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

XXIX. The mechanism of enhancement in cool tolerance by raising water temperature before the critical stage

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Abstract : Potted rice plants were grown in the phytotron with different water temperatures in a range from 18 to 25°C during the period from the spikelet differentiation stage to just before the young microspore stage (most critical stage to cool temperature), then cooled at 12°C for 3 days at the critical stage to test their cool tolerance. The cool tolerance was enhanced by raising water temperature and the enhancement in cool tolerance was closely associated with an increase in the number of engorged pollen grains per anther at anthesis. The increase in the pollen number per anther caused an increase in the number of pollen shedding on the stigma, resulting in enhancement in the percentage of fertilization. The increase in the engorged pollen grains at anthesis by raising water temperature was primarily originated from an increased differentiation of microspores. A raise of the water temperature during the critical stage also caused an increase in engorged pollen grains at anthesis. In this case, the increase in the number of pollen grains resulted from a decreased abortion of microspores. On the basis of these results, causal sequence from deep water irrigation at the booting stage to enhancement in spikelet fertility was discussed.
Key words : Cool injury, Cool tolerance, Microspore, Pollen, Rice, Sterility, Water temperature.

イネの小胞子初期冷温処理による雄性不稔　第29報 前歴水温上昇による耐冷性向上の機構：佐竹徹夫（農林水産省北海道農業試験場）

要　旨：ポットで土耕栽培のイネをファイトトロン自然光室（昼24/夜19°C）内で前歴水温（頴花分化期から小胞子初期までの水温）を変えて栽培し，小胞子初期に冷温処理（12°C3日間）を行って耐冷性を検定した．前歴水温の上昇（18°Cから25°Cの範囲で）に伴う耐冷性の向上は開花期の葯当り充実花粉数の増加と高い相関関係を示し，充実花粉数の増加は柱頭上受粉数の増加を介して受精率の向上をひき起こした．前歴水温の上昇による充実花粉数の増加は小胞子分化数の増加によるものであり，分化後花粉成熟までの過程で退化および発育不全となる小胞子数は前歴水温にほとんど影響されなかった．他方，小胞子初期の水温上昇は発育不全小胞子数を減少させることによって充実花粉数を増加させた．以上の結果に基づいて，先に提唱した前歴深水灌漑および古くから唱導されてきた危険期深水潅漑から受精率向上に至るまでの因果関係を明らかにした．
キーワード：イネ，花粉，小胞子，水温，不稔，冷温，冷害．

The most critical stage for spikelet sterility due to cool temperature is the young microspore stage[6,8]. We reported in the previous paper[9] that cool tolerance in rice at the critical stage was enhanced by raising water temperature before the critical stage. On the basis of this fact, we proposed the water management practice with a depth of 10cm during the period from the spikelet differentiation stage to the critical stage as a new countermeasure against cool injury.

The present paper reports that enhancement in cool tolerance by raising water temperature before the critical stage was caused by an increase in the number of pollen grains resulting from an increase in the number of differentiated microspores.

Materials and methods

Abbreviation

WT : water temperature, WD : water depth, p : previous period to the critical stage (from the spikelet differentiation stage to just before the young microspore stage), c : critical stage (the young microspore stage). For example, water temperature during the previous period is abbreviated as WTp, water depth during the critical stage as WDc, and so on.

Experiment 1. *Changes in spikelet fertility and floral characters with the WTp.*

A paddy rice variety Hayayuki (cool tolerant) was used. Twenty seeds were directly sown in a circular pattern in each 4-liter plastic pot and grown in the naturally lit room with day/night temperature regime of 24/19°C. Each pot was provided with 0.9g each of N, P and K. To facilitate production of uniform main culms, tillers were removed as they appeared. Only the spikelets taken from the 3rd to 5th locations on the uppermost 3 primary branches on the panicles of the main culms were sampled (the specified spikelets : 9 spikelets per panicle). Water temperature in the pot during the previous period was controlled to 4 levels of 18, 20, 22 and 25°C, with a depth of 10cm, by submerging the pot into water baths in the growth room at 24/19°C. At the young microspore stage, 3 pots from each treatment were taken out from the water bath and cooled for 3 days in the 12/12°C naturally lit room. After the cooling treatment the pots were transferred back to the 24/19°C room. Two pots from each treatment were remained in the 24/19°C room as control after taking out from the water bath.

The following treatments and estimations were applied for each of the above mentioned plots. The specified spikelets from 3 panicles were fixed with 50% ethanol at heading time. The lengths of anthers and stigmas were measured for 10 spikelets and the number of engorged pollen grains stainable with iodine-potassium iodide solution per anther was measured for 15 to 20 anthers. The specied spikelets from 15 to 20 panicles were examined for fertility. The experiment with the same design was repeated for 3 years from 1983 to 1985.

Experiment 2. *Effect of the WTp on the pollen growth, pollination and fertilization.*

Hayayuki was grown as in the previous experiment in the naturally lit room at 24/19°C until the spikelet differentiation stage. During the previous period, plants were grown under 8 different conditions in which 4 levels of the WTp (18, 20, 22 and 25°C) were combined with 2 levels of the WDp (3 and 10cm). As in the experiment-1, 3 pots from each treatment were cooled at 12/12°C for 3 days at the young microspore stage and 2 pots from each treatment were remained in the 24/19°C room as control. The following treatments and estimations were applied for each of the above mentioned plots. The specified spikelets from 3 panicles were fixed with 50% ethanol at the beginning of the middle microspore phase. The number of microspores was counted for 15 anthers. The spikelets at heading time were also fixed in the same manner and the number of engorged pollen grains was determined as an average of 15 anthers. To determine the number of pollen grains shed on the stigma, stigmas were excised from spikelets in the afternoon of the day of anthesis, stained with acetocarmine and observed under the microscope. The actual number of pollen grains per stigma was recorded up to 200 grains. Stigma with more than 200 pollen grains were recorded as 200. The number of pollen grains shed on the stigma was detemined as an average of 50 to 60 spikelets. At maturity, the specified spikelets from about 20 panicles were examined for fertility.

Experiment 3. *Effect of the WTc on the occurence of abortive microspores..*

A paddy rice variety Shimahikari (cool susceptible) was grown in the naturally lit room with day/night temperature regime of 25/19°C. Water temperature in the pots dur-

Table 1. Changes in spikelet fertility and floral characters with different WTp.

WTp	Fertility		Fertility index	Stigma length		Anther length		Number of pollen/anther	
	C	T		C	T	C	T	C	T
°C	%	%	%	mm	mm	mm	mm		
25	98	89	85	1.13	1.08	2.22	2.12	1,182	1,039
22	97	72	71	1.14	1.04	2.00	1.80	825	563
20	78	29	49	1.08	1.05	1.79	1.63	496	223
18	23	1	11	1.08	1.08	1.61	1.31	238	36

C : Control(not cooled), T : Cooled(12°C 3days).
Each figure in the table is an average of 3 determinations from 1983 to 1985.

ing the previous period was controlled to 2 levels of 25 and 22°C, at a depth of 10cm. Plants were divided into 6 groups at the critical stage and 5 groups of them were transferrerd to the other water baths controlled at 12, 15, 17, 19 and 21°C, respectively. The water level of these baths was kept at 20cm for the purpose of regulating the temperature around the specified spikelets at the water temperatures. After 5 days, plants were taken out from the water baths and grown in the control room at 25/19°C until seed ripening. Plants not submerged in the water bath were regarded as control. The numbers of microspores and pollens per anther were determined in the same manner as described above.

Results

The percentage of fertility decreased with a fall in the WTp in each plot of the control and the cooled (Table-1). Fertility index was calculated acording to the following equation to compare the degree of cool tolerance among plants grown at the different WTp.

$$\text{Fertility index } (\%) = \frac{\arcsin\sqrt{\text{Fertility}(\%) \text{ in the cooled}}}{\arcsin\sqrt{\text{Fertility}(\%) \text{ in the control}}} \times 100$$

The fertility index decreased with falling WTp. Anther length and the number of engorged pollen grains per anther decreased with falling WTp and also by the cooling treatment at the young microspore stage, while stigma length was hardly affected by these treatments. Highly positive correlation ($r = 0.97^{**}$) was observed between the anther length and the number of engorged pollen grains per anther. Spikelet fertility increased with an increase in the number of pollen grains per anther, and it was a little higher in the control than in the cooled even when the number of pollen grains per anther was equal (Fig.1). From Fig.1, the number of pollen grains for obtaining 90% fertility was estimated at about 620/anther in the control and around 1000/anther in the cooled.

Fig.2 and Fig.3 show the mutual relations among the pollen number per anther, pollen shedding and spikelet fertility. When more pollen grains were produced in an anther by raising WTp, more pollen grains shed on the stigma (Fig.2) and this resulted in the increase of spikelet fertility (Fig.3). The number of

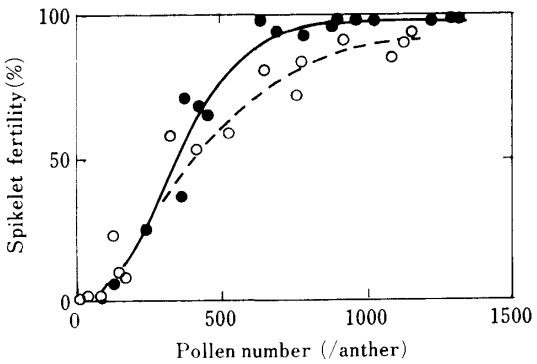

Fig. 1. Relation between spikelet fertility and the number of pollen grains per anther.
—●— : Control
···○··· : Cooled

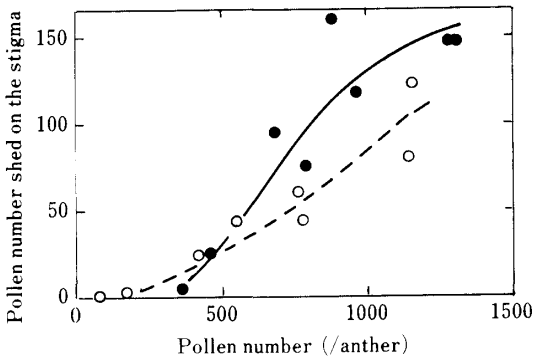

Fig. 2. Relation between the number of pollen grains shed on the stigma and the number of pollen grains per anther.
—●— : Control
···○··· : Cooled

pollen grains shed on the stigma was larger in the control than in the cooled even when the number of pollen grains in an anther was equal (Fig.2). In addition, the percentage of spikelet fertility was higher in the control than in the cooled even when the same number of pollen grains were shed on the stigma (Fig.3). As a result, the fertility index was closely correlated with the number of engorged pollen grains per anther in the control plants (Fig.4), as well as in the cooled plants.

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[6]. Thus, the difference in the numbers between microspores at the beginning of middle microspore

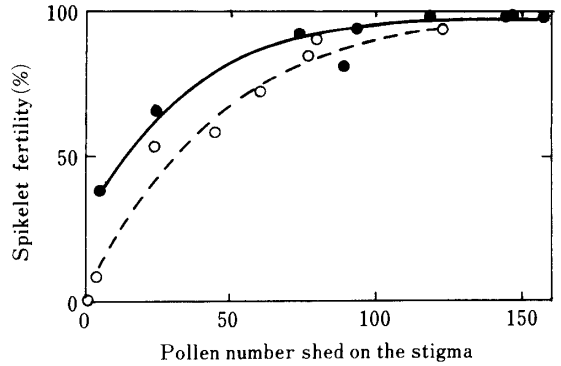

Fig. 3. Relation between spikelet fertility and the number of pollen grains shed on the stigma.
—●— : Control
--○-- : Cooled

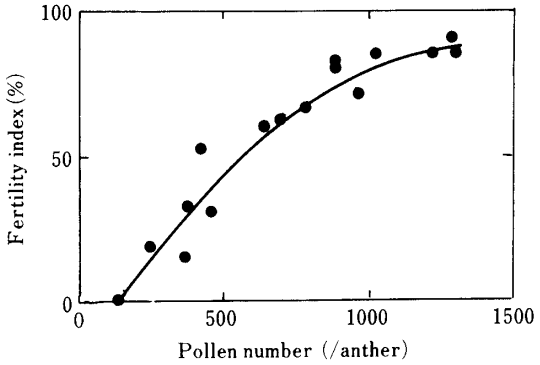

Fig. 4. Relation between the fertility index and the number of pollen grains per anther in the control.

phase and engorged pollen grains at anthesis was taken as the number of aborted or degenerated microspores on the way of pollen maturation. Fig.5 shows the number of differentiated and aborted microspores in plants grown at the different WTp. The number of differentiated microspores increased with rising WTp ; for example, the number of differentiated microspores per anther in the plant grown at 25°C-WTp (1460/anther) exceeded two times that in the plant grown at 20°C-WTp (690/anther) and the difference of the actual number between them reached around 770 per anther. The number of aborted microspores in the control plants was negligible compared with the number of differentiated microspores (Fig.5). Aborted microspores increased by the cooling treatment, however, the actual number of them was not much different among the WTp treatments (Fig.5). Consequently, variation in the

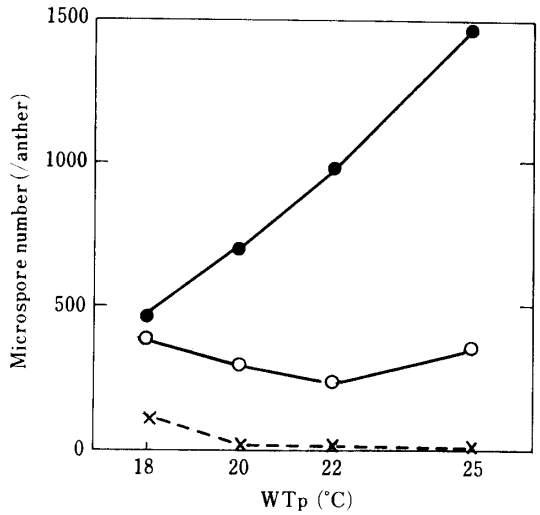

Fig. 5. The number of differentiated micorespores in plants grown under different WTp and the number of aborted microspores caused by cooling treatment at the critical stage.
—●— : Differentiated microspores in the control.
—○— : Aborted microspores in the cooled.
× : Aborted microspores in the control.

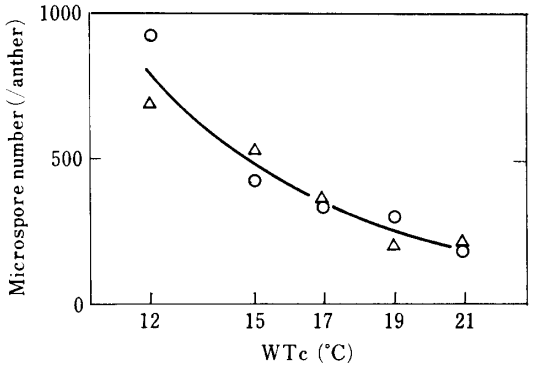

Fig. 6. Changes in the number of aborted microspores by the WTc treatment.
—○— : Plants grown at 25°C-WTp
—△— : Plants grown at 22°C-WTp

number of engorged pollen grains with the WTp depended mainly on the difference in the number of differentiated microspores per anther.

The number of aborted microspores increased with falling WTc and this resulted in the decrease of number of engorged pollen grains at anthesis (Fig.6). The number of aborted microspores caused by the WTc treatment was not greatly different between the plants grown at 25°C-WTp and 22°C-WTp, although the number of differentiated mi-

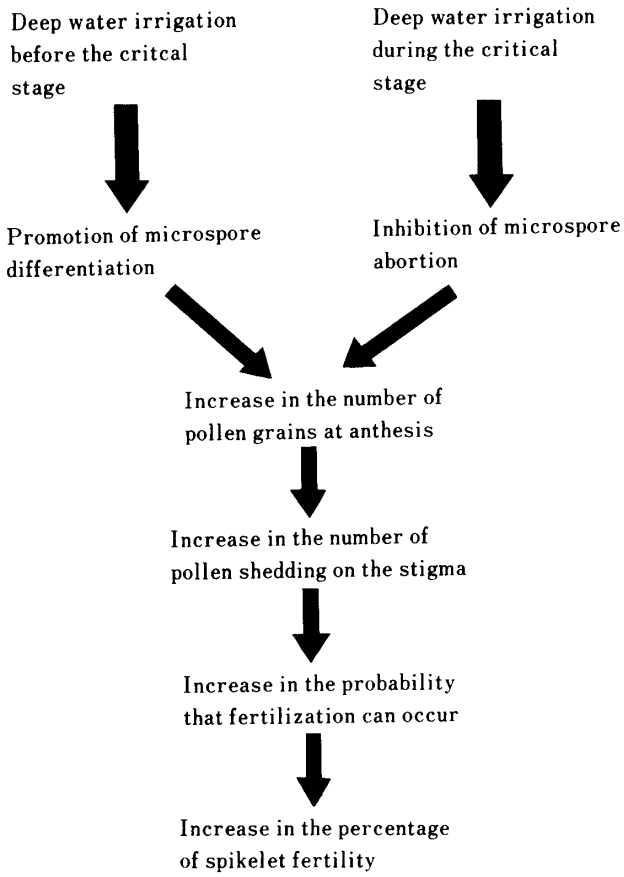

Fig. 7. Causal sequence from deep water irrigation at the booting stage to increase in spikelet fertility.

crospores was significantly larger in the plants grown at 25°C-WTp (1090/anther) than at 22°C-WTp (790/anther). This result indicates again that changes in the number of engorged pollen grains with the WTp depends mainly on changes in the number of differentiated microspores.

Discussion

Hashimoto[1] reported for the first time in 1961 a positive correlation between anther length and cool tolerance at the booting stage among rice varieties. His results were recently reconfirmed by several researchers[2,7,10,11] who revealed that cool tolerance was closely correlated with the number of pollen grains per anther and anther length but not with stigma length. Nishiyama and Satake[4] demonstrated that the spikelets on the upper part of the panicle are most susceptible to coolness for sterility induction at the booting stage than those on the lower part. Nishiyama[3] clarified that the difference in susceptibility among spikelets on a panicle was significantly correlated with anther length and pollen number per anther. In addition to these reports, enhancement in cool tolerance by raising WTp also closely associated with increases of anther length and pollen number per anther (Table-1, Fig.5). These results clearly indicate that cool tolerance for sterility is closely related to the number of engorged pollen grains per anther at anthesis. The reason can be explained as follows : if a large number of pollen grains are produced in an anther the number of pollens shed on the stigma increases (Fig. 2), and if a large number of pollen grains are shed on the stigma fertilization can occur with a higher probability (Fig.3).

The number of engorged pollen grains at anthesis is the difference between the numbers of differentiated and aborted microspores. A rise in the WTp promoted microspore differentiation resulting in an increase of pollen number (Fig.5), while a rise in the WTc inhibited microspore abortion resulting in an increase of pollen number (Fig.6). These results are understandable because the period of WTp treatment includes the stages of pollen mother cell differentiation to tetrad, while the WTc treatment starts at the young microspore stage just after the end of microspore differentiation. Deep water irrigation during the booting stage as a countermeasure against cool injury is affected by water temperature which is generally 3-4°C higher in daily average than air temperature[5,9]. Considering the results described above, causal sequense from deep water irrigation to enhancement in spikelet fertility is summerized in Fig.7.

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** In Japanese, the titile was tentatively translated by the present author.
*** In Japanese with an English summary.