Percentage of Dehisced Thecae and Length of Dehiscence Control Pollination Stability of Rice Cultivars at High Temperatures

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Abstract: Global warming may reduce rice yield through poor pollination caused by high temperatures at flowering. The dominant parameter controlling the pollination stability in rice cultivars at high temperatures was studied. We examined the effects of a high daytime temperature (35.0ºC, 37.5ºC, 40.0ºC) and its duration (1, 3, 5 days) on the percentage of dehisced thecae, the length of dehiscence in the basal part of the theca for pollen dispersal, and pollination stability. The percentage of sufficiently pollinated florets (%SPF) decreased with the increase in daytime temperature and the duration of treatment. At a daytime temperature of 37.5ºC, %SPF varied widely among the cultivars and was highly correlated with the length of dehiscence formed at the basal part of the theca (r = 0.930, P < 0.01, n = 6) and the percentage of dehisced thecae (r = 0.868, P < 0.05, n = 6). The factor that better explained the variation in %SPF shifted from the length of the basal dehiscence to the percentage of dehisced thecae with increasing duration of high-temperature treatment. Thus, the process preventing pollination shifted from pollen release to anther dehiscence with the increase of exposure to a high temperature.

Key words: Anther dehiscence, Global warming, Heat tolerance, High temperature, Pollination, Rice (Oryza sativa L.)

Crop simulation models project that global warming may increase the occurrence of floret sterility in rice, reducing yields even in temperate parts of Asia, such as Japan (Horie et al., 1996; Nakagawa et al., 2003). Indeed, the rice yield in the Yangtze River region of China was seriously reduced because of floret sterility in the summer of 2003, when the daily maximum temperature exceeded 39ºC during the flowering period (Wang et al., 2004). Thus, high-temperature-induced floret sterility is a pressing issue in Asian rice production.

The adoption of cultivars tolerant to high temperatures is a most effective countermeasure to maintain stable, high rice production in Asia, even under the anticipated climate change (Horie et al., 1996; Nakagawa et al., 2003). An improvement in the high-temperature tolerance of floret fertility by 1.5ºC would markedly mitigate the negative effects of climate change (Horie et al., 1996; Nakagawa et al., 2003).

The mechanism controlling the tolerance of rice to high temperatures needs to be identified. Rice plants tolerant to high-temperature-induced floret sterility have the ability to maintain sufficiently high levels of successful pollination to produce a high seed set at high temperatures during flowering (Satake and Yoshida, 1978; Matsui et al., 2001; Tian et al., 2010). Pollination stability seems to be determined by three processes in pollination.

The first process is septum rupture, which causes stomium cracking (Matsui et al., 1999). The rupture determines whether the anther dehisces or not (Matsui et al., 1999). Satake and Yoshida (1978) reported that the anthers of a heat-tolerant rice cultivar dehisced at the beginning of floret opening, while those of a heat-intolerant cultivar either dehisced late or failed to dehisce at a high temperature. Morphological studies of rice anthers suggest that some intolerant cultivars possess an apparently strong anther septum (Matsui and Omasa, 2002), supporting the theory that the process of septum rupture is involved in tolerance. Therefore, the difference among cultivars in the percentage of dehisced thecae would represent the difference in fragility of the anther septa at dehiscence.

The second process is the release of pollen grains from the dehisced anther. On septum rupture and stomium cracking, pollen grains are held in the thecae until the
stomium crack widens, when they are released. Pollination of cultivars with long basal dehiscence of the anther was found to be stable (Matsui and Kagata, 2003), even under hot conditions (Matsui et al., 2005), and the seed-set percentages were high under those conditions (Tian et al., 2010). These findings suggest that large basal dehiscence helps to release pollen grains from the anthers and promotes stable pollination at high temperatures. A high temperature at flowering makes the pollen grains sticky (Satake and Yoshida, 1978; Matsui, 2005), which may also affect pollen release.

The third process is the transport of pollen from anther to stigma. The number of pollen grains that reach the stigma is determined by the number released and by transport efficiency, which may be affected by many factors (Fægri and van der Pijl, 1979), such as the size of the stigma (Matsui and Kagata, 2003), the distance between anther and stigma, their relative positions, and the microclimate around the florets.

The purpose of this study was to determine the dominant parameter controlling variation among rice cultivars in pollination stability at high temperatures. We examined the percentage of dehisced thecae, the length of dehiscence, and pollination stability under different durations of high-temperature treatment. The stickiness of pollen grains and the factors controlling pollen transport that may also affect the pollination stability at high temperatures were not examined due to difficulty in measurement and quantification. In contrast, the dehiscence of anther is visible and thus suitable for breeding.

Materials and Methods

1. Plant materials and treatment

Six rice cultivars with great differences in the length of dehiscence in the basal part of the thecae under normal conditions (Matsui et al., 2005) were used (Table 1). Koshihikari, Nipponbare, and IR72 are leading cultivars in Japan and Southeast Asia. Takanari, IR65564-44-2-2 (referred to as a New Plant Type: NPT), and WAB450-1-B-P-38-HB (referred to as WAB) were bred as high-yielding cultivars at the National Institute of Crop Science (Japan), the International Rice Research Institute (the Philippines), and the Africa Rice Center (Côte d’Ivoire), respectively.

Surface-sterilized seeds were germinated at 32°C for 24 h. Uniformly germinated seeds, 20 seeds per pot, were sown in pots (20 cm in height, 15 cm in diameter) containing soil equivalent to 3.6 kg dry weight (air-dried Andosol and a granitic saprolite Cambisol mixture, 1:1 by volume) on 17 March 2003, using the circular dense-culture method (Satake, 1972).

Plants were grown outdoors before the start of treatment. Koshihikari and Nipponbare were grown under a 16-hr daylength supplemented with incandescent lamps for sufficient vegetative growth. The pots were watered to field capacity for 10 days after sowing, and later kept flooded to a depth of 2−3 cm. Liquid fertilizer was applied every week until the start of treatment: 0.075 g N, 0.046 g K, and 0.033 g P at the first application, and 0.15 g N, 0.092 g K, and 0.065 g P subsequently. The tillers that appeared during the vegetative stage were removed to obtain uniform plants. The panicles emerged from early July to late August.

| Cultivar     | Ecotype                | Origin          |
|--------------|------------------------|-----------------|
| Koshihikari  | japonica               | Japan           |
| Takanari     | indica                 | Japan           |
| NPT*         | indica × tropical japonica | Philippines  |
| Nipponbare   | japonica               | Japan           |
| IR72         | indica                 | Philippines     |
| WAB**        | O. glaberrima × tropical japonica | Côte d’Ivoire |

* IR65564-44-2-2 (New Plant Type).
** WAB450-1-B-P-38-HB.
2. High-temperature treatments

When about 25% to 35% of plants had headed, 12 pots of each cultivar were randomly divided into three groups of four pots each. Growth chambers with artificial light (high-pressure sodium lamps and metal halide lamps) were used for high-temperature treatments. Each group was exposed to 35.0, 37.5, or 40.0ºC for 8 hr (0830−1630) a day over 5 consecutive days (Fig. 1). Daylength was 12 hr (0600−1800, 330 µmol m⁻² s⁻¹) and the night (1900−0600) temperature was 25.0ºC in all treatments. Relative humidity was 60% in the high-temperature period and 80% in the low-temperature period. Air temperature and humidity from 0600 to 0830 and from 1630 to 1800 were changed in steps (Fig. 1). All cultivars flowered during the high-temperature period from 0830 to 1630 at all daytime temperature conditions.

3. Sampling and measurements

To examine pollination stability, we sampled more than 10 florets that had completed flowering from the primary branches in each pot (>40 florets per group) at 1600 h on days 1, 3, and 5 of treatment. Stigmata were extracted from the florets and stained with cotton blue, and pollen grains on them were counted under an optical microscope.

To examine the percentage of dehisced thecae and the length of basal dehiscence, we sampled five florets from five plants in each group at 1600 on days 1, 3, and 5 of treatment in each group. Then, we counted the indehisced thecae and measured the length of dehiscence in the basal part of dehisced thecae under a digital microscope (VH-5000; Keyence Corporation, Osaka, Japan).

4. Data analysis

The percentage of florets having ≥20 pollen grains on the pair of stigmata in each treatment was calculated for each cultivar. Since many florets with ≥20 pollen grains on the stigma after anthesis can become fertile even at 38.0°C (Satake and Yoshida, 1978) or 37.5°C (Matsui et al., 2001), at which temperatures poor pollination is the primary cause of sterility, the percentage of florets having ≥20 pollen grains indicates the reliability of pollination, and is called here the "percentage of sufficiently pollinated florets" (%SPF).

We conducted a three-way analysis of variance to examine the effects of daytime temperature, duration of high-temperature treatment, and cultivar on the length of dehiscence in the basal part of the thecae, the percentage of dehisced thecae, and %SPF. Differences between the mean values of samples were analyzed by Tukey’s HSD test at P=0.05. Regression analysis was conducted to examine the relationships among the anther characteristics and the reliability of pollination.

Results

1. Effects of high-temperature treatments on %SPF

Cultivar (P<0.001), daytime temperature (P<0.001), and their interaction (P<0.05) significantly affected the percentage of florets having ≥20 pollen grains on the stigma (%SPF). The mean %SPF in Koshihikari was significantly higher than that in NPT, Nipponbare, IR72, and WAB, and that of WAB was significantly lower than that of Koshihikari, Takanari, and NPT (Table 2). %SPF decreased as the daytime temperature increased (Table 2). The range of %SPF among the cultivars was greatest at 37.5°C (Table 2). Even at 35.0°C, values of %SPF in all cultivars except Koshihikari were <80%, and the difference among cultivars was large (Table 2). By contrast, significant differences in %SPF were not detected among the cultivars at 40.0°C (Table 2).

The effect of duration of treatment on %SPF was
significant ($P<0.01$), but the interaction between duration and cultivar was not ($P=0.062$). The mean %SPF across the three temperatures was significantly greater on day 1 than on days 3 ($P<0.01$) and 5 ($P<0.0001$) (Table 3).

2. Effects of high-temperature treatment on thecal dehiscence

Cultivar, daytime temperature, and their interaction significantly affected the percentage of dehisced thecae ($P<0.001$). The mean percentages of dehisced thecae were significantly higher in Koshihikari, NPT, and Takanari than in IR72 and WAB across all temperatures (Table 4). The mean percentage of dehisced thecae across all cultivars decreased with an increase in temperature (Table 4). At 35.0°C, the values in all cultivars except WAB were >95%. At 37.5°C, the mean value was around 90% in Koshihikari and Takanari; that in IR72 at 37.5°C was markedly lower than that at 35.0°C. The range of values among the six cultivars was greatest at 37.5°C among the three temperatures.

The duration of high-temperature treatment and the

### Table 3. %SPF under different durations of treatment.

| Duration of treatment (days) | %SPF  |
|-----------------------------|-------|
| 1                           | 54.4  |
| 3                           | 37.5  |
| 5                           | 33.2  |

Values followed by the same letters are not significantly different at $P=0.01$.

### Table 4. Effect of daytime temperature on the mean percentage of dehisced thecae across all sampling days in each rice cultivar.

| Cultivar | Dehisced thecae (%) |
|----------|----------------------|
|          | Day temperature (°C) | Mean     |
|          | 35.0 | 37.5 | 40.0 |
| Koshihikari | 100.0 a | 91.6 a | 58.5 bc |
| Takanari   | 97.0 a | 88.7 a | 48.5 cd |
| NPT        | 99.8 a | 84.5 ab | 52.6 bc |
| Nipponbare | 99.4 a | 77.0 ab | 25.7 d |
| IR72       | 96.1 a | 43.3 cd | 27.1 d |
| WAB        | 77.2 abc | 57.7 bc | 21.8 d |

Mean 94.81 73.82 39.33 69.2

Range 22.8 48.3 36.7 31.2

Values followed by the same letters are not significantly different at $P=0.05$, and means followed by the same numbers are not significantly different within the row or column at $P=0.05$ by Tukey’s HSD test.

### Table 5. Effect of duration of treatment on the mean percentage of dehisced thecae across all temperatures in each rice cultivar.

| Cultivar | Dehisced thecae (%) |
|----------|----------------------|
|          | Duration of treatment | Mean     |
|          | 1 day | 3 days | 5 days |
| Koshihikari | 88.1 ab | 82.5 abc | 79.5 ab cd |
| Takanari   | 87.5 ab | 76.0 ab cde | 68.8 bc def |
| NPT        | 96.9 a | 80.6 ab cde | 59.4 cdef |
| Nipponbare | 74.0 abcde | 71.7 b cde | 53.4 fg |
| IR72       | 57.0 defgh | 44.5 gh | 66.9 b cdef |
| WAB        | 72.1 abcde | 34.1 h | 50.4 g fg |

Mean 79.31 65.12 63.0 69.2

Values followed by the same letters are not significantly different at $P=0.05$, and means followed by the same numbers are not significantly different within the row or column at $P=0.05$ by Tukey’s HSD test.
interaction of cultivar and duration also significantly affected the percentage of dehisced thecae (P < 0.001). The mean percentage of dehisced thecae among the six cultivars was higher on day 1 than on days 3 and 5 (Table 5), although the decrease in percentage varied among cultivars.

The length of basal dehiscence significantly differed with the cultivar (P < 0.001), but not the duration of treatment or temperature. Dehiscence was longest in Koshihikari, followed by NPT, and was shortest in WAB (Table 6).

### Discussion

1. **Effect of high temperature on pollination**

   The decrease in %SPF with increase in daytime temperature shows that a high daytime temperature led to poor pollination. As judged from the range of %SPF among cultivars, 37.5°C appears to be ideal for estimating the tolerance of the cultivars to a high temperature (Table 2). A temperature of 40.0°C had a severe effect, and no significant difference was detected in %SPF among the cultivars. Satake and Yoshida (1978) proposed that daytime temperatures of 35ºC and 38ºC were appropriate for the selection of cultivars intolerant and tolerant to a high temperature, respectively. Our results support their proposal.

   The decrease in %SPF with increasing duration of high-temperature treatments (Table 3) suggests a cumulative effect of high temperature just before flowering on pollination. Although flowering time is the most sensitive stage to high temperatures (Satake and Yoshida, 1978), the cumulative effect indicates another sensitive stage before flowering day.

   The percentage of dehisced thecae decreased with increase in both daytime temperature (Table 4) and duration of treatment (Table 5), showing that high temperatures caused poor thecal dehiscence. The daytime temperature of 37.5°C seemed ideal to estimate the

### Table 6. Length of dehiscence in the basal part of the theca in each rice cultivar.

| Cultivar    | Mean (mm) |
|-------------|-----------|
| Koshihikari | 0.516 a   |
| Takanari    | 0.391 c   |
| NPT         | 0.455 ab  |
| Nipponbare  | 0.428 bc  |
| IR72        | 0.343 ed  |
| WAB         | 0.313 d   |

Values followed by the same letters are not significantly different at P=0.05 by Tukey’s HSD test.

### Table 7. Coefficients of correlation between the percentage of florets having ≥20 pollen grains (%SPF) and the percentage of dehisced thecae in each rice cultivar (n=9).

| Cultivar    | r          |
|-------------|------------|
| Koshihikari | 0.951 ***  |
| Takanari    | 0.906 ***  |
| NPT         | 0.785 *    |
| Nipponbare  | 0.902 ***  |
| IR72        | 0.825 **   |
| WAB         | 0.846 **   |

*, P=0.05; **, P=0.01; ***, P=0.001.

### Table 8. Coefficients of correlation between the percentage of florets having ≥20 pollen grains (%SPF) at each daytime temperature and each of percentage of dehisced thecae and mean length of dehiscence in the basal part of the theca across all rice cultivars (n=6).

| Temperature (ºC) | % dehisced thecae | Length of dehiscence |
|------------------|-------------------|----------------------|
| 35.0             | 0.715 ns          | 0.740 ns             |
| 37.5             | 0.868 *           | 0.990 **             |
| 40.0             | 0.815 ns          | 0.925 ns             |

*, P=0.05; **, P=0.01; ns, not significant at P=0.05.
tolerance of anther dehiscence to high temperatures because of the wide range of cultivars. The effect of the duration of treatment on the percentage of dehisced thecae shows the cumulative effect of high temperature on dehiscence. Matsui et al. (2000) reported a similar cumulative effect on the decrease in anther dehiscence. A high temperature before flowering seemed to decrease the fragility of the anther septum to pollen pressure, which drives anther dehiscence, while high temperatures at flowering seemed to decrease the pollen pressure (Matsui et al., 2000). Jagadish et al. (2007) also reported the cumulative effect of high temperature on fertility on flowering day.

Since indehiscence of the anther decreases pollen dispersal, part of the negative cumulative effect of high temperature on %SPF would be due to a decrease in the number of dehisced anthers. Sato et al. (1973) reported that floret sterility increased on day 3 of high-temperature treatments, demonstrating a negative cumulative effect on fertility, but Satake and Yoshida (1978) refuted this cumulative effect. The interaction between cultivar and duration of high temperature on the percentage of dehisced thecae that we observed suggests that the susceptibility of thecal dehiscence to high temperatures before flowering day varies with the cultivar. The difference in the cumulative effect in previous studies may have depended on the variation in this susceptibility among cultivars.

In contrast to the percentage of dehisced thecae, the effect of cultivar on the length of basal dehiscence was significant, but the effects of duration of treatment and temperature were not. This agrees with the strong correlation of the length of basal dehiscence under normal conditions with that under hot conditions (Matsui et al., 2005). The length of dehiscence seems to be under strong genetic control.

2. Causes of poor pollination induced by a high temperature

Satake and Yoshida (1978) assumed that delayed dehiscence and indehiscence of the theca were responsible for poor pollination induced by a high temperature. Although the percentage of dehisced thecae was strongly correlated with %SPF in each cultivar (Table 7), pollination was insufficient in five cultivars even at 35°C (Table 2), at which the percentage of dehisced thecae was ≥95% (except for WAB) (Table 4). Moreover, %SPF was ≤25% at 40°C (Table 2), at which temperature about 20%
to 60% of the thecae dehisced (Table 4). These results suggest that the direct cause of poor pollination is not solely indehiscence of thecae. We observed that many pollen grains sometimes remained in dehisced thecae at high temperatures. Pollination stability seems to depend on pollen release from dehisced thecae in addition to dehiscence of the thecae. This idea is supported by the high correlation between %SPF and the length of basal dehiscence with a maximum range of %SPF among the cultivars at 37.5°C (Table 8). A similar correlation between the length of dehiscence and pollination stability has been observed under humid conditions (Matsui et al., 2005) and field conditions (Tian et al., 2010).

The range of %SPF and the percentage of dehisced thecae among the cultivars were greatest at 37.5°C. Hence, we examined the correlation between %SPF and the percentage of dehisced theca or the length of dehiscence at this temperature so as to ascertain which of these two parameters is more responsible for the variation in pollination stability among the cultivars. As Fig. 2 shows, the influence shifted from the length of basal dehiscence to the percentage of dehisced theca with increasing duration of treatment. Thus, the main direct cause of poor pollination changed with the duration of high-temperature treatment. The correlation between the length of dehiscence and %SPF suggests that the size of dehiscence after widening of the stomium crack strongly controls the pollination stability, while the correlation between the percentage of dehisced thecae and the %SPF suggests the septum rupture before stomium widening is a dominant factor.

In conclusion, our results show that both anther dehiscence and pollen release from dehisced anther are involved in poor pollination at high temperatures. Long basal dehiscence would enhance pollination stability through guaranteed pollen release when the duration of high temperature is short. However, indehiscence would directly limit pollen release and induce poor pollination when the high temperature persists, as suggested by Satake and Yoshida (1978). The cumulative negative effect of the high temperature before flowering on anther dehiscence made the effect of indehiscence dominant.

The length of basal dehiscence seems suitable as a visible and simple selection marker for tolerance to a brief exposure to a high temperature. Since this length is not affected by a high temperature, we can also use it as a marker at normal temperatures. However, it may not be enough for breeding tolerance to a long duration of high temperatures.

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