Stability of Rice Pollination in the Field under Hot and Dry Conditions in the Riverina Region of New South Wales, Australia

Tsutomu Matsui¹*, Kazuhiro Kobayasi², Mayumi Yoshimoto³ and Toshihiro Hasegawa³

¹Experimental Farm, Graduate School of Agriculture, Kyoto University, 12-1, Hatchournawate, Takatsuki, Osaka 569-0096, Japan; ²Faculty of Life and Environmental Science, Shimane University, 1060, Nishikawatsu-chou, Matsue, Shimane 690-8504, Japan; ³National Institute for Agro-Environmental Sciences, 3-1-3, Kannondai, Tsukuba, Ibaraki 305-8604, Japan

Abstract : Even under extremely hot (40°C) conditions during anthesis, heat-induced floret sterility does not appear to be a serious issue for Australian rice growers. This contradicts previously reported temperature thresholds for floret sterility. To determine the factors associated with stable rice production under hot and dry conditions in the Riverina region of New South Wales (Australia), we examined rice (cv. 'Langi¹) pollination at different distances from the windward edge of a paddy field and its association with canopy microclimate. With an air temperature of 34.5°C and a relative humidity of 20.7% during anthesis, poor pollination of florets occurred at the windward edge, but pollination remained stable farther from the edge. The temperature difference between the air and the panicles in the canopy reached as high as 6.8°C under these conditions because of low humidity and strong transpirational cooling. Moreover, the length of the dehiscence at the base of the thecae during anthesis was long; this is a desirable trait for heat tolerance. The long basal dehiscence of the thecae of this cultivar and the lower panicle temperatures relative to the ambient temperature caused by high transpirational cooling appear to be the key factors responsible for stable pollination under the extremely high temperatures of the Riverina region.

Key words : Anther dehiscence, Floret sterility, Heat stress, Oryza sativa L., Transpirational cooling.

Projected global warming is likely to have an enormous impact on rice production around the world. For this reason, crop scientists are attempting to estimate the effects of global warming on the rice production using crop simulation models (Horie et al., 1996; Nakagawa et al., 2003) and controlled environments (Kim et al., 1996a, b; Ziska et al., 1997). Some of them have pointed out that floret sterility induced by high temperatures during anthesis can decrease the yield of rice, and that this may become a problem under global warming, even in the temperate regions such as Japan (Horie et al., 1996; Kim et al., 1996b; Nakagawa et al., 2003).

Ecophysiological analysis has revealed the mechanism responsible for heat-induced floret sterility. High temperatures (above 35°C) at the time of anthesis impede the swelling of pollen grains in the locules (Matsui et al., 2000) that is the driving force for anther dehiscence (Matsui et al., 1999a, b), and this can result in indehiscence of the anther and defective pollen release (Matsui et al., 2000). The resulting poor pollination is the main cause of the floret sterility observed at these temperatures (Satake and Yoshida, 1978; Matsui et al., 2001), especially for heat-susceptible cultivars (Satake and Yoshida, 1978). Matsui et al. (2005) showed that the tolerance to heat-induced spikelet sterility of rice cultivars is closely related to the length of dehiscence at the basal part of thecae. In general, the longer the basal dehiscence, the larger the number of pollen grains on the stigma; this reduces the risk of floret sterility. The length of basal dehiscence, therefore, is the key trait for stable pollination under heat conditions.

Previous experiments in controlled-environment chambers revealed that temperatures above 34°C (Satake and Yoshida, 1978) or 35°C (Matsui et al., 2001) at the time of anthesis induce floret sterility. These threshold temperatures coincided with the temperature ranges that induced floret sterility of field-grown rice in Thailand (Osada et al., 1973). Angus (1997), however, indicated that in paddy fields in Australia, the maximum daily air temperature during the anthesis period sometimes reaches 40°C, but that no serious yield losses due to heat-induced spikelet sterility have been reported; this contradicts previously reported temperature thresholds for floret sterility. Understanding the conditions that induce floret sterility would help researchers to predict the impacts of climate change on rice production and to develop countermeasures against any yield losses that might result from high temperatures.

One possible reason for this seemingly stable pollination under extremely high temperatures in Australia may be that the high transpiration that...
results from the large vapor-pressure deficit in this environment cools the rice canopy below the ambient air temperature, and thus reduces the risk of the panicles being exposed to heat. Another reason may be that the cultivars grown in this area are more heat-tolerant than other cultivars. However, neither hypothesis has been examined in any detail. The purpose of the present research was thus to determine why the extremely high temperatures that occur during the rice anthesis period do not induce floret sterility in rice plants growing in the Murrumbidgee Irrigation Area (MIA) of the Riverina region, a major rice-growing area in Australia. For this purpose, we measured panicle temperatures, pollination stability, and anther dehiscence in a paddy field in MIA. Although the plants did not encounter extremely high temperatures (above 40°C) during our measurements, we were nonetheless able to observe the features of pollination in the paddy field in relation to panicle temperatures and anther dehiscence, both of which could partially explain why the high air temperatures do not induce floret sterility in the MIA.

Materials and Methods

1. Field and plant material

We conducted our experiment at the Leeton Field Station (soil type: a self-mulching heavy clay) of the Yanco Agricultural Institute (146° 21’ 40.7” E, 34° 35’ 53.3” S, elev. 138 m), located in the southeastern part of MIA, during the 2004-2005 growing season. The total area of the paddy fields was 15.26 ha (279 m from east to west and 547 m from north to south). Because the prevailing wind direction was from west to east, we used an east–west strip within the field (279 m from east to west × 5 m from north to south) for observation. Seeds of the long-grain cultivar ‘Langi’ (Parentage, YC 73/M 7/ PELDÉ) were drill-sown on 14 October 2004 at a density of 140 kg ha⁻¹ using a disc seeder. Prior to sowing, a chemical fertilizer (Granulok 12Z lite, Incitec Pivot Ltd., Southbank, Victoria, Australia) was applied as a basal dressing at rates of 13.8, 9.16, and 5.76 kg ha⁻¹ for N, P and S, respectively. After seeding, the field was supplied with four flushes of water before November 11 (28 days after planting, DAP). The first flush submerged the bay to a depth of about 10 cm for about 3 hr, followed by drainage to

Fig. 1. Changes in (a) air temperature (T_a) and relative humidity (RH), and (b) wind speed (U) measured at the windward edge of a rice community at Leeton, NSW, Australia on 7 and 8 February 2005. Shaded zones show the flowering time.
initiate germination. The field was supplied with three subsequent flushes at approximately 1-week intervals to supply soil moisture for seedling growth. On 24 November (41 DAP), 150 kg N ha\(^{-1}\) was applied as urea to the soil surface. The field was then flooded to a depth of 10 cm, and the water was held at approximately this depth for the remainder of the growing season. The heading date for this crop was 5 February (114 DAP), and plant height was about 80 cm on the dates of our temperature and humidity measurements.

2. Measurements

We measured the site’s microclimate on two clear days (7 and 8 February) in 2005, which corresponded to the anthesis period in this community. We measured general meteorological variables such as air temperature, relative humidity, and wind speed at the western (windward) edge of the rice community. The paddy field was isolated and only about 30% of the land in the surrounding district was irrigated, and there were no paddy fields west of it, so that the measurements represent typical surface atmospheric conditions in this region with limited effects of the rice community. We installed a temperature and humidity sensor (HMP45D, Vaisala Inc., Helsinki, Finland) just inside the bay 132 cm above the soil surface, and installed an anemometer and wind vane (Model 03001 –5 Wind Sentry, R.M. Young Co., Traverse City, MI, USA) at a height of 165 cm. We measured the wind velocity, wind direction, air temperature, and air humidity every 10 seconds, and recorded 10-minute averages using a datalogger (CR10X, Campbell Scientific Inc., Logan, UT, USA).

We measured panicle temperatures (\(T_p\)) at the time of anthesis at distances of 0, 5, 10, 15, and 20 m from the western edge of the community using an infrared thermometer (Model TA-0510F, Konica-Minolta Co. Ltd., Tokyo, Japan) with a fixed emissivity factor of 1. To avoid biases due to direction of the sun and wind, we measured the temperatures of panicles located north, east, south, and west of each sampling location (four plants) three times for each compass direction at each spot, which took about 45 seconds to complete. We started these measurements from the western edge of the paddy field towards the 20-m distance, and moving back from the 20-m distance to the western edge. Thus during the anthesis period of about 1 hr, we replicated these measurements twice. We then calculated the mean \(T_p\) at each spot based on these 12 \(\times\) 2 readings.

For our observation of pollination, we sampled five florets on the primary rachis branches that had completed anthesis at the same spots used for our observations of pollination, and measured the length of the basal dehiscence of the anthers. First, we counted the number of indehiscent thecae and the number of thecae whose stomium had dehisced completely from the apex to the base of the theca ("longitudinally dehisced thecae"). Then, for thecae whose stomium had dehisced separately on the apical and basal parts ("cylindrically dehisced thecae"), we measured the length of the dehiscence that formed on the basal part using a digital microscope at 100 \(\times\) magnification (NH-5000, Keyence Corporation, Japan).

For our observation of pollination, we sampled five florets on the primary rachis branches that had completed anthesis at each of three distances (0, 5, and 20 m) from the western edge of the community. To do so, we detached the stigma from the florets and stained them with cotton blue. We then counted the number of pollen grains on the stigma under an optical microscope (DSM-1, Nippon Optical Works Co., Ltd., Tokyo, Japan) at 100 \(\times\) magnification.

To observe dehiscence of the thecae, we sampled five florets on the primary rachis branches that had completed anthesis at the same spots used for our observations of pollination, and measured the length of the basal dehiscence of the anthers. First, we counted the number of indehiscent thecae and the number of thecae whose stomium had dehisced completely from the apex to the base of the theca ("longitudinally dehisced thecae"). Then, for thecae whose stomium had dehisced separately on the apical and basal parts ("cylindrically dehisced thecae"), we measured the length of the dehiscence that formed on the basal part using a digital microscope at 100 \(\times\) magnification (NH-5000, Keyence Corporation, Japan).

![Panicle temperatures, \(T_p\) (a), and temperature difference between the air and panicle, \(T_a-T_p\) (b), measured at different distances from the windward edge at the time of flowering, measured on two clear days in the field. Bars indicate ± standard error of the mean of two replicates. Analysis of covariance with date as a fixed variable and distance as a covariate variable indicated that the effects of both date (D=1 for the first day and 2 for the second) and distance on \(T_p\) were significant (\(P<0.01\)), and that of distance only was significant for \(T_a-T_p\) (\(P<0.01\)). The interaction between distance and date was not significant on both days.](image)
Osaka, Japan).

We tested the effects of the dates of measurements and the distance from the windward edge on $T_p$, basal dehiscence and percentage of the longitudinally dehisced thecae for significance by means of analysis of covariance with distance as a covariate variable, using the SPSS for Windows 7.5J statistical software (SPSS, Inc., Chicago, Illinois, USA).

Results

1. Microclimate and panicle temperatures

The sky was clear from the morning of the first day to the noon of the second day of our measurements, and daily maximum air temperatures reached 35.9 and 34.7°C, respectively (Fig. 1). The air temperature ($T_a$) during anthesis was 33.3°C on the first day and 34.5°C on the second day. The relative humidity (RH) during anthesis was 22.5% on the first day and 20.7% on the second day: The corresponding vapor pressure deficits were 3.96 and 4.33 kPa, respectively. The wind direction was mainly from the west, and the wind velocity ($U$) at the time of anthesis was 3.2 m s$^{-1}$ on the first day and 4.2 m s$^{-1}$ on the second day.

Anthesis occurred between 1300 and 1400 h on the first day and between 1145 and 1245 h on the second day. We did not observe noted differences in the time or date of anthesis amongst five distances (0, 5, 10, 15, 20 m) from the windward edge. Accordingly, we selected panicles at full anthesis at each spot and we used SPSS for Windows 7.5J to determine the number of pollen grains and the distance from the windward edge of the community. The frequency distribution for the number of pollen grains was generally longer on the second day than on the first day though the difference was not significant ($P=0.059$ for the distance effect, Table 1).

2. Anther dehiscence

In general, all of the thecae dehisced, except at the windward edge of the community on the second day, where 16% of the thecae failed to dehisce. The mean dehiscence of the basal part of the thecae was generally longer on the second day than on the first day though the difference was not significant (0.53 vs. 0.48 mm, $P=0.052$; Table 1). The dehiscence was generally longer at the windward edge of the community than inside the community (Table 1). The longest dehiscence (0.60 mm) was recorded at the windward edge on the second day. The percentage of longitudinally dehisced thecae was also larger at the edge of community on the second day (10.7% vs. less than 8% at all other time–distance combinations; $P=0.018$ for the date effect and $P=0.059$ for the distance effect, Table 1).

3. Pollination

The number of pollen grains on the stigma of each floret ranged from 6 to 437 (Fig. 3). We did not observe any clear relationship between the mean number of pollen grains and the distance from the windward edge of the community. The shape of the frequency distribution for the number of pollen grains on the stigma at different distances was generally similar at both days, with the peak appearing between 80 and 160 grains per stigma. However, the distribution at 0 m from the edge on the second day had the largest range of values. As a result, the coefficient of variation on this occasion was nearly twice the next largest value.

Table 1. The length of the dehiscence at the basal part of the thecae and the percentage of the longitudinally dehisced thecae of cultivar ‘Langi’ grown in the field. The results of analysis of covariance (ANCOVA) with date as a fixed variable and distance as a covariate variable are presented.

| Distance from the edge of the community (m) | Length of the basal dehiscence (mm) | Longitudinally dehisced thecae (%) |
|-------------------------------------------|-------------------------------------|-----------------------------------|
|                                           | 7 Feb. | 8 Feb. | 7 Feb. | 8 Feb. |
| 0                                         | 0.51   | 0.60   | 1.9    | 10.7   |
| 5                                         | 0.48   | 0.48   | 2.2    | 7.7    |
| 20                                        | 0.44   | 0.50   | 0      | 3.6    |

ANCOVA

- Date: $P=0.018$
- Distance (Covariate): $P=0.059$
1. Panicle temperature

One of the hypotheses we developed to explain the stable pollination observed under extremely hot temperatures in the Riverina region was that the canopy or panicles would be substantially cooler than the air as a result of transpirational cooling. Unfortunately, we did not observe a $T_a$ higher than $40^\circ$C during our measurements, so we could not confirm this hypothesis for extreme temperatures. Our results nonetheless demonstrated that under hot and dry conditions, $T_p$ was much lower than $T_a$, even at the edge of the crop canopy, and that the $T_a$-$T_p$ difference became larger with increasing distance from the edge of the rice community; the greatest difference was $6.8^\circ$C, and the smallest difference was $4.0^\circ$C.

Only limited information has been available on temperatures of the rice panicles. Nishiyama (1981) measured temperature inside rice spikelets under various conditions including chambers and fields (in the Philippines and India), and showed that temperature inside the spikelet can be either higher or lower than just outside the spikelet by 1-2$^\circ$C, depending on the outside temperature; the temperature inside can be lower than outside where outside temperature is higher than about $30^\circ$C and vice versa. Note that in his study, the temperature outside the spikelet were measured just around the spikelets, which were influenced by the transpiration.

Fig. 3. Frequency distribution of number of pollen grains per stigma after anthesis at distances of 0, 5, and 20 m from the windward edge of the rice community on two clear days.
cooling by the canopy, so that the direct comparison with the present results would be difficult because we measured $T_a$ outside the canopy with the limited effect of canopy transpirational cooling. Yoshimoto et al. (2005) measured the panicle temperature in Wuxi, Jiangsu, China and showed that the panicle temperature was similar to or slightly lower than air temperature inside the canopy (at the panicle height), but lower than above-canopy air temperature by 0.4–1.5°C. In wheat, Ayeneh et al. (2002) showed that the spike temperature measured in northwestern Mexico can be lower than ambient air by 2.8–5.2°C. Thus, the $T_a-T_p$ difference observed in the present study is generally larger than those in the previous studies.

The panicle temperature inside the community is determined by heat exchange between the panicles, leaves, and atmosphere in and above the canopy. Yoshimoto et al. (2005) developed a simple heat-budget model to calculate $T_p$ in the rice canopy. We applied this model to two values of $T_a$ (30 and 34°C) and two values of RH (20 and 60%) to estimate the transpirational cooling effects, defined here as the difference between $T_a$ and $T_p$ (i.e., $T_a-T_p$), under different climatic conditions. We used the same values for solar radiation and wind speed in all simulations: 800 W m$^{-2}$ and 1 m s$^{-1}$, respectively. The transpirational cooling effect estimated by the model was 0.6°C at 30°C $T_a$ and 60% RH, which is a typical microclimate for paddy rice fields in Japan on a clear day. The cooling effect is more pronounced under hotter and drier conditions: 2.1°C at 34°C/60%, 4.6°C at 30°C/20%, and 6.9°C at 34°C/20%. The last of these three conditions is similar to those measured during the current study in Australia. Thus, the cooling effect (the temperature difference of more than 6°C that we observed in the present study) could easily be explained by the energy balance (evaporative cooling) hypothesis. The hot and dry air that enters the community is cooled by the rice canopy, and forms a steep $T_p$ gradient from the edge of the community to the interior of the community; this likely explains the observed $T_p$ difference between plants inside and at the edge of the community.

2. Dehiscence of thecae and stability of pollination

'Langi' is a long-grain variety of polination that is commonly planted in the Riverina region. Its heat tolerance is unknown, but the basal dehiscence measured in this study averaged 0.5 mm, which was as long as the value reported by Matsui and Kagata (2003) for japonica-type cultivars tolerant of high temperatures (Fig. 4). The coefficient of variation for the number of pollen grains observed on the stigma of 'Langi' in the present study was also similar to that of the tolerant japonica cultivars, and was much smaller than that reported for the heat-susceptible cultivars (Matsui and Kagata, 2003). Because the stability of pollination increases with the length of the basal dehiscence (Matsui and Kagata, 2003) and long basal dehiscence is also advantageous at high temperatures (Matsui et al., 2005), 'Langi' can be considered tolerant to high temperatures during anthesis. Interestingly, the regression line for the relationship between the length of the basal dehiscence and the coefficient of variation in the number of pollen grains on stigma that was obtained in a previous study (Matsui and Kagata, 2003; Fig. 4) agreed well with the data obtained in the present study for 'Langi'. This supports the hypothesis of a robust relationship between the length of the dehiscence and the stability of pollination.

The length of the basal dehiscence of thecae is under strong genetic control (Matsui and Kagata, 2003; Matsui et al., 2005), but the present results suggest that the external environment also affects this length: Basal dehiscence was longer at the edge of the rice canopy than inside the community, and tended to be longer on the second day than on the first day. Despite the long basal dehiscence and high percentage of longitudinally dehisced thecae at the edge of the community on the second day, the percentage of florets with a small number of pollen grains on the stigma was high and the coefficient of variation for the number of pollen grains was large at the community edge on the second day (Fig. 3), which is a typical symptom of poor pollination. The reason for the
unstable pollination at the edge of the community on the second day is not clear, but higher temperatures, lower humidity, and stronger wind observed at the edge of the community on the second day might be a reason for this. Matsushima et al. (1982) also observed a negative correlation between RH during the anthesis period and floret sterility under the hot and dry climate of central Sudan. A heavy water loss from florets might have impeded a major process of pollination such as swelling of the pollen grains (Matsui et al., 1999a), but further study is needed to determine the interactive effects of high Tp and water loss on pollination processes.

**Conclusion**

Our study has revealed two important features of 'Langi' rice growing in the Riverina region of Australia that are related to stable pollination under the area's hot and dry climate. First, 'Langi' had a long dehiscence at the basal part of the thecae, which is a trait commonly observed for cultivars that are tolerant of high temperatures and that show reduced floret sterility at these temperatures. Second, strong transpirational cooling of the canopy due to high vapor pressure deficit around the time of anthesis on clear days can reduce panicle temperatures by as much as 6.8°C compared with the ambient air temperature. These features contribute to stable pollination and stable crop yield under the high temperatures that occur in this region by reducing the occurrence of floret sterility induced by high temperatures. The results suggest that accurate prediction of relative humidity associated with global warming is also needed to estimate the occurrence of heat-induced spikelet sterility under future climates. To confirm the effects of a long dehiscence on pollination stability under extremely hot and dry conditions, we need to perform studies using varieties that are genetically different in their dehiscence length. We should also determine the effects of dry winds on anther dehiscence under both milder and more extreme temperature conditions.

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