High Temperature at Flowering Inhibits Swelling of Pollen Grains, a Driving Force for Thecae Dehiscence in Rice (*Oryza sativa* L.)

Tsutomu Matsui, Kenji Omasa* and Takeshi Horie**

*(Experimental Farm, Kyoto University, Takatsuki, Osaka 569-0006, Japan; *Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan; **Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan)*

**Abstract**: To clarify the mechanism of high temperature-induced floret sterility in rice (*Oryza sativa* L.), we studied the effects of high temperature at flowering on the ability of thecae to dehisce and on pollen-grain swelling which causes thecae dehiscence. Two japonica rice cultivars, grown in four L pots under submerged soil conditions were subjected to high (39°C) and moderate (34°C) temperatures from 10:00 to 16:00 for three consecutive days at the flowering stage. The percentage of the thecae dehisced in response to artificial opening of the florets by removing lemma and the mean diameter of the pollen grains measured during four minutes after the artificial opening were examined to determine the ability of the thecae to dehisce and that of the pollen grains to swell, respectively. The high temperature given on the day of flowering decreased both the percentage of the dehisced thecae and the diameter of the pollen grains but did not affect the relationship between them. The thecae of the plants which had been subjected to the high temperature for one or two days before flowering, however, showed a lower dehiscence percentage than those of the plant treated on the day of flowering against the same pollen-grain diameter. We concluded that high temperature given on the day of flowering decreased the ability of the pollen grains to swell resulting in poor theca dehiscence, and that, besides this decrease, high temperature given just before the days of flowering lowered the function of thecae themselves to dehisce, causing poorer theca dehiscence.

**Key words**: Floret sterility, High temperature, *Oryza sativa* L., Pollen grain swelling, Pollination, Rice, Theca dehiscence.

Increasing concentration of greenhouse-effect gas in the atmosphere may have a significant impact on crop productivity by increasing both the surface temperature and the amount of carbon available for photosynthesis. The effects of the global environmental change on rice yield have been studied using simulation models (Bachelet and Gay, 1993; Horie, 1993; Horie et al., 1996). In particular, Horie et al. (1996) showed that suboptimal temperatures negate the positive effect of elevated CO₂ on the rice yield and fluctuates the yield mainly through high temperature-induced florets sterility even in a temperate region.

Various experiments and observations show that a day temperature of over 35°C at the flowering stage induces the floret sterility in rice (Osada et al., 1973; Satake and Yoshida, 1978; Kim et al., 1996; Matsui et al., 1997a,b; Matsui et al., 2000). The main cause of the high-temperature-induced sterility is the failure of the pollination caused by the poor theca dehiscence at flowering (Sato et al., 1973; Satake and Yoshida, 1978; Matsui et al., 1997a;b; Matsui et al., 2000). However, how the high temperature induces the poor theca dehiscence is unknown.

We previously reported that the driving force for the theca dehiscence in rice is the rapid swelling of the pollen grains (Matsui et al., 1999a,b), and assessed the heat tolerance of japonica-rice cultivars (Matsui et al., 2000). In this paper, we report the effect of high temperature on the swelling of pollen grains and the anther dehiscence in high-temperature-tolerant and susceptible cultivars, using the property of rice that the floret opening triggers the process of the pollen swelling and that the theca dehiscence is driven by the pollen swelling. We also discuss the mechanism of the high-temperature-induced sterility and the high-temperature tolerance.

**Materials and Methods**

1. **Plant materials and treatment**

Two japonica rice cultivars, Hinohikari (heat susceptible) and Nipponbare (heat tolerant) were used. Seedlings at the 5.2 leaf stage were transplanted in a circular pattern into four L pots, 20 seedlings per pot, on June 15 for Hinohikari and on June 25 for Nipponbare and grown under submerged soil conditions outdoors (Tsukuba, Japan). The heading of both cultivars were around August 25. Each pot was provided with 0.5 g each of N, P₂O₅ and K₂O as a top-dressing at about 45 days before heading. Tillers were removed during the vegetative stage when they appeared.

Two sun-lit phytotrons were used for the temperature treatments. Each chamber was assigned one of two different day temperatures (10:00-16:00), 34°C (mod-
erate temperature) or 39°C (high temperature). Night temperature (18:00-8:00) was 26°C, and air humidity was 60 (day) and 80 (night) % R.H. in both chambers. Air temperature and humidity from 8:00 to 10:00 and from 16:00 to 18:00 were linearly changed. The plants at the mid-heading stage were treated for three consecutive days late in August. Treatments started and ended at 18:00.

2. Experimental procedure

The florets expected to open next were marked at 18:00 every day during the treatment period. Ten marked florets were detached from the plants by cutting rachis-branch several times from 7:00 to just before natural flowering from both chambers every day during the treatments. Immediately after the detachment, the florets were artificially opened by removing lemma and maintained under the condition of 25°C, 75% R.H. Five florets were used for the measurement of the mean pollen grain diameters for four minutes after the artificial opening. The diameters of two pollen grains from an anther were measured under a stereomicroscope about every eight seconds (60 pollen grains from 30 anthers in total). The remaining five florets were used for the examination of percentage of the thecae dehisced in response to the artificial opening. The percentage of the dehisced thecae was examined five hours after the opening of florets.

After flowering, 21 florets per treatment were sampled to count the number of pollen grains deposited on the stigmas. The number of pollen grains on each stigma was counted under a microscope after staining with cotton blue.

Results

In both cultivars, the percentage of thecae dehisced in response to the artificial opening of the florets just before the time of flowering was lower in the plants exposed to a high temperature than those exposed to the moderate temperature, and the percentage decreased with the increase in the days exposed to a high temperature before artificial floret opening (Fig. 1). Moreover, the percentage in Hinohikari was lower than that in Nipponbare in both temperature conditions. The percentage of the dehisced thecae was significantly correlated with the number of the pollen grains deposited on the stigma (Fig. 2).

The mean diameter of the pollen grains measured for four minutes after the artificial opening of the florets just before the flowering time under the high-temperature condition was lower than that under the moderate-temperature condition during the treatment (Fig. 3). The effect of the duration in days of the high-temperature treatment on the pollen diameter was unclear. The high-temperature treatment decreased the diameter by 4.30 ± 0.22% (mean of the values measured on three days ± SE) in Hinohikari and by 2.88 ± 0.67% in Nipponbare.

The percentages of the dehisced thecae determined several times from early morning to just before the time of flowering every day during the treatments was plotted.
against the diameter of the pollen grains (Fig. 4). In both cultivars, the relationship between the diameter and the percentage of the dehisced thecae on the first day of the high-temperature treatment was similar to that under the moderate-temperature condition. In contrast, the percentage of dehisced thecae against the same pollen-grain diameter on the second and third days of the high temperature treatment were lower than that under the moderate temperature condition.

Under the moderate-temperature condition and at the first day under the high-temperature condition, the thecae of two cultivars began to dehisce when the diameter of the pollen grain was around 48.5 µm. The percentage of dehisced thecae under these conditions in Nipponbare increased rapidly with the increase of pollen grain diameter and reached the maximum value of almost 100% at about 49 µm in the pollen grain diameter. In contrast, the percentage increased gradually and reached the maximum value of about 80% at around 50.5 µm in the pollen grain diameter in Hinohikari under the same conditions. Such slow increase in the percentage of dehisced thecae plotted against the pollen diameter in Hinohikari was also observed on the second and third day under the high temperature condition.

**Discussion**

The artificial opening of florets triggers the pollen-grain swelling and the thecae dehiscence as in natural opening (Matsui et al., 1999a,b). The diameter of pollen grains just before natural flowering increase to the maximum within a few minutes after the artificial floret opening, and maintained the maximum level for over 20 minutes (Matsui et al., 1997a). The pollen grains early in the morning repeat swelling and contraction after the artificial floret opening for periods of a few minutes and the maximum value of the pollen grain diameter is smaller than that just before flowering time (Matsui et al., 1999a). The swelling of pollen grains is the driving force for the opening of thecae (Matsui et al., 1999a, b). Therefore, the mean diameter of pollen grains measured for four minutes after the artificial floret opening and the percentage of dehisced thecae in response to the artificial opening indicate the ability of pollen grains to swell and the thecae to dehisce at the time of the artificial floret opening.

A number of observations suggested that the indehiscence of thecae is the direct cause of poor pollination.
induced by high temperature at flowering, especially in high-temperature-susceptible cultivars (Sato et al., 1973; Satake and Yoshida, 1978; Matsui et al., 1997b). In the present experiment, the high temperature lowered the percentage of the dehisced thecae and the percentage was positively correlated with the number of pollen grains deposited on the stigmas. Considering the uncertainties of the anther dehiscence under the stressed condition, these results suggest that the poor pollination was caused by the poor thecae dehiscence induced by high temperature, supporting the above ideas.

In the present experiment, the percentages of the dehisced thecae under the moderate temperature condition and on the first day of the high-temperature treatment were plotted on nearly the same line against the diameter of the pollen grains. This means that the high temperature on the day of flowering had no effect on the relationship between the pollen diameter and the percentage of the dehisced thecae. The main cause of the poor dehiscence caused by the high temperature on the day of flowering will be the insufficient swelling of the pollen grains. Since the floret is the most sensitive to high temperature on the day it flowers (Satake and Yoshida, 1978; Matsui et al., 1992), the insufficient swelling of the pollen grains would be the main cause of the high-temperature-induced sterility.

On the other hand, the percentage of dehisced thecae decreased with the increase in the days of exposure to the high temperature before artificial floret opening. This is inconsistent with the report of Satake and Yoshida (1978) who showed that the sterility is induced by the high temperature given on the flowering day, but not on other days in indica rice. However, the present result is in agreement with the reports of Nabeshima et al. (1988), Sato et al. (1973), and Matsui et al. (1992) who used japonica rice. There may be a difference between indica and japonica rice in the length of period susceptible to high temperature.

In contrast to the percentage of the dehisced thecae, the swelling of pollen grains was not affected by the high temperature given before the day of flowering. The percentage of dehiscence plotted against the pollen grain diameter of the thecae subjected to the high temperature from one or two days before flowering (2nd and 3rd day of the high-temperature treatment) was, therefore, lower than those which were not subjected to high temperature before the flowering (Fig. 4). These results suggest that the high temperature before the day of flowering disturbs the function of the thecae itself for the dehiscence rather than the swelling of pollen grains. For example, Keijzer et al. (1996) reported that the cell wall lysis occurs in septum and stamium (dehiscent slk in the future) from three days to one day before anthesis in maize, and that either artificially dehydrated or over-hydrated premature anthers with the closed septum never rupture the septum. This indicates that the thecae and pollen cannot generate the force to open the septum without the lysis.

The cell wall lysis in the septum and the stomium is one of the indispensable process for the thecae dehiscence. The high-temperature treatment decreased the diameter of pollen grains at the flowering time by 4.3% in Hinohikari and by 2.9% in Nipponbare, suggesting that Nipponbare is more tolerant than Hinohikari to a high temperature in the pollen-grain swelling. Furthermore, the rate of increase in the percentage of the dehisced thecae against the pollen-grain diameter in Nipponbare was higher than that in Hinohikari, although there was little difference in the pollen-grain diameter at which the thecae began to dehisce. Moreover, the maximum percentage of the dehisced thecae in Hinohikari was about 80% while that in Nipponbare was almost 100%. These results suggest that in Hinohikari the thecae are heterogeneous in the function for the dehiscence, some of them being indehiscent irrespective of the pollen grain size. The easy and homogeneous dehiscent characteristic of the thecae would be a factor causing the high-temperature tolerance in Nipponbare. The fact that the percentage of the dehisced thecae in Nipponbare induced at the floret opening just before the time of flowering was higher than that in Hinohikari in both temperature conditions supports the theory that the well-dehiscent characteristic of the thecae themselves is a cause of the high-temperature tolerance. Mackill et al. (1982) reported that the number of pollen grains per stigma at flowering time under the control temperature is highly associated with the fertility at high temperatures. Their data may also show that the well dehiscent characteristic of the thecae increases the fertility percentage under the high-temperature condition.

On the other hand, Satake and Yoshida (1978) showed that even day temperature of 32°C affects the fertility of the high-temperature-susceptible indica-cultivar. Therefore, we can not exclude the possibility that the day temperature of 34°C set as the control affected the faculty of the anther to dehisce, although the midday temperature in Tsukuba is commonly 34°C in late August where the materials were grown. The similarity between the solid line in Fig. 4A and the broken line in B may imply that the anther of Hinohikari was potentially affected by the high temperature in the outdoor and the control conditions.

In conclusion, (1) the high-temperature-induced floret sterility is mainly caused by the poor swelling of pollen grains due to the high temperature on the day of flowering; (2) the high temperature given before the flowering day reduces the function of the thecae to dehisce rather than reduce the swelling of the pollen grains; (3) the successful pollination under high-temperature conditions in Nipponbare may be attributed to the easy and homogeneous dehiscent characteristic of the thecae and to the high-temperature tolerance in pollen grain swelling.
References


Matsui, T., Omasa, K. and Horie, T. 2000. The difference in sterility due to high temperature during the flowering period among japonica-rice cultivars. (in submission to PPS)


*In Japanese.

**In Japanese with English abstract.