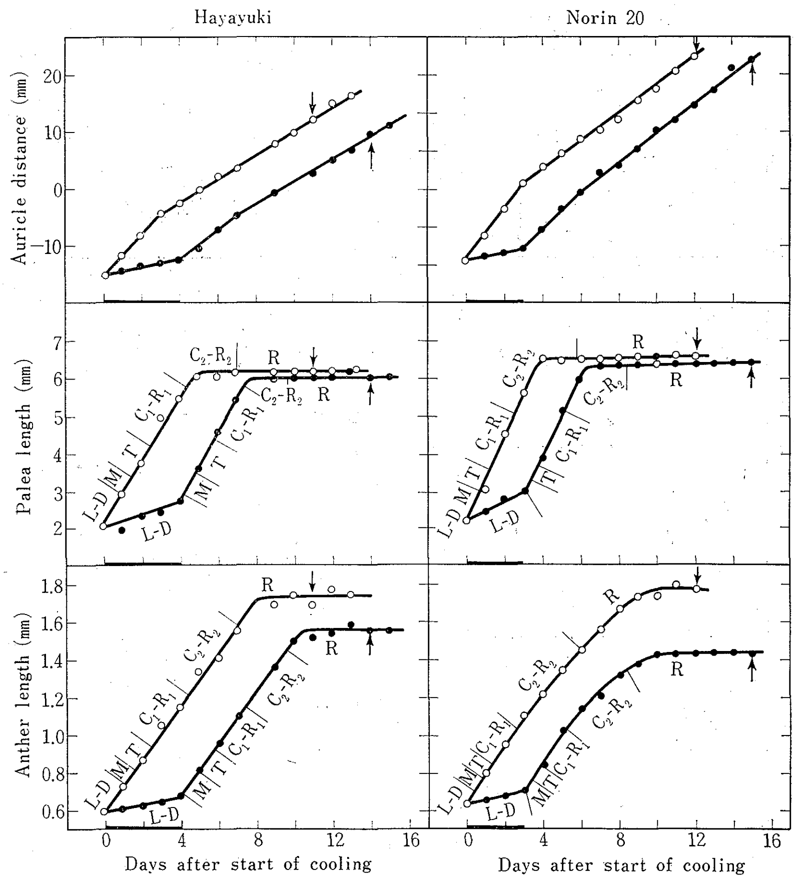

Fig. 1. Progresses of auricle distance, palea length, anther length and pollen developmental stage in the special location of spikelets.
——○—— : control. ——●—— : cooled. ———— : cooling period.
L～D : Leptotene ～ Diakinesis.
C_1～R_1 : 1 st contraction ～ recovering phese.
C_2～R_2 : 2 nd contraction ～ recovering phase.
M : 1 st and 2 nd division.
T : tetrad. ↑ : heading. R : ripe stage.

spores(6 to 7 days before heading) and those of anthers at the early ripening stage of pollen (2 to 3 days before heading). The elongation of cooled paleae proceeded normally after the treatments up to their full lengths. Full lengths of paleae were almost equal between control and cooled plants, while those of anthers were shorter in cooled plants by 10% in Hayayuki and by 20% in Norin 20 than in control plants. Such shortening of anthers appeared at the early ripening stage. From the above-mentioned facts it may be said that cooling treatment did not change the relation of pollen developmental stage to palea and anther length although it

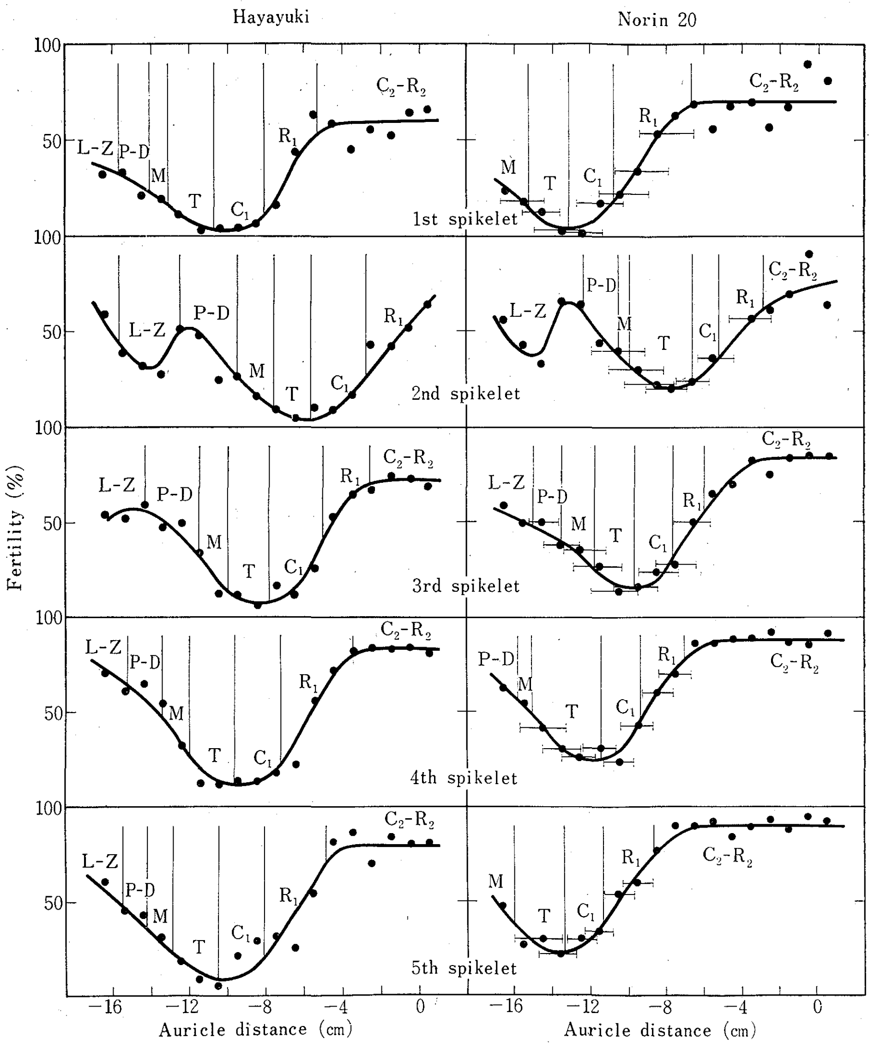

Fig. 2. Relation among fertility, auricle distance and pollen developmental stage of the five apical spikelets on the upper primary branches.

Auricle distances were measured at the start of cooling.

L~Z : leptotne ~ zygotene. C_1 : 1 st contraction phase.

P~D : pachytene ~ diakinesis. R_2 : 1 st recovering phase.

M : 1 st and 2 nd divisions. C_2 : 2 nd contraction phase.

T : tetrad ~ just before C_1. R_2 : 2 nd recovering phase.

|—●—| : the range of progress of pollen developmental stage during each treatment in Norin 20.

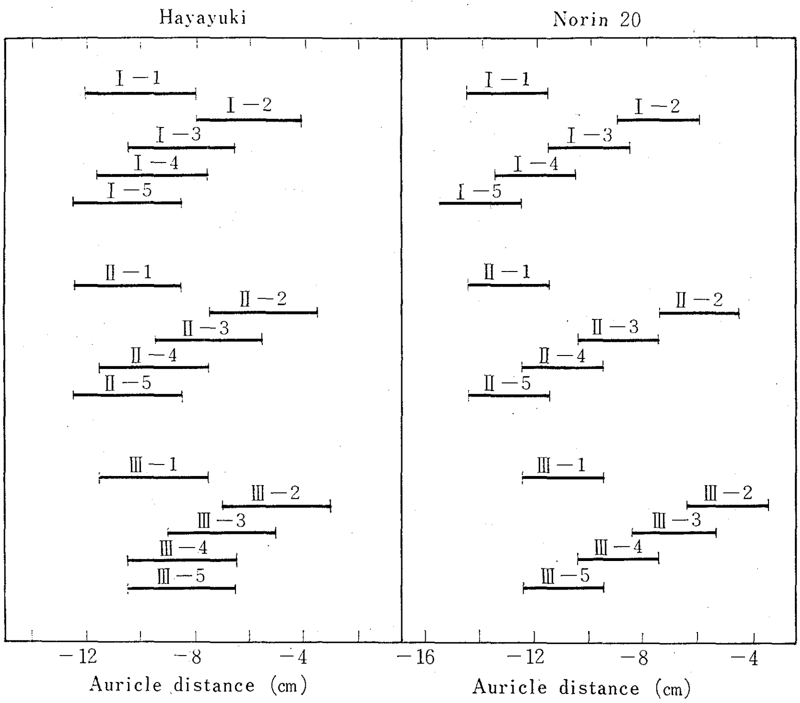

Fig. 3. The site of troughs in fertility of five apical spikelets on the three primary branches.
Auricle distances were measured at the start of cooling.
Roman figures show the order of primary branches.
Arabic figures show the location of spikelets.

lowered their growing rates.

2. The most sensitive stage to coolness.

Fig. 2 shows the relation among fertility, auricle distance and pollen developmental stage. The range of progress of pollen developmental stage during each treatment is shown. The site of trough in fertility differed with the spikelet location, but pollen developmental stage in those troughs were almost the same among them: young microspore stage from tetrad to the first contraction phase. At the second location of spikelets the secondary troughs of fertility were observed at the leptotene to zygotene stages of the meiosis in these two varieties.

Fig. 3 shows the site of troughs in fertility on five apical spikelets of the three upper primary branches. The site of troughs in fertility moved with the location of spikelets and the moving order in auricle distances coincides with the order of spikelet maturation in a panicle (1 st→5 th→4 th→3 rd→2 nd spikelets).

DISCUSSION

The auricle distance and the length of spikelets have been used to judge pollen developmental stages in rice plants[5,7]. The results of the present studies showed that these criteria are also applicable to the plants cooled at the meiotic stage. The relation of palea length to pollen developmental stage was not practically disturbed by the cooling treatment, though the growing rates of those two characters were lowered. The pollen developmental stage can be estimated from the meiosis to the second contraction phase by palea length. By using anther length as the criterion, the estimation can be extended as far as the early ripening stage. But the measurement of anther lengths required much labour and is not recommended as a practical method.

Earlier studies on the cool injury in rice plants have been made on the basis of panicle unit. The age of spikelets in a panicle, however, usually differs by more than 7 days. This large dispersion had prevented the precise analysis of the causal changes in the problem. For the purpose of reducing this dispersion, we have used the following procedures[2,6]: (1) use only main stems, (2) select synchronously growing panicles with the auricle distance, and (3) restrict the location of sampling spikelets to the upper three primary branches excluding two apical spikelets and all the spikelets on the secondary branches from each of them. The dispersion in spikelet age was reduced to less than 2 days by adopting these procedures.

Kakizaki and Kido showed for the first time that the most sensitive stage to coolness was the meiotic division stage of pollen mother cells[4]. Thereafter, many researchers have obtained the same results on the basis of a panicle unit, though Sakai noticed tapetal hypertrophy occurred during the stage from the first contraction phase to the second contraction phase of microspores[7,8]. Therefore, this idea has been accepted to be true.

In the course of our studies, we had a doubt on the exactness of these results and considered that this problem should be precisely re-examined in the light of every location of spikelets on the selected panicle groups. The present results showed that the most sensitive stage to coolness is not the meiotic division stage, but the young microspore stage from tetrad to the first contraction phase.

According to the above-mentioned results we changed the title of this series from "Male sterility caused by cooling treatment at the *meiotic stage* in rice plants" to "Male sterility caused by cooling treatment at the *young microspore stage* in rice plants" from the present paper.

The present study showed that the sites of secondary troughs in fertility were always at the second spikelets of three primary branches in the two varieties tested. This suggests the existence of the secondary sensitive stage to coolness before the meiotic division of pollen mother cells. We will investigate this suggestion in our further study.

Summary

The elongation of palea and anther and the development of the pollen much delayed during the cooling treatment, but recovered their normal rates soon after transfer to the normal warm rooms. The estimation of pollen developmental stage by palea length can be practically made both in control and cooled plants during the period from the early stage of meiosis to the second contraction phase of microspores.

To determine precisely the most sensitive stage to coolness, the dispersion in spikelet age sampled was minimized by the following procedures: (1) group uniform panicles belonging to every 1 cm range in auricle distance, (2) estimate pollen developmental stage at every location of five apical spikelets at the cooling time, and (3) compare the pollen developmental stage with the fertility at every location of spikelets.

The present studies showed that the most sensitive stage to coolness was not the meiotic stage of pollen mother cells, but the young microspore stage from tetrad to the first contraction phase.

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〔和 文 摘 要〕

イネの小胞子初期冷温処理による雄性不稔

第5報　花粉発育時期および冷温感受性のもつともたかい時期の推定

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減数分裂期に冷温処理を行ない，頴花，葯および花粉の発育経過をしらべた．これらの形質の生長速度は処理期間中いちじるしく抑制されたが，処理終了後は正常なものと同じ速度に回復した．正常なものも処理されたものも減数分裂初期から小胞子の第2収縮期までは，頴花長によつて花粉の発育時期を推定することができる．

冷温感受性のもつとも高い時期を正確に判定するため，冷温処理時に 1 cm ごとに葉耳間長の印をつけ，処理時の花粉の発育時期と稔実歩合との関係を，葉耳間長別，穂の最先端5頴花の位置別にしらべた．冷温感受性のもつともたかい時期は，これまでいわれてきた減数分裂盛期ではなく，4分子期から第1収縮期までの小胞子初期である．