Correlation between Viability of Pollination and Length of Basal Dehiscence of the Theca in Rice under a Hot-and-Humid Condition

Tsutomu Matsui\textsuperscript{1}, Kazuhiro Kobayasi\textsuperscript{2}, Hisashi Kagata\textsuperscript{1} and Takeshi Horie\textsuperscript{3}

\textsuperscript{1}Experimental Farm, Kyoto University, Hatchonawate 12-1, Takatsuki, Osaka 569-0096, Japan; \textsuperscript{2}Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan; \textsuperscript{3}Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Abstract: Anticipated global warming may increase the floret sterility of rice \textit{(Oryza sativa L.)}. For selection of genotypes tolerant to high temperatures during the flowering period, it is important to identify morphological traits associated with tolerance to temperature stress. This study investigated the relationship between the length of dehiscence at the basal part of thecae and the viability of pollination in 18 cultivars of rice subjected to a hot-and-humid condition (37/25 \degree C, day/night, >90% relative humidity) for three days at flowering. Control plants were left under the ambient conditions in a semi-cylindrical house covered with cheesecloth (30% shading; temperature range: 24-35 \degree C). The length of basal dehiscence of thecae and the number of pollen grains on the stigmata were examined with a light microscope after flowering. The length strongly correlated with the percentage of florets having more than 80 pollen grains on the stigmata under the ambient condition ($r = 0.72$, $P < 0.001$), and with the percentage of florets having more than 20 pollen grains on the stigmata under the hot condition ($r = 0.93$, $P < 0.001$). In other words, the length correlated with pollination viability or reliability under both conditions. In addition, basal dehiscence was shorter in the non-\textit{japonica}-type cultivars than in many of the \textit{japonica}-type cultivars under both conditions. We concluded that the low pollination viability in the non-\textit{japonica}-type cultivars is associated with their small basal dehiscence on the thecae, and the length of basal dehiscence can be used as a selection marker of high temperature tolerance.

Key words: Dehiscence of anther, Flowering, High temperature, Hot-and-humid condition, Pollination, Rice, Tolerance.

As the increase in greenhouse gases is anticipated to continue, the period under high-temperature stresses is expected to be prolonged, which may have a significant impact on crop production. The effects of increasing temperatures and high CO$_2$ concentrations in the atmosphere on the rice yield have been analyzed, using crop simulation models (Jin et al., 1995; Horie et al., 1996; Matthews et al., 1997) and under controlled environments (Collins et al., 1995; Horie et al., 1996; Kim et al., 1996a,b; Matsui et al., 1997; Ziska et al., 1997). An anticipated high temperature (> 34 \degree C daily maximum temperature at the time of flowering) may induce floret sterility and decrease rice yield even in temperate regions such as western Japan, if the cropping seasons are unchanged (Kim et al., 1996b; Horie et al., 1996).

Cultivar differences in the tolerance to high temperatures at flowering have been reported (Satake and Yoshida, 1978; Matsuishima et al., 1982; Matsui et al., 2001b). Satake and Yoshida (1978) reported that the temperature at which the percent floret fertility decreased to 50\% was 39 \degree C for a tolerant cultivar, 36.5 \degree C for a moderately tolerant cultivar and 33.5 \degree C for a high-temperature-susceptible cultivar. Horie et al. (1996) demonstrated, using a crop simulation model, that adoption of high-temperature-tolerant cultivars, with a fertility higher than 50\% even when the daily maximum temperature of flowering is 38.2 \degree C, is one of the most effective countermeasures to maintain high productivity and stability of rice production under the anticipated climate change in western Japan.

Differential responses of genotypes to a high temperature suggest the possibility of genetic improvement in high-temperature tolerance (Mackill et al., 1982). To produce high-temperature-tolerant cultivars, it is important to identify the characteristics that determine the tolerance and to explore the genetic resources with these characteristics. Ecophysiological analysis of sterility induced by temperatures over 35 \degree C during the flowering period showed that the direct cause of the sterility is the reduction of germinated pollen grains on the
stigmata induced by the high temperature at the time of flowering (Satake and Yoshida, 1978). This was caused by the indehiscence of thecae and the delay of pollen release, and a decrease in the percentage of germinated pollen grains deposited on the stigmata (Satake and Yoshida, 1978; Matsui et al., 2000; Matsui et al., 2001).

Matsui and Omasa (2002) showed that some high-temperature-tolerant cultivars that have over 80% fertility under a day temperature of 37.5°C have well-developed lacuna for the anther dehiscence between the septum and the stomium of theca. The well-developed lacuna may weaken the septum and promote theca dehiscence, thus increasing the tolerance of pollination to high temperatures (Matsui et al., 2001a; Matsui and Omasa, 2002). The well-developed lacuna, however, is not an appropriate marker for exploration of genetic resources and breeding of high-temperature-tolerant cultivars because the lacuna does not appear outward and it takes much time to observe the lacuna.

For efficient selection in a breeding program, visible markers of high-temperature tolerance are required. Matsui and Kagata (2003) reported that, using japonica rice cultivars in Japan, long basal dehiscence of theca for pollen dispersal increased the stability and sureness of pollen transport to the stigmatic under normal conditions. Genetic improvement for large basal dehiscence may increase the sureness of the pollen transport, compensating for the decrease of theca dehiscence and delay of the pollen release caused by high temperatures at the flowering time, and improve the tolerance to high temperatures. It is easy to measure the dehiscence size.

On the other hand, the size of the basal dehiscence of rice thecae largely varies with the origin (Morinaga and Kuriyama, 1944). Morinaga and Kuriyama (1944) reported that the basal dehiscence was not smaller than the apical dehiscence in the cultivars from Japan and Russia, but was not larger than the apical dehiscence in the cultivars from mainland China and Taiwan. If such variation in the size of basal dehiscence among cultivars with different origins correlates with the viability of the pollination in a wide range of genotypes, we can use the size of basal dehiscence as a useful morphological marker of pollination viability.

The objective of this study was to determine if the length of dehiscence at the basal part of the theca can be used as a potential marker of pollination viability for selection of genotypes tolerant to high temperatures. We examined the correlation between the length of the dehiscence at the basal part of the thecae and the viability of pollination in a wide range of cultivars including japonica-type cultivars, indica-type cultivars, tropical japonica-type cultivar and *Oryza glaberrima* × *O. sativa* hybrid under a normal and hot-and-humid conditions. The humid condition was used to avoid the drying damage of the plants under the high-temperature condition.

### Materials and Methods

#### 1. Plant materials and treatments

The experiment was conducted at the Experimental Farm of Kyoto University (34°51′N, 135°38′E, 10 m above sea level) located in Takatsuki, Japan. Twelve *japonica* type cultivars (three modern cultivars + nine old cultivars with wide range in stability of pollination under normal condition (Matsui and Kagata, 2003) and six non-*japonica* type cultivars of rice were used (Table 1). Seeds were sown on 3 May 2002. Seedlings at the 5th leaf stage were transplanted in a circular pattern into pots (20 cm in height and 15 cm in diameter), 20 seedlings per pot, and grown in water-logged soil. The soil was obtained from a paddy field on the Experimental Farm of Kyoto University. The soil was clay loam. The soil pH was 6.40. Plants were grown in a semi-cylindrical house covered with cheesecloth (30% shading) throughout the experimental period to avoid wind damage. Plants in each pot were supplied with 0.6 g P2O5 as basal dressing. Top dressing of 0.1 g N and 0.1g K2O per pot was applied on 24 June and 9 July to maintain a healthy leaf color. During the vegetative stage, we removed tillers as they appeared to obtain uniform plants. Panicles emerged from late July to early September.

When 50% of the plants headed, six pots with uniformly grown plants were selected from each cultivar, and were then randomly divided into

| Cultivar | Ecotype | Origin |
|----------|---------|--------|
| Shanguichao | Indica type | China |
| WAB450-I-B-P-38-HB | *O. glaberrima* × *O. sativa* | Cote d'Ivoire |
| Takanari | Indica × japonica | Japan |
| IR72 | Indica type | Philippines |
| IR6564-44-2-2 | *Indica* × *tropical japonica* | Philippines |
| Banten | Tropical *japonica* type | Indonesia |
| Somewake | japonica type | Japan |
| Homura3 | japonica type | Japan |
| Koshihikari | japonica type | Japan |
| Nipponbare | japonica type | Japan |
| Kokuryoumiyako | japonica type | Japan |
| Ginhouzu | japonica type | Japan |
| Husakushirazu † | japonica type | Japan |
| Takenari | japonica type | Japan |
| Tairaippon † | japonica type | Japan |
| Magatama † | japonica type | Japan |
| Kameji2 † | japonica type | Japan |
| Kimmaza | japonica type | Japan |

† Seeds were obtained from the Gene Bank of the National Institute of Agrobiological Sciences (Japan).
two groups. One group was exposed to a high air temperature of 37 °C for 6 h (1000 – 1600) for 3 d in a growth chamber with a 10 h (0800 – 1800) photoperiod. Light was provided from white fluorescent tubes (270 µmol m⁻² s⁻¹). Night temperature (1800 – 0800) was controlled to 25.0 °C. From 0800 to 1000 and from 1600 to 1800, the air temperature was changed stepwise. The relative humidity was controlled over 90% during the high temperature treatment. The other group was left in the semi-cylindrical house covered with cheesecloth (temperature range 24-35 °C). This is referred to as ambient condition in this paper. The pots in both groups were rotated in each condition every day during the treatment. The flowering time was 1100 to 1400 under the high-temperature condition and 1000 to 1300 under the ambient condition.

2. Sampling and measurements

Seventeen florets that completed flowering were sampled from the primary branches on panicles of each cultivar every day at 1600 during the treatment under the high-temperature condition, and at 1300 for more than 3 d during the flowering periods under the ambient condition.

Anthers from five florets randomly selected from 17 florets of each cultivar were used for counting the number of indehisced thecae, and in the dehisced thecae, the length of the dehiscence at basal parts of the thecae was also measured using an ocular micrometer under a stereomicroscope (Fig. 1). The stigmata from all 17 florets were then stained with cotton blue and pollen grains on the stigmata were counted under a light microscope.

3. Data analysis

The percentage of florets having more than 80 pollen grains on the stigmata (referred to as “>80-pollen-grain florets” hereafter) in the ambient condition and that with more than 20 pollen grains (>20-pollen-grain florets) in both high-temperature and ambient conditions were calculated for each cultivar. Since most of the >20-pollen-grain florets are fertilized even under the high-temperature condition (Satake and Yoshida, 1978; Matsui et al., 2001b), the percentage of >20-pollen-grain florets indicates the viability of pollination under the high-temperature condition. On the other hand, the percentage of >80-pollen-grain florets strongly correlated with the number of pollen grains deposited on the stigmata and its coefficient of variation under the ambient condition (Matsui and Kagata, 2003). The percentage of >80-pollen-grain florets was used as the reliability of the pollination under the ambient condition.

The means of the values (the percentages of indehisced thecae, the length of the basal dehiscence, the percentages of >80-pollen-grain florets and the percentage of >20-pollen-grain florets) on the all sampling days were calculated for each cultivar under each condition. Regression analysis on the mean data was then conducted to examine the relationship between the means of the values.

Results and Discussion

Under the ambient condition, the percentage of >20-pollen-grain florets ranged from 48.5 to 100% in the cultivars examined, and was 100% in nine out of 18 cultivars (Table 2). In all cultivars, the percentage of >20-pollen-grain florets was lower under the high temperature than under the ambient condition. The percentage under the high-temperature condition was 3.9 to 88.2% and was below 50% in 10 out of 18 cultivars. This indicates that the viability of pollination for fertilization was decreased by the high-temperature treatment at the time of flowering.

The length of basal dehiscence strongly correlated with the percentage of >80-pollen-grain florets under the ambient condition (Fig. 2). Matsui and Kagata (2003) reported a strong correlation between the length of basal dehiscence and the percentage of florets with a large number of pollen grains on the stigmata under a normal condition in old japonica cultivars. They assumed that since the basal dehiscence is located just above the stigmata and opens at the floret opening, the pollen grains in thecae with a long basal dehiscence fall easily onto the stigmata, and thus a long basal dehiscence ensures pollination (Matsui and Kagata, 2003). The present results support their assumption and indicates that the correlations hold in a wide range of genotypes including non-japonica type cultivars.

The length of basal dehiscence also strongly correlated with the percentage of >20-pollen-grain
Table 2. Percentage of florets with more than 20 pollen grains on the stigmata after the anthesis (>20-pollen-grain florets) under ambient and high temperature conditions.

| Cultivar     | % of >20-pollen-grain florets under ambient condition | % of >20-pollen-grain florets under high temperature |
|--------------|------------------------------------------------------|-----------------------------------------------------|
| Shanguichao  | 100.0 ± 0.0                                          | 33.3 ± 27.8                                         |
| WAB450-1-B-P-38-HB | 96.0 ± 5.0                                           | 7.8 ± 9.0                                           |
| Takanari     | 90.6 ± 9.8                                           | 3.9 ± 5.4                                           |
| IR72         | 48.5 ± 17.6                                          | 19.6 ± 14.8                                         |
| IR65564-44-2-2 | 100.0 ± 0.0                                        | 56.9 ± 23.8                                         |
| Banten       | 76.4 ± 8.5                                           | 31.4 ± 22.3                                         |
| Somewake     | 94.1 ± 8.3                                           | 27.5 ± 23.8                                         |
| Homura3      | 100.0 ± 0.0                                          | 84.3 ± 12.2                                         |
| Koshihikari  | 100.0 ± 0.0                                          | 74.5 ± 13.6                                         |
| Nipponbare   | 98.5 ± 2.9                                           | 62.7 ± 35.5                                         |
| Kokuryomiyako| 98.5 ± 2.9                                           | 43.1 ± 3.4                                          |
| Ginhouzu     | 94.1 ± 6.8                                           | 23.5 ± 21.2                                         |
| Husakushirazu| 100.0 ± 0.0                                          | 27.5 ± 22.3                                         |
| Takenari     | 100.0 ± 0.0                                          | 27.5 ± 14.8                                         |
| Tairaippon   | 98.5 ± 2.9                                           | 62.7 ± 9.0                                          |
| Magatama     | 100.0 ± 0.0                                          | 70.6 ± 5.9                                          |
| Kameji2      | 100.0 ± 0.0                                          | 88.2 ± 10.2                                         |
| Kimnaze      | 100.0 ± 0.0                                          | 78.4 ± 9.0                                          |

Each value is the mean ± standard deviation of sampling days (n ≥ 3).

Florets under the high-temperature condition (Fig. 3). Our data suggest that the long basal dehiscence increases the percentage of sufficiently pollinated florets under a high-temperature condition and thus increases the high-temperature tolerance of the pollination.

The percentage of indehisced thecae was also significantly but negatively correlated with the percentage of >20-pollen-grain florets under the high-temperature condition (r = −0.689, P < 0.005). In the japonica-type cultivars, however, the percentage of indehisced thecae was not correlated with the percentage of >20-pollen-grain florets under the high-temperature condition (r = −0.548, not significant at 5% level). Since the pollen grains are released only from the dehisced thecae, the total number of pollen grains released from the thecae in the floret is proportional to the number of dehisced thecae. Therefore, the dehiscence of thecae would decrease the number of pollen grains deposited on the stigmata at the end of anthesis. Indeed, Matsui et al. (2000), using japonica-type cultivars, showed that the thecae of a high-temperature tolerant cultivar retained the high ability to dehisce even under the high-temperature condition. In the present experiment, the percentage of indehisced thecae was below 23% and its standard deviation was 7.0% in the japonica-type cultivars, whereas the percentage in all 18 cultivars ranged from 5.8 to 47% and the standard deviation was 13.9%. When a large range of data in percent indehisced thecae is included, the correlation between the viability of pollination and the percentage of dehisced thecae became stronger.

The non-japonica-type cultivars had a small basal dehiscence under the ambient condition (Fig. 2). This is coincident with the report of Morinaga and Kuriyama (1944). Hector (1913) reported that, in indica-type cultivars, the percentage of natural hybridization was about 4% whereas Akemine and Nakamura (1924) reported that the percentage in japonica-type cultivars was below 2.5%. The high percentage of natural hybridization in indica-type cultivars suggest low viability of self-pollination and is consistent with our result that non-japonica-type cultivars have low viability of pollination. The close correlation between the length of basal dehiscence and the percentage of >80-pollen-grain florets under the ambient condition (Fig. 2) suggests that the small basal dehiscence in the non-japonica-type cultivars resulted in the low viability of pollination in the ambient condition. The small basal dehiscence may allow the high percentage of hybridization in indica-type cultivars.

Both the length of basal dehiscence and the percentage of >20-pollen-grain florets under the high-temperature condition in the non-japonica-type
cultivars were smaller than in many of the *japonica*-type cultivars (Fig. 3). These results indicate that pollination of the non-*japonica* type cultivars was susceptible to high temperatures and suggest that the susceptibility was caused by the small dehiscence at the basal part of theca. Genetic improvement for a large basal dehiscence would increase the high-temperature tolerance of these cultivars. Some *japonica*-type cultivars with long basal dehiscence may be a useful genetic resource for the improvement of heat tolerance in rice.

The length of basal dehiscence under the ambient condition was positively correlated with that under the high-temperature condition (Fig. 4). This correlation shows that character of the long dehiscence is stably expressed both under the high temperature and normal environments at flowering. Therefore, we can estimate the length of the basal dehiscence under a high-temperature condition from that under a normal condition.

In this study, we found that the long basal dehiscence at the basal part of the thecae raised the viability of the pollination under the hot-and-humid condition as well as under the normal condition in a wide range of cultivars, and that the length of the basal dehiscence under the ambient condition was parallel to that under the high-temperature condition. Since the length of dehiscence is a visible trait, the length under normal conditions may be useful as an appropriate morphological marker for selection of a high-temperature tolerant genotype.

Fig. 3. Relationship between the length of dehiscence at the basal part of thecae and the percentage of >20-pollen-grain florets under the high-temperature condition (day temperature = 37°C, >90% R. H.). ***P < 0.001. Symbols and numbers are the same as those in Fig. 1.

Fig. 4. Relationship between the dehiscence at the basal part of thecae under the ambient condition (in a semi-cylindrical house covered with cheesecloth) and that under the high-temperature condition (day temperature = 37°C, >90% R. H.). ***P < 0.001. Symbols and numbers are the same as those in Fig. 1.

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