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Heat-Induced Floret Sterility of Hybrid Rice (*Oryza sativa* L.) Cultivars under Humid and Low Wind Conditions in the Field of Jianghan Basin, China

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Abstract: Projected global warming is expected to increase the occurrence of heat-induced floret sterility (HIFS) in rice. However, there are few field-scale studies that could aid in predicting the potential risks to rice yield and developing countermeasures against yield losses. The aim of this study was to elucidate the factors that induce floret sterility under high temperature conditions during the flowering season in the field condition in China. Studies were conducted in irrigated paddy fields with the regional hybrid-rice cultivars grown in Jianghan Basin where air temperature is not so high during the flowering season but HIFS frequently occurs. The microclimate, panicle temperature, floret sterility, pollination, and size of dehiscence formed at the base of anthers were investigated. Significant losses in seed set were observed under the high temperature condition. Although the maximum atmospheric temperature was approximately 35°C, the relative humidity was very high (around 70% at the time of maximum temperature), with low wind speeds, occasionally below 1 m s^{-1}. Under such conditions sunlit panicle temperature exceeded atmospheric temperature by as much as 4°C. Moreover, the anthers of some cultivars exhibited short basal dehiscence, and the dehiscence length was positively correlated with the percentage of sufficiently pollinated florets ($r=0.859$, $P<0.05$, $n=7$) and with seed set ($r=0.827$, $P<0.05$, $n=7$) across the cultivars. The results suggest that the combination of hot, humid, and windless climatic conditions with short basal dehiscence of anthers induces HIFS in hybrid rice grown in this region.

Key words: Anther dehiscence, Heat-induced floret sterility, Humidity, Panicle temperature, Pollination stability, Rice.

Severe rice (*Oryza Sativa* L.) sterility occurred in the Yangtze Valley of China during the summer of 2003, which was the hottest summer recorded for this region (Wang et al., 2004). There is a large amount of scientific evidence for increasing global surface temperatures (Trenberth et al., 2007), and if this trend continues, extreme heat events are likely to occur more frequently, which may, in turn, increase the occurrence of heat-induced floret sterility (HIFS) in rice in Asia. Some crop model predictions have pointed out that a climate warmer than the present condition could decrease rice yield by increasing the occurrence of HIFS, even in temperate regions (Horie et al., 1996; Cruz et al., 2007).

Information on the conditions that induce floret sterility would help researchers to predict the impacts of climate change on rice production in Asia and to develop countermeasures against yield losses that might result from high temperatures. Previous experiments in controlled-environment chambers revealed that high temperature above 34°C (Satake and Yoshida, 1978; Jagadish et al., 2007) or 35°C (Matsui et al., 2001) at the time of anthesis could induce floret sterility. However, information about HIFS in the field is limited and the conditions under which it is induced are unclear.

In the Yangtze valley, the temperature during the flowering period can become high, but not exceeding 40°C, even in the recent hot summer of 2003, where HIFS was recorded in many locations (Matsui, 2009). This is in contrast with reports of no serious yield losses due to HIFS in paddy fields in Australia where the daily maximum temperature sometimes reaches 40 °C during the flowering period (Angus, 1997). In this regard, factors that

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induce floret sterility of rice in Yangtze Valley are assumed to be different from that in Australia. Matsui et al. (2007) suggested that the relatively stable pollination under high temperatures in Australia could be explained by high transpiration resulting from the large vapor pressure deficit due to dry winds that cooled the rice canopy relative to air temperature. In that study, panicles were cooler than air by as much as 6–7°C in the middle of the paddy field, which would reduce the risk of the panicles being subjected to excess heat. However, where relative humidity is high and wind speed is low, as typically seen in monsoon Asia, panicle temperatures may become higher than air temperatures, which may induce HIFS, even where air temperature is not extremely high. Therefore, we hypothesized that the limited transpiration cooling of the panicle under high humidity and low wind conditions may play a role in inducing HIFS in the Yangtze Valley.

In addition to environmental conditions, genetic factors can have a strong influence on the magnitude of HIFS. The hybrid-rice cultivars that have contributed to large increases in yield in China (Peng et al., 2008) are predominant in the Yangtze Valley; however, it is still not clear whether these cultivars are susceptible to high temperatures. Previous studies under controlled environments proposed a mechanism of tolerance to high temperatures. High temperatures at the time of anthesis impede the swelling of pollen grains in the locules (Matsui et al., 2000) that is a driving force for anther dehiscence (Matsui et al., 1999a, 1999b), and this can result in indehiscence of anther (Matsui et al., 2000). In addition, the high temperatures make pollen grains sticky (Satake and Yoshida, 1978), and thereby difficult to shed which cause defective pollen release (Matsui, 2005). The resulting poor pollination is the main cause of the floret sterility observed under the controlled chamber condition (Satake and Yoshida, 1978; Matsui et al., 2001). Although poor pollination is the main direct cause of HIFS, pollination of some cultivars was found to be stable even under high temperatures (Satake and Yoshida, 1978; Matsui et al., 2001). Moreover, such high-temperature-tolerant cultivars with stable pollination under controlled hot and humid conditions formed a longer dehiscence at the basal part of the thecae than did susceptible cultivars at the time of pollination (Matsui et al., 2005). The long basal dehiscence helps to ensure the release of pollen grains from the thecae at the beginning of flowering, and thus, enables stable pollination under high temperatures (Matsui et al., 2005). If poor pollination is a direct cause of HIFS and long basal dehiscence strongly controls tolerance under field conditions, we can use the length of dehiscence as a simple gauge to estimate the HIFS tolerance of cultivars.

In this study, we examined how high temperature in the Jianghan Basin induced severe floret sterility in rice. Our hypotheses are 1) the humid and low wind make the panicle hotter than air and 2) short dehiscence at base of anther of some regional hybrid rice cultivars are responsible for the difference in HIFS across the cultivars. To examine these hypotheses, we conducted studies in irrigated paddy field in the Janghan Basin of the middle Yangtze valley where heat-induced floret sterility frequently occurs with regional hybrid cultivars.

Table 1. Heading date of cultivars in two observation periods in 2006

Cultivar	First observation period		Second observation period
	Crop I	Crop II	Crop III
Guofeng No.1		28 July	
Fengliangyou No.1		30 July	15 August
Jinyou 63		29 July	11 August
Jinyougui 99		28 July	
Shanyou 63	25 July		12 August
Jinyou 725	27 July		12 Augus
II You 838	30 July		12 August
II You 084	31 July		15 August
II You 725	1 August		16 August
Honglianyou No.6	5 August		20 August
Liangyoupeijiu	5 August		17 August
II You Ming 86	30 July		16 August

Materials and Methods

1. Field and plant materials

The experiment was conducted in the experimental paddy field of Yangtze University (Jingzhou City, 112°09′E, 30°21′N, elev. 32 m) located in the western part of Jianghan Basin in China, in the summer of 2006. The total area of the paddy field examined was about 600 m^2. The field was first divided into two blocks, and then each block was divided into three planting times. This enabled us to observe pollination of cultivars that require different number of days to the heading at the same time in a range of temperatures during the hottest season (late-July to mid-August). Twelve hybrid-rice cultivars that are common in this region were used (Table 1). Each plot was 3 (east–west) m by 2 (north-south) m and was again divided into western and eastern parts, in which panicle fertility and floret pollination were examined, respectively. The experiment followed a split-plot design with two blocks. Each block was bordered by three rows of rice (about one meter for three rows). The seeds were sown in the seed beds on 19 April (Crop I) and 26 April (Crop II), and 10 May (Crop III). Seedlings were transplanted into the plots in the field after 30 days at a density of 33.3 hill m^{-2} (20×15 cm) with two plants per hill. The soil was clay-loam with mineral N at 76.3 μg g^{-1}, available P at 15.2 μg g^{-1} and exchangeable K at 153.8μg g^{-1}. The soil pH was 6.71. Prior to paddling, ammonium bicarbonate and superphosphate were applied as a basal dressing at rates of 106 kg of N ha^{-1} and 38 kg of P ha^{-1}, respectively. Seven days after transplantation, 78 kg of K ha^{-1} was applied as potassium chloride. The field soil was kept submerged until the ripening stage. The heading dates (50% heading) of the cultivars are listed in Table 1. The plant heights of the cultivars at heading ranged from 115 to 125cm. The panicle densities of the cultivars ranged from 220 to 230 m^{-2}.

2. Microclimate and panicle temperature

The site's microclimate (air temperature, relative humidity, velocity and direction of wind, and solar radiation of paddy field) was measured at the southern edge of the experimental field during daytime (0700 to 1800). When heavy rainfall occurred, the measurement was stopped. The wind was mainly from the south during the experimental period. A 50 m rice paddy field extended to the south from the edge of the experimental field.

We installed a temperature and humidity sensor (HMP45D, Vaisala Inc., Helsinki, Finland) with radiation shield on the southern border of the experimental field, 140 cm above the soil surface (30 cm above the top of the canopy), and installed an anemometer wind vane at 160 cm above the soil surface. Measurements were taken every 10 s and 10-min averages were recorded in the data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA).

The panicle temperatures of each cultivar were measured at the time of anthesis, with an infrared thermometer (Model TA-0510F, Konica-Minolta Co. Ltd., Tokyo, Japan) with the fixed emissivity of 1.00. Flowering of rice,occurs in midmorning and the time varies slightly with the day. The peak of flowering was between 1000 to 1045 on 29 July and around 1100 to 1130 from 14 to 16 August, during which we made the measurement. To avoid biases due to direction of the sun and wind, we measured the panicle temperatures from two directions, north and south, in each plot. Five panicles on which florets were flowering were randomly selected and measured their temperature in each direction. The distance between the panicle and thermometer was around 50–100 cm. The measurement angle to the panicle was around 90º. Since we could not find statistically significant differences in panicle temperatures between the cultivars, the 10 min averages of panicle temperature of different cultivars were used corresponding to climatic data recorded in the logger without regard to cultivars.

3. Pollination and seed set

The pollination and seed set were examined during two heading seasons, 28–30 July (first observation period), 14–19 August (second observation period). Cultivars with the same flowering periods were used for the examinations.

For examination of the seed set, panicles that mainly flowered during the first and the second observation periods were tagged and sampled at maturity. Thirty panicles were tagged in each plot for each observation period and their seed sets were examined by manual inspection of ovary development. During the first observation period, 'Guofen No.1', 'Fenglianyou No.1', 'Jinyou 63' and, 'Jingyougui 99' from Crop II and the other eight cultivars from Crop I were examined. During the second observation period, 'Guofeng No.1' and 'Jingyougui 99' were not used because their flowering stage had already finished. The other 10 cultivars from Crop Ⅲ were examined.

For the observation of dehiscence of thecae, florets on the primary rachis branches were sampled just after flowering. Seven cultivars which flowered during the observation periods were used for this observation. Sampling was conducted once in each observation period. On 29 July in the first observation period, 'Jinyou 725', 'Shanyou 63', and 'II you 838' in Crop I, and 'Jinyougui 99', 'Fengliangyou No.1', 'Jinyou 63', and 'Guofeng No. 1' in Crop II were used. On 15 August in the second observation period, 'II you 838', 'Fengliangyou No. 1', 'Jinyou 63', 'Shanyou 63', 'II you 084', 'II you 725', and 'II you Ming 86' in Crop III were used. Five florets were collected from dispersed hills in each plot in each observation period. We measured the length of the dehiscence that formed at the base of the thecae (Matsui and Kagata, 2003) using a digital microscope (VH-5000, Keyence Corporation, Osaka, Japan).

For observation of pollination, 12 florets on the primary rachis branches were sampled from each cultivar in each block everyday from 14 to 19 August, covering the second observation period. The seven cultivars in Crop III that were used for observation of anther dehiscence were used. About 2 hr after anthesis, the florets were sampled, following which the stigmata were detached from the florets and stained with cotton blue. After staining, the numbers of total and germinated pollen grains on the stigmata in each floret were counted with an optical microscope (Eclipse E600, Nikon Corporation, Tokyo, Japan).

4. Data analysis

The effects of cultivars and observation periods on the percentage of seed set were examined with analysis of variance. Then, differences between the mean of seed set percentage of samples were analyzed by Tukey's HSD test at a probability level of 0.05. Statistical analysis was conducted after arcsine conversion of seed set percentage.

The percentages of florets having more than 10 pollen grains and more than five germinated pollen grains on the stigma were calculated for the seven cultivars in the second observation period. The effects of sampling dates and cultivars were examined with analysis of variance. The

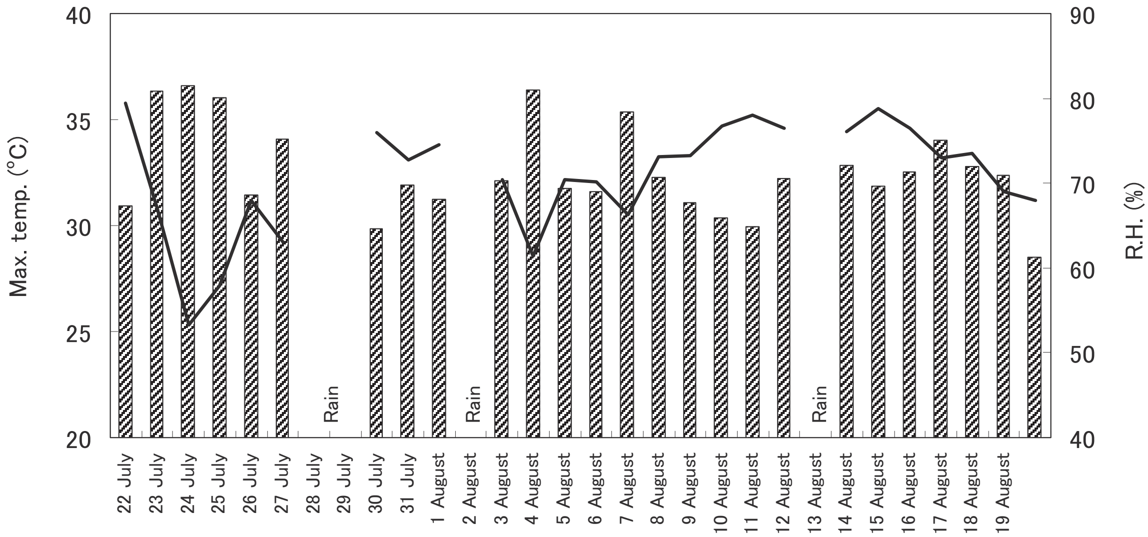

Fig. 1. Change in daily maximum air temperature (Max. temp., line) and relative humidity (R.H., bars) at the time of maximum temperature at the windward edge of the rice community examined in Jingzhou (western part of Jianghan Basin) in the hottest period of 2006.

Table 2. Panicle temperature and microclimate in the field during flowering

Date	Time	Temperature (°C)			R.H.	Solar radiation	Wind velocity
		Panicle	Air	Panicle - Air	(%)	(W m^{-2})	(m s^{-1})
29 July	958–1000	29.5	29.7	–0.2	79.4	362.7	0.645
	1000–1010	29.9	29.4	0.5	81.2	327.1	0.786
	1010–1020	29.8	29.3	0.5	81.5	444.9	0.897
	1020–1030	33.1	30.2	2.9	80.2	656.5	0.557
	1030–1040	31.1	30.7	0.4	77.1	476.5	0.563
	1040–1048	29.8	30.6	–0.8	77.0	573.4	0.872
14 August	1056–1100	37.1	32.8	4.3	79.0	677.8	0.629
	1110–1120	37.2	33.2	4.0	77.2	626.8	0.550
	1120–1130	36.2	33.0	3.1	78.0	768.8	1.029
15 August	1115–1120	32.1	32.1	0.0	74.0	737.1	0.014
	1120–1130	33.1	32.6	0.5	73.0	793.3	0.230
	1130–1133	33.4	32.8	0.7	74.5	810.1	0.374
16 August	1057–1100	29.9	29.9	0.0	85.2	507.1	0.985
	1100–1110	29.4	30.0	–0.6	85.0	401.6	0.973
	1110–1120	30.0	29.9	0.1	86.2	464.5	0.980
	1120–1130	32.1	30.4	1.7	84.0	538.7	0.730

Panicle temperature was not different among cultivars.

differences between the mean of the percentages of florets were analyzed by Tukey's HSD test at a probability level of 0.05. Statistical analyses were conducted after arcsine conversion of floret percentage.

The correlation between the length of basal dehiscence, the percentage of seed set and the floret with more than 10 pollen grains on the stigma were analyzed with statistical software, STATISTICA ver.6J (StatSoft JAPAN Inc., Tokyo, Japan).

Table 3. Seed set percentages of panicles that flowered during the 1st (28–30 July) and 2nd (14–16 August) observation periods in the 12 cultivars

Cultivar	Observation period*		Mean*
	1st	2nd	
Fengliangyou No.1	91.5[ab]	84.6 [ab]	88.1[a]
IIYou 838	91.5[ab]	69.9[cde]	80.7[ab]
IIYou 084	91.2[ab]	69.5[cde]	80.3[abc]
IIYou Ming 86	90.7[ab]	67.1[cde]	78.9[abc]
IIYou 725	92.4[a]	59.9[de]	76.2[bc]
Shanyou 63	87.0[ab]	68.8[cde]	77.9[bc]
Honglianyou No.6	83.0[abc]	68.6[cde]	75.8[bc]
Liangyoupeijiu	82.2[abc]	58.7[e]	70.4[cd]
Jinyou 725	78.7[abc]	57.4[e]	68.1[cd]
Jinyou 63	77.6[bcd]	52.0[e]	64.8[d]
Jingyougui 99	89.0±2.6	N.A.	
Guofeng No.1	87.7±1.1	N.A.	
Mean**	86.6	65.7	76.1

Note: Ten cultivars, except Jingyougui 99 and Guofeng No.1 were used for statistical analysis. *Within the columns of Observation period or Mean, values with the same letters are not significant at $P<0.05$ (Tukey's HSD test). **Seed set percentages in 1st and 2nd observation periods are significantly different at $P=0.00001$. Arcsin-values were used for statistical analysis. N.A.: not available.

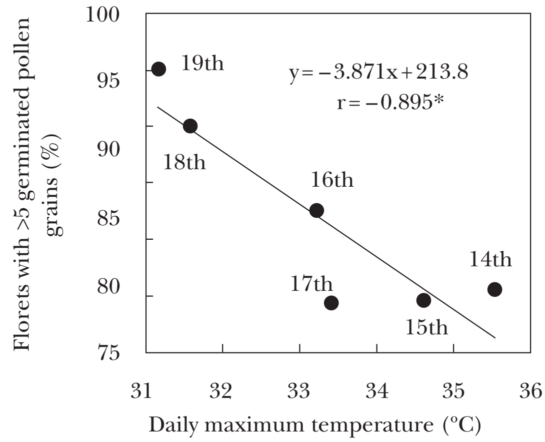

Fig. 2. Relationship between the daily maximum temperature and the percentage of florets having more than five germinated pollen grains on the stigma during the second observation period (14–19 August). *significant at $P<0.05$. Numbers adjacent to the symbols represent date of flowering in August.

Results

1. Microclimate

The daily maximum atmospheric temperature during the flowering period (July to August), except for rainy days, ranged from 25.3 to 35.7°C (Fig. 1), with relative humidity at the times of daily maximum temperature ranging from 61.2 to 81.5% which were common in the last decade in this region.

On 28 July, the first day of the first observation period, we did not monitor the microclimate in the field because of heavy rainfall (Fig. 1). On 29 and 30 July, the daily maximum temperature was 34.4 and 33.1°C, respectively, and relative humidity 64.6 and 70.0%, respectively. The air temperature at the time of flowering was about 31°C on both days and relative humidity was about 75%.

During the second observation period, i.e., 14, 15 and 16 August, the daily maximum temperature was 35.5, 34.6, and 33.2°C, respectively, and was somewhat higher than that in the first observation period, while relative humidity was 69.7, 71.3, and 75.0%, respectively The air temperature on 14, 15 and 16 August was 33.5, 32.5, and 30.5°C, and was also higher than that in the first period. The relative humidity was around 75–85%.

2. Panicle temperature

The effect of cultivar on panicle temperature was not significant at $P=0.05$. The mean panicle temperature was equivalent to air temperature or higher (Table 2). In particular, the temperature difference between the panicle and atmosphere reached 4°C, and the panicle temperature was 37°C at the time of flowering on 14 August when solar radiation, wind velocity and relative humidity were approximately 650 W s^{-1}, 0.6 m s^{-1} and 78%, respectively. On 29 July, the temperature difference between the panicle and atmosphere reached 2.9°C when cloudy conditions became suddenly clear, resulting in conditions of high solar radiation (657 W m^{-2}), low wind speed (0.56 m s^{-1}), and high humidity (80.2%).

3. Seed set

The effects of cultivars and observation periods on the percentage seed set were significant at $P=0.001$ and the interaction between them was also significant at $P=0.041$. The percentage seed set of florets that mainly flowered in the first observation period ranged from 78 to 92% across cultivars and those in the second observation period ranged from 52 to 85% (Table 3). The seed set percentage averaged over cultivars was lower in the second observation period than in the first by 21% and variation between cultivars was larger in the second observation period by 20%.

4. Pollination and anther dehiscence

From 14 to 19 August, the effect of date on percentage of floret having more than five germinated pollen grains on the stigma in each floret was significant (P=0.0016). The percentages of florets on 18 and 19 August were significantly higher than those on 13, 14 and 16 August at $P=0.05$. The percentage correlated with the daily maximum temperature (Fig. 2). In the second observation

Table 4. Pollination traits of florets collected about two hours after anthesis from 14–16 August for seven cultivars in Crop III

Cultivar	Florets with ≤ 5 germinated pollen grains			Florets with > 5 germinated pollen grains		
	Total	Florets with ≤ 10 pollen grains	Florets with > 10 pollen grains	Total	Florets with ≤ 10 pollen grains	Florets with > 10 pollen grains
Jinyou 63	52.8[a]	41.7[a]	11.1[a]	47.2[a]	6.9[a]	40.3[a]
Shanyou 63	26.4[ab]	18.1[ab]	8.3[ab]	73.6[ab]	0.0[b]	73.6[b]
II You 838	21.2[ab]	12.8[ab]	8.5[ab]	78.8[ab]	0.0[b]	78.8[b]
II You 725	16.7[ab]	13.9[ab]	2.8[ab]	83.3[ab]	0.0[b]	83.3[b]
II You 084	15.7[b]	15.7[ab]	0.0[ab]	84.3[b]	1.4[b]	83.0[b]
II You Ming 86	13.0[b]	10.0[b]	3.0[b]	87.0[b]	1.4[b]	85.6[b]
Flyou No.1*	8.5[b]	4.3[c]	4.2[c]	91.5[b]	1.4[b]	90.2[b]

Values are mean percentages of blocks (n=2). Within a column, values with the same letters are not significant at P<0.05 (Tukey's HSD test). *Fengliangyou No.1.

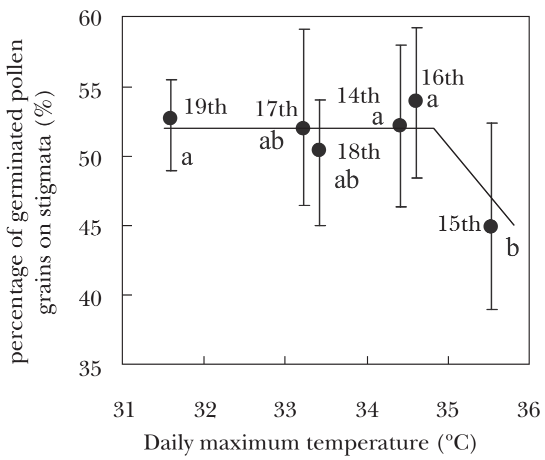

Fig. 3. Relationship between the daily maximum temperature on the day before flowering and the percentage of germinated pollen grains on the stigma during the second observation period (14–19 August). Symbols with the same letters are not significantly different at P<0.05 (Tukey's HSD test). Numbers adjacent to the symbols represent date of flowering in August. Bars represent the range of percentage of germinated pollen grains on the stigma among cultivars.

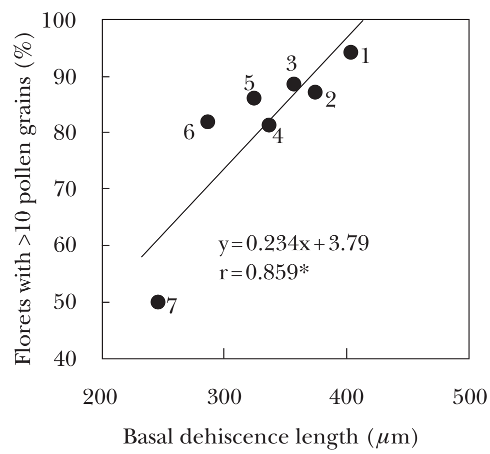

Fig. 4. Relationship between the length of dehiscence formed at the basal part of the thecae (15 August) and the mean percentage of florets having more than 10 pollen grains on the stigma in the second observation period (14–16 August). *significant at P<0.05. Cultivars: 1, Fengliangyou No.1; 2, IIYou 838; 3, IIYou Ming 86; 4, IIYou 084; 5, IIyou 725; 6, Shanyou 63, 7, Jingyou 63.

period, the effect of cultivars on the percentage of florets having more than five germinated pollen grains was significant (P=0.014) and the seed set percentage in the second observation period significantly correlated with the percentage of florets across the cultivars (r=0.746, n=7, P=0.045). Moreover, the total number of pollen grains on the stigma in the floret with five or fewer germinated pollen grains was 10 or less in many cases, and many florets with more than five germinated pollen grains had more than 10 pollen grains (Table 4). The effect of date on the percentage of germinated pollen grains on the stigma was significant (P=0.020), but the effect of cultivars on the percentage was not. The germination percentage on 15 August when the daily maximum temperature was 35.5 ˚C the day before was lower than the other days, but the percentage was more than 45% in average of cultivars and around 40% even in the lowest cultivars (Fig. 3). The percentage of florets having more than 10 pollen grains on the stigma significantly correlated with the length of dehiscence at the basal part of the thecae on 15 August (Fig. 4). The basal dehiscence lengths significantly correlated with the seed set percentage of florets on panicles that mainly flowered in both observation periods (Fig. 5).

Discussion

1. Environmental factors related to the occurrence of HIFS in Jianghan Basin

The daily maximum air temperatures and the air temperatures at the times of flowering were higher in the second than in the first observation period. The panicle

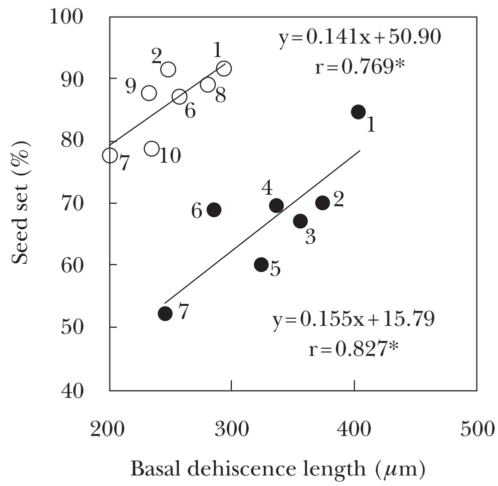

Fig. 5. Relationship between the length of dehiscence formed at the basal part of the thecae and the percentage of seed set in the first observation period (29–30 July, ○) and in the second observation period (14–16 August, ●). *significant at $P<0.05$. Cultivars: 1, Fengliangyou No.1; 2, IIYou 838; 3, IIYou Ming 86; 4, IIYou 084; 5, IIyou 725; 6, Shanyou 63; 7, Jingyou 63; 8, Jingyougui 99; 9, Guofeng No.1; 10, Jinyou 725.

temperatures at the times of flowering in the second period reached 37.0°C (Table 2). Percentage seed set in the second observation period was significantly lower than that in the first observation period (Table 3). A rice floret is most susceptible at the time of flowering (Satake and Yoshida, 1978) and the panicle temperatures were beyond the reported panicle temperature thresholds of 33.7°C (Jagadish et al., 2007) and 33°C (Weerakoon et al., 2008) for HIFS in rice. We therefore conclude that the reduced percentage seed set in the second observation period was due to high panicle temperature.

The hypothesis we developed to explain the occurrence of severe sterility at high normal temperatures in the Yangtze Valley was that the panicle temperature would become higher than air temperature under humid air conditions in contrast to the dry air conditions prevalent in Australia. Our results demonstrated that the panicles can be warmer than the atmosphere. The difference was as large as 4°C under high solar radiation with low wind speed and high atmospheric humidity. Such a large difference has not been reported previously. In contrast, Matsui et al. (2007) reported that the panicle temperature was lower than air temperature by as much as 6.8°C at maximum in the dry atmosphere of Australia. A heat budget model for estimation of panicle temperature (Yoshimoto et al., 2005), however, did reproduce the extreme cases of differences between the panicle temperature and the air temperature observed under very different climatic conditions (Yoshimoto et al., 2007), which justifies the current observations.

Low humidity and high wind have been thought to promote the occurrence of HIFS (Matsushima et al., 1982). Nevertheless, the low seed set percentage in the second observation period at high normal temperatures suggests that humid and windless conditions in China can exacerbate HIFS, largely due to increased panicle temperatures. This is in contrast to the hot and dry conditions prevalent in Australia.

2. Tolerance of hybrid-rice cultivars to sterility under high temperature conditions in Jianghan Basin

We confirmed that germination of sufficient pollen grains on the stigma strongly correlated with the seed set percentage across the cultivars tested in this experiment. For successful fertilization, more than five or ten germinated pollen grains are required on the stigma in each floret (Satake and Yoshida, 1978; Satake and Koike, 1981). Significant correlation between the seed set percentage and the percentage of florets having more than five germinated pollen grains on the stigma after flowering suggests that the reduction in germinated pollen grains on stigma is largely responsible for the occurrence of sterility under hot and humid field conditions. Moreover, we observed that the percentage of florets having more than five germinated pollen grains significantly correlated with the daily maximum temperature in the period covering the second observation. This supports the view that the high temperature during the second observation period decreased the percentage of floret having more than five germinated pollen grains.

Many florets with five or fewer germinated pollen grains on the stigma in each floret had 10 or fewer total pollen grains, and most florets with more than five germinated pollen grains had more than 10 total pollen grains (Table 4). This indicates that pollination strongly controls the percentage of florets with five or fewer germinated pollen grains. Under controlled hot conditions, insufficient pollination was found to be the main direct cause for reduction in numbers of florets with a sufficient number of germinated pollen grains on the stigmata (Satake and Yoshida, 1978; Matsui et al., 2001), and this is confirmed by our field observations.

The percentage of germinated pollen grains on the stigma seemed to be correlated with the maximum temperature on the day before flowering (Fig. 3). Nabeshima et al. (1988) reported about the damage from exposure to 40°C for two hours at a flowering time. The main cause of sterility was poor pollination of the florets that flowered at the high temperature, and no germination of pollen grains for the florets that flowered on the following day. The present results agree with theirs on the point that high temperatures on the day before flowering affect the percentage of pollen grains. However, the germination percentage in present experiment was around 40%, which seemed adequate for seed fertilization.

A previous experiment in a controlled-environment chamber revealed that longer basal dehiscence of the anther makes pollination stable under a high temperature condition (Matsui et al., 2005). The percentage of florets with more than 10 pollen grains on the stigma was strongly correlated with the length of basal dehiscence (Fig. 4). Moreover, we found that the length of anther dehiscence affects the seed set even under field condition (Fig. 5). It has been proposed that long basal dehiscence is a useful marker for selection of high temperature tolerant cultivars (Matsui et al., 2005) on the basis of experiments with growth chambers. Our results in the field strongly support this assumption.

Even in the first observation period when the daily maximum temperature was below 34.5°C and seed set percentage was more than 78%, the correlation between the length of basal dehiscence and seed set percentage was statistically significant. This suggests that excessively short basal dehiscence of some hybrid-rice-cultivars grown in this region is a source of yield loss under humid conditions even at ordinary temperatures, probably because of unstable pollination.

Matsui et al. (2005) reported that the lengths of basal dehiscence in *japonica* rice ranged from 350 to 650 μm and that in *indica* rice from 300 to 500 μm under controlled hot and humid conditions. Moreover, Matsui et al. (2007) reported that the length of basal dehiscence of a *japonica* cultivar 'Langi' in Australia with stable rice production under extremely hot condition was around 500 μm. The hybrid-rice cultivars used in this experiment seem to have rather short basal dehiscence: thus we estimate that these hybrid-cultivars were more susceptible to a high temperature at flowering than the *japonica* cultivars.

Short basal dehiscence decreases the drop of pollen grains from the thecae at the beginning of floret opening, which results in scatter of pollen grains from apical dehiscence out of the florets after the stamen bends down. Therefore, the short dehiscence would increase the chance that pollen grains reach the stigmata of the other florets (Matsui and Kagata, 2003). Although there is no information on the basal anther dehiscence of the staminate parental lines of hybrid rice, their length may play a role in seed production efficiency that depend on the success in pollination from staminate parent to pistillate. If the short basal dehiscence in the staminate parent is necessary for efficient seed production and the short dehiscence of hybrid rice necessarily results from efficient seed production, long basal dehiscence is not available to increase their high temperature tolerance. To confirm whether the long basal dehiscence is available characteristic to increase the tolerance, we need to examine the mode of inheritance of dehiscence length from staminate parent to hybrid rice.

3. Conclusion

Our observations have revealed two outstanding factors that may contribute to the occurrence of HIFS in hybrid rice cultivation in the Jianghan Basin: humid conditions and low wind speed. A third factor is short basal dehiscence of the anthers in some regional hybrid-rice cultivars. The short basal dehiscence seems to make pollination unstable under hot, humid, and low wind conditions. Other factors may also be important in the occurrence of HIFS. For example, we have not compared the hybrid cultivars with any others with respect to transpirational conductance, which could affect the occurrence of sterility. Even though the observations reported here did not cover all the factors potentially contributing to HIFS, the results strongly suggest that we have to consider the tolerance of regional cultivars and the meteorological factors that can affect the panicle temperature for prediction of the impacts of climate change on rice production. The present study considered the variation in the lengths of basal dehiscence, which can be a source of variation in the occurrence of floret sterility even under high temperature conditions. The long basal dehiscence seems to be a useful marker for high temperature tolerance of florets in the field.

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