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Factors determining the occurrence of floret sterility in rice in a hot and low-wind paddy field in Jianghan Basin, China

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ABSTRACT

Global warming may increase the occurrence of heat-induced floret sterility (HIFS) in rice. The effects of meteorological factors and plant traits on the occurrence of floret sterility have only been examined independently and under controlled or simplified conditions. The aim of this study was to comprehensively clarify the role and impact of these factors in HIFS in rice under practical field conditions. We observed pollination and seed set of 12 rice cultivars with wide variation in traits potentially related to HIFS for 3 years in a paddy field in the Yangtze Valley in China where HIFS sometimes occurs. Floret sterility increased as temperature increased under moderately high temperatures (32–36.5 °C) and low-wind conditions (<3.5 m s^{-1} at flowering time). Path analysis with multiple regression revealed that high temperatures increased the occurrence of floret sterility through processes after pollen germination on the stigma and that wind lessened the occurrence of floret sterility; the magnitude of the wind effect was equivalent to that of high temperature. Multiple regression analysis estimated that low and erect panicles, and short anthers have positive effects on tolerance to HIFS and known heat tolerance traits, e.g., long anther dehiscence at the base of the anther and early morning flowering. The results clarified the factors that control HIFS in the paddy field and identify the processes and traits that scientists and breeders should focus on to mitigate HIFS under global warming.

1. Introduction

In the past 20 years, severe rice (*Oryza sativa* L.) sterility events occurred several times in the Yangtze Valley of China—in the summers of 2003 (Wang et al., 2004), 2007 (Liu et al., 2013), 2010 (Yoshimoto et al., 2012), and 2013 (Yan et al., 2015)—probably due to high temperatures at flowering time. There is a large amount of scientific evidence for increasing global surface temperatures (IPCC, 2013), and if this trend continues, extreme heat events are likely to occur more frequently, which may, in turn, increase the occurrence of HIFS in rice in

Abbreviations: AL(μm), anther length; ADL(μm), length of dehiscence for pollen dispersal formed at the apical part of the anther; BDL(μm), length of dehiscence for pollen dispersal formed at the basal part of the anther; FCdate, Flowering commencement date; FD(cm), depth of florets at anthesis in canopy; FH(cm), height of florets in anthesis; FS, percentage of floret sterility; FT(hours), flowering time; ID(%), percentage of indehisced thecae; LD(%), percentage of longitudinally dehisced thecae; LH(cm), leaf height; Logit(FS), logit-transformed FS; Logit(ID), logit-transformed ID; Logit(LD), logit-transformed LD; Logit(LT10GP), logit-transformed LT10GP; Logit(LT20TP), logit-transformed LT20TP; LT10GP(%), percentage of florets with less than 10 germinated pollen grains on the stigma after anthesis; LT20TP (%), percentage of florets with less than 20 total pollen grains on the stigma after anthesis; PA(°), angle of panicle from the vertical; PD, panicle depth in canopy (cm); PH, panicle height (cm); T_{10-12}(°C), mean temperature from 10:00 h to 12:00 h; $T_{flowering}$(°C), mean temperature over the 1-h period from 30 min before to 30 min after flowering time; T_{max}(°C), daily maximum temperature; T_{mean}(°C), daily mean temperature; W_{10-12}(m s^{-1}), mean wind speed from 10:00 h to 12:00 h; $W_{Flowering}$(m s^{-1}), mean wind speed over the 1-h period from 30 min before to 30 min after flowering time.

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Asia (IPCC, 2014; Horie, 2020). Some crop model predictions have indicated that a climate warmer than present could decrease rice yield by increasing HIFS occurrence even in temperate regions (Horie et al., 1996; Cruz et al., 2007).

The mechanism of HIFS has been examined mainly under controlled conditions such as a phytotron or growth chamber. Satake and Yoshida (1978) concluded that florets are most susceptible to high temperatures (>35 °C) at flowering time, which induce floret sterility. They also showed that the percentage of sterile florets correlated with the percentage of poorly pollinated florets (defined as <10 germinated pollen grains or <20 total pollen grains on the stigma after anthesis) in *indica* rice (Satake and Yoshida, 1978). Matsui et al. (2001) confirmed the same correlation between floret sterility and pollination using *japonica* rice at 37.5 °C day temperature. Shi et al. (2018) demonstrated with a microscopy technique that failure of the pollen tube to reach the embryo sac was the major cause of HIFS. Anther dehiscence inhibition and low pollen shedding onto stigma are thought to be main reasons for floret sterility at high temperatures in enclosed environments (Wang et al., 2019). However, the mechanism of occurrence of HIFS has not yet been clarified under field conditions. Recently, Zhang et al. (2018) showed that high temperature of (40 °C for 2 h) disturbs pollen tube elongation in the pistil and cause pollination failure. Coast et al. (2016) related heat susceptible pollen germination to susceptibility of floret fertility in a cultivar, IR64. Matsui et al. (2014) observed low germination percentage of pollen grains on the stigma under hot, dry windy conditions in the field. Could factors peculiar to the real paddy field, such as diurnal changes in temperature and wind, affect the occurrence of HIFS?

Adoption of heat-tolerant rice cultivars seems an effective countermeasure to global warming (Nakagawa et al., 2004). Such cultivars may increase the chance for production in extremely hot regions and seasons, and thereby contribute to stable rice production under global warming in near future. A few traits that decrease the chance of HIFS have been reported. One is early morning flowering (Bheemanahallia et al., 2017). Rice is very sensitive to high temperatures at flowering time (Satake and Yoshida, 1978; Jagadish et al., 2007) and thus florets that finish flowering in the early morning can escape from the high temperature damage (Nishiyama and Blanco, 1980; Yoshida et al., 1981; Ishimaru et al., 2012). Another trait is morphologically long anther dehiscence. One direct cause of HIFS under artificially controlled conditions is poor pollination (Satake and Yoshida, 1978). Long dehiscence of anthers helps the pollen grains to drop onto the stigma (Matsui and Kagata, 2003) and makes the pollination stable under high temperatures (Matsui et al., 2005). The positive effects of early morning flowering and long anther dehiscence on fertility have been confirmed under controlled (Kobayasi et al., 2011; Matsui and Hasegawa, 2019; Ishimaru et al., 2010) and hot field (Matsui et al., 2009; Tian et al., 2010; Zhao et al., 2010; Ishimaru et al., 2012) conditions.

Some other morphological traits are also associated with success or failure of pollination under unfavorable weather conditions and/or in the open field. For instance, a long anther is an important trait when rice is exposed to low temperature at the microspore stage. This is because the number of pollen grains in a rice anther is proportional to the anther length (Nishiyama, 1981; Suzuki, 1982); thus, the large number of pollen grains in long anthers compensates for the damage to pollen grains by the low temperature. Moreover, the position of the panicle at flowering relative to the canopy (Matsui et al., 2020) and inclination of the panicle from the vertical (Win et al., 2019, 2020) affect pollination stability and sterility in field conditions, although the underlying mechanisms are unclear. The optimal vertical position for pollination and seed set at flowering is around the height of the canopy surface, and a higher or lower position negatively affects those processes. Moreover, wind negativly affects the optimal height: i.e., strong wind makes the optimal position lower (Matsui et al., 2020). A panicle inclination of over 30° significantly decreases pollination stability and seed set (Win et al., 2019, 2020). Since poor pollination is an important cause of HIFS, the above traits may also relate to the occurrence of sterility in rice under hot conditions. Contributions of these traits to pollination and grain setting under hot and open field conditions are yet to be determined.

Although many possible factors and processes that determine the occurrence of HIFS have been proposed by studies conducted under controlled or simplified conditions, their impacts have not yet been examined under practical paddy field conditions at the same time. The purpose of this study was to clarify the framework of occurrence of HIFS: i.e., (a) determine if high temperatures affect the seed set mainly through poor pollination as the preceding studies suggest; (b) determine if the meteorological (air temperature, wind velocity), morphological (plant height, panicle or flowering height, panicle angle and anther morphology), and ecological factors (flowering time) that preceding studies found mainly in controlled environments affect the seed set; and (c) to estimate their magnitude on seed set under hot field condition. The study was conducted in a hot, humid, and low-wind paddy field using 12 rice cultivars with wide variation in traits potentially related to heat tolerance or susceptibility over 3 years in the Jianghan Basin in the Yangtze Valley. The Jianghan Basin is an important rice-producing region in China and its rice production is vulnerable to heat stress (Wang et al., 2016). We comprehensively describe the role of these traits, pollination variables, and meteorological variables in occurrence of HIFS.

2. Materials and methods

2.1. Field and plant materials

The experiment was conducted in a paddy field at the Yangtze University's farm (Jingzhou City, 112° 09′ E, 30° 21′ N, elevation 32 m) located in the western part of Jianghan Basin in the Yangtze Valley, China, in the summers (July to August) of 2015–2017 (meteorological data in Jingzhou City during the study period are shown in Table S1). The total area of paddy field examined was about 1000 m^2 (55 m east–west by 18 m north–south). The field was first divided into two blocks of randomized block design (North and South blocks, because the main wind direction in summer is fixed and wind affects pollination and seed set (Matsui et al., 2020)); then each block was divided into four parts with four different flowering periods. In each flowering period, the sowing dates of the cultivars were adjusted for synchronized heading. This enabled us to simultaneously observe pollination of cultivars that require different numbers of days to heading many times during the hot period (late July to mid-August). Eleven rice cultivars with wide variation in plant height, flowering time, and anther morphology were selected from the mini core-collection of rice in Hubei, China (Zhang et al., 2011), and an additional cultivar (Xiaomazhan) with early morning flowering (Kobayasi et al., 2009) was also used (Table S2). Each part of flowering period in each block contained 12 plots of cultivars. The plot for each cultivar was 3 m (east–west) by 2 m (north–south) and was located randomly in each season. Each plot was subdivided into western and eastern parts, in which panicle fertility and floret pollination were examined, respectively; this was to avoid any accidents whereby sample florets for pollination were obtained from the panicles used for the seed set test. The edge of the field had a 1.5-m wide border of five rows of rice plants. The seeds were sown in the seed beds four times for each cultivar following a staggered sowing plan for the four flowering seasons. Prior to puddling, ammonium bicarbonate (106 kg N ha^{-1}) and superphosphate (38 kg P ha^{-1}) were applied to the paddy field as a basal dressing. About 30 days after sowing, the seedlings were transplanted into the field at a density of 2 plants per hill (20 × 15 cm) and 33.3 hills m^{-2}. The soil was a Haplic fluvisol of a clay-loam texture with mineral N at 76.3 μg g^{-1}, available P at 15.2 μg g^{-1}, and exchangeable K at 153.8 μg g^{-1}. The soil pH was 6.7. About one week after transplantation, potassium chloride (78 kg of K ha^{-1}) was applied. The field soil was kept submerged until the ripening stage.

2.2. Microclimate

The microclimate (air temperature, relative humidity, wind speed, and wind direction) at the site was measured using an all-in-one weather sensor (WXT510 Vaisala Inc., Helsinki, Finland) at the southern edge of the experimental field during the flowering period. The sensor was installed about 240 cm above the ground surface. Solar radiation was measured with a pyranometer (LI-200sa, LI−COR, Lincoln, NE, USA) at 150 cm above the ground surface. 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. Measurements of air temperature, relative humidity, wind speed, wind direction, and solar radiation were taken every 10 s and 1-min averages were recorded in the data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA).

Daily maximum temperature (T_{max} [°C]), daily mean temperature ($T_{mean,}$ [°C]), mean temperature and mean wind speed for the 1-h period from 30 min before to 30 min after flowering time ($T_{flowering}$ [°C], $W_{flowering}$ [m s^{-1}]), and mean temperature and wind speed from 10:00 h to 12:00 h (T_{10-12} [°C], W_{10-12} [m s^{-1}]) were used for analyses to clarify the effects of temperature and wind on sterility, pollination stability, and flowering time. We adopted T_{10-12} [°C] and W_{10-12} [m s^{-1}] as additional meteorological variables because many cultivars flower from 10:00 h to 12:00 h under normal conditions.

2.3. Pollination, anther morphology, and seed set

Flowerings of cultivars continued for around 3 weeks from the end of July (Fig. S1, Tab. S3). By the staggered sowings, the flowering periods of cultivars were overlapped. To examine seed set, panicles in which anthesis had started, but the lowest floret that had finished anthesis was less than 30 % of the panicle length from the apex were tagged with the date (hereafter, “flowering commencement date, FC date”) in the early evening every other day, except rainy days, during the flowering period. Tagging was not conducted on rainy days and conducted 11, 11, and 9 times during the flowering period in 2015, 2016, and 2017, respectively. Thirty panicles were tagged each day in each plot. The daily number of cultivars in which enough panicles could be tagged was 7–12 in 2015, 5–11 in 2016, and 8–12 in 2017. The panicles were collected at maturity and their seed sets were examined by manual inspection of ovarian development. The average sterility of panicles was calculated with respect to FC date.

To observe the morphology of anthers after anthesis, florets were sampled just after flowering. Five florets were collected from five hills in each plot. Florets were collected randomly across the rage of vertical positions on the panicles. First, we counted indehisced and longitudinally dehisced thecae and measured the length of all anthers (Fig. 1). For cylindrically dehisced thecae, we measured the length of the dehiscences that formed at the basal and apical parts of the thecae (Matsui and Kagata, 2003) and the length of the thecae by using a digital microscope (KH-7700, Hyrox, Tokyo, Japan).

To observe pollination, 15 florets were sampled at about 2 h after anthesis from 15 hills in each plot. Florets were collected randomly across the range of vertical positions on the panicles. The stigmata were detached from the florets and stained with cotton blue solution. After staining, the numbers of germinated pollen grains and total (germinated and ungerminated) pollen grains on the stigmata in each floret were counted with optical microscopes. Rice requires 10 or more germinated pollen grains or 20 or more total pollen grains on the stigmata in each floret for fertilization to occur (Satake and Yoshida, 1978; Matsui et al., 2001). We then calculated the percentage of the florets with less than 10 germinated pollen grains (LT10GP) or less than 20 total pollen grains (LT20TP) on the stigmata after anthesis, which we used as indices of pollination stability.

During the flowering period, samplings for observation of anther dehiscence and pollination were conducted every day except rainy days. Samplings were not conducted on rainy days. The daily numbers of cultivars from which enough florets could be sampled for observation of pollination was 7–12 in 2015, 5–12 in 2016, and 11–12 in 2017

Fig. 1. Dehiscences for pollen dispersal. (a) Dehiscences formed on the apical and basal parts of cylindrically dehisced thecae. ADL, length of dehiscence formed at the apical part of the theca; BDL, length of dehiscence formed at the basal part of the theca. Cultivar, Zhengdao No.5. (b) Longitudinally dehisced anther. Cultivar, Aijiaozao. (c) Indehisced anther. Cultivar, Zhengdao No.5. Bars, 300 μm.

(Fig. S1).

2.4. Plant posture for pollination

When 80 % of plants in a plot reached heading stage, the following plant posture parameters were measured for nine tillers, three each from three randomly selected hills excluding outermost two rows of each plot. Leaf height (LH) was defined as the height of the highest leaf tip of the tiller; panicle height (PH) was defined as the height of the tip of panicle; panicle angle (PA) was measured as the vertical angle of the center of the panicle part at anthesis; flowering height (FH) was defined as the height of florets at anthesis. All heights were measured as cm above the ground surface. Panicle depth (PD) was calculated as LH minus PH and flowering depth (FD) was calculated as LH minus FH. PA was measured with a split level and a protractor.

2.5. Flowering time of the day

The state of flowering in each plot was observed and recorded every 15 min from 8:30 to 14:00 every day except rainy days. The times of five phases of flowering were recorded for each plot: phase 1, when the first floret opened in the plot; phase 2 when florets started opening on more than half (observer's impression) of the hills, phase 3, when number of opening florets reached maximum, phase 4 when more than half (observer's impression) of the once opened florets started to close; and phase 5, when more than half (observer's impression) of the florets were closed. Detection of the time of phase 3 was sometimes difficult, especially when it was cloudy and the florets opened gradually, because the observer could not accurately estimate how many florets would open on that day before the end of flowering. In such cases, the time of phase 3 was recorded several times depending on the observer's impression; after phase 4 was recorded, the latest entry for phase 3 was determined as phase 3. Flowering time (FT) for each plot on each day was defined as the time when the number of opening florets reached the maximum (phase 3) in this study.

2.6. Data analysis

To assess the effects of genotype and environment on variables thought to be related to high temperature tolerance, we conducted ANOVA with combination of randomized block design and split-plot design. For sterility, pollination variables, anther morphology variables and flowering time, we adopted a split plot design with genotype as the main plot factor, date (FC date of panicle for sterility, sampling date for pollination and anther morphology variables, and measuring date for flowering time) as the sub-plot factors, randomly arranged in two blocks. Date was used as a categorical variable that represented the environment factor. We ignored year and season in analysis of the effects of genotype and environmental factors (FC date, sampling date, or flowering date) on the variables. For plant posture variables, we adopted a split-plot design with season as the main plot factor, and genotype as the subplot factor. Since we could not obtain samples from all 12 cultivars on the day depending on the state of the flowering of the plants, the cultivars sampled (i.e., the genotypes examined) differed from day to day. Thus, e did not study interactions between the factors. After confirming that one or more factors had a significant effect in ANOVA, Tukey's HSD tests were conducted; *P* values less than 0.05 were classed as statistically significant.

We conducted Pearson correlation analyses for floret sterility (FS), LT10GP, and LT20TP against variables that might be related to them: i.e., pollination variables, meteorological variables, and rice traits. Multiple regression analyses for path analysis were then conducted for the response variables FS, LT10GP, LT20TP, and FT with subsets of the above variables as explanatory variables. Finally, multiple regression analysis for FS without pollination variables (i.e., without LT10GP and LT20TP) was conducted. This enabled us to quantify the effect of rice traits on FS by avoiding the dilution of path coefficients caused by sampling errors of FS, LT10GP and LT20TP. Variables for multiple regressions were selected by the forward-backward stepwise selection method with F_{in} (the minimum partial F value that a variable must have to enter the analysis) and F_{out} (the smallest partial F value that a valuable cannot be removed from the model) set at 5.0, equivalent to *P* value of about 0.025.

All analyses were conducted with STATISTICA version 10 (Statsoft Inc., Tulsa, OK, USA). FS, LT10GP, LT20TP, percentage of longitudinally dehisced thecae (LD), and percentage of indehisced thecae (ID) were logit transformed prior to analysis (logit(FS), logit(LT10GP), logit (LT20TP), logit(LD), and logit(ID)); because LT10GP, LT20TP, LD, and ID include the value 0, empirical logit transformation was adopted for their analysis. Since, in our study, vigorous flowering continued 3 or 4 days on the panicle on after heading day, we used 3-day averages of all variables on and after heading day except FS for the regression analysis.

3. Results

3.1. Summary of microclimate

In 2015, it was windy and hot in the first half and cool in the latter half of the observation period (Fig. 2). In 2016, it was hot at the start and in the latter half of the observation period. In 2017, it was very hot in the first half of the observation period (i.e., T_{max} was >36 °C for 5 consecutive days), and it became cool in the latter half of the observation period. T_{max} ranged from 26 to 36.8 °C in the three observation periods. Daily minimum relative humidity ranged from 42.7%–84.7%, and the 10th and 90th percentiles were 50.0 % and 71.9 % in the tree observation periods, respectively. The W_{10-12} ranged from 0 to 7.3 m s^{-1}, and the 20th and 80th percentiles were 0.94 and 2.02 m s^{-1} in the three observation periods, respectively.

3.2. Floret sterility and pollination

According to ANOVA results, both genotype and FC date had significant effects on logit(FS) (Table 1). Mean FS values ranged from 11.9%–41.7% across the genotypes, and from 13.2%–43.8% across the FC dates (Fig. 3). The mean FS was high around the start of August in 2016 and 2017 and around mid-August in 2016 when the T_{max} reached over 35 °C.

Genotype and sampling date also had significant effects on logit-transformed pollination variables, LT10GP and LT20TP (Table 1). Across genotypes, mean LT10GP ranged from 9.2%–59.7%, and mean LT20TP ranged from 7.0%–51.7%, whereas across dates, mean LT10GP ranged from 1.7%–46.3% and mean LT20TP ranged from 2.4%–51.3% (Fig. 3). LT10GP and LT20TP were low around the start of August in 2015, on August 21 and 22 in 2016, and at the end of July in 2017 when the W_{10-12} was high (>2 m s^{-1}).

According to the Tukey's HSD tests (Table 1), genotype V9 had a significantly lower logit(FS) than any other genotype. Genotype V11 had a significantly lower logit(LT10GP) than any other genotype, and V9 and V10 had a significantly lower logit(LT10GP) than any other genotype except V3. Genotypes V11, V10, and V9 had a significantly lower logit(LT20TP) than any other genotype except V3. Genotypes V9, V10, and V11 correspond to Huangsiguizhan (modern *indica* cultivar), Chengnongshuijing (modern *indica* cultivar), and Zhengdao No.5 (modern *indica* cultivar), respectively. On the other hand, two genotypes form Yunnan province (V4 and V5) showed significantly higher FS than almost all other genotypes.

3.3. Anther morphology

The effects of genotype and date on the morphological variables of dehisced anther (i.e., apical dehiscence length (μm) [ADL], basal dehiscence length (μm) [BDL], logit(LD), and logit(ID) were also

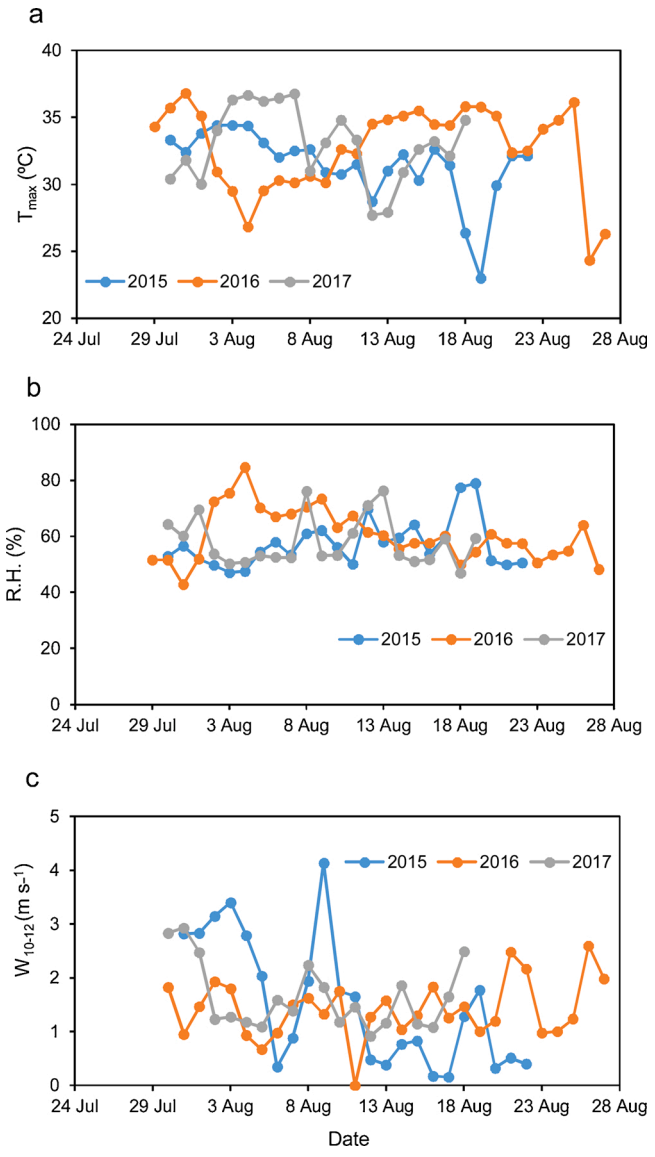

Fig. 2. Time courses of daily maximum temperature (T_{max}) (a), daily minimum relative humidity (R.H.) (b), and mean wind speed from 10:00 h to 12:00 h (W_{10-12}) (c) at the paddy field used for the present study (Yangtze University in Jingzhou, China) during the sampling periods in 2015–2017. The meteorological variables were measured at 240 cm above the ground on the south edge of the paddy field. T_{max} and W_{10-12} were selected by the forward-backward stepwise selection method in multiple regression analysis in the present study and are used here as indicators of the heat and wind of the day.

significant in ANOVA (Table 2). AL, ADL, and BDL ranged from 1816 to 2484, 399–700, and 265–463 μm, respectively, across the genotypes (Table 2) and from 1891 to 2166, 489–674, and 324–439 μm, respectively, across the sampling dates (data not shown). ID ranged from 0.8 % to 2.2 % across genotypes (Table 2) and from 0.06%–8.52% across sampling dates (data not shown).

In the Tukey's HSD tests (Table 2), genotypes V5, V8, V10, and V12, and had significantly longer AL than other genotypes; the range in AL among these four genotypes was 443 μm, while the range in AL among the other 8 genotypes was 123 μm. Genotypes V4, V5, V9, V11, and V12 had significantly smaller ADL than other genotypes; the range in ADL values among these five genotypes was 169.5 μm, while the range among the other 7 genotypes was 72.6 μm. Compared with AL, BDL values of the various genotypes were distributed more evenly across their range. Genotypes V1–V3, V6, and V7, which showed a high

Table 1
Effects on floret sterility (FS) and pollination variables (LT10GP and LT20TP): Summary of ANOVA examining the effects of genotype and date (flowering commencement for FS and sampling date for pollination variables) on these variables and Tukey's HSD test of the effects of individual genotypes on these variables.

		FS		LT10GP		LT20TP	
ANOVA F value (DF)	Factor						
	Genotype	40.4 *** (11,11)		112.3 *** (11,11)		52.1 *** (11,11)	
	Date	12.8 *** (32,512)		15.2 *** (52,1014)		13.5 *** (52,1014)	
Tukey's HSD test	Genotype						
	V1	24.8	cd	25.7	c	25.1	cde
	V2	23.5	cd	25.5	c	25.8	cde
	V3	22.8	cd	17.7	ef	17.8	ef
	V4	37.6	ab	59.7	a	51.7	a
	V5	41.7	a	39.7	b	38.4	ab
	V6	26.8	bcd	28.3	c	28.8	bcd
	V7	27.9	bcd	24.4	cd	26.4	bcde
	V8	22.4	cd	18.5	de	19.0	de
	V9	11.9	e	13.1	f	13.0	f
	V10	29.7	abc	13.5	f	11.6	fg
	V11	18.6	d	9.2	g	7.0	g
	V12	27.5	bc	37.4	b	36.8	bc

LT10GP, percentage of florets with fewer than 10 germinated pollen grains on the stigma after anthesis; LT20TP, percentage of florets with fewer than 20 pollen grains in total on the stigma after anthesis.
For FS, LT10GP and LT20TP, tests were conducted after logit transformation, but the means of the raw data are presented here.
Tukey's HSD tests: within each column, values labeled with the same letters were not significantly different from each other.
ANOVA:
*** $P < 0.001$. DF, degrees of freedom. Date indicates heading date for FS and sampling date for LT10GP and LT20TP.

dehiscence length as a proportion of anther length ((ADL + BDL)/AL, > 53 %), had significantly larger LD than the other genotypes.

3.4. Plant posture for pollination

The ANOVA analysis showed that both genotype and season had a significant effect on the variables related to plant posture for pollination (i.e., LH, PH, PD, FH, FD, and PA) (Table 3). LH ranged from 94.0–158.1 cm and PH ranged from 86.8–167.6 cm across genotypes. FH was closely correlated with PH (Table 3).

The cultivars used in this study were divided into two groups: those with around 95 cm PH (86.8–110.5 cm) and those with around 150 cm PH (133.6–167.6 cm). In high-panicle cultivars (genotypes V1–V6, V12), the PD varied from −2 to −9.5 cm, and in low-panicle cultivars (genotypes V7–V11), PD varied from 7.2–15.2 cm. The results of the Tukey's HSD tests showed that PD was significantly higher for the V7–V11 genotypes, corresponding to the modern indica varieties, Huangsiguizhan, Gu154B, Chengnongshuijing, Zhengdao No.5, Dianrui409B, respectively, than for all other genotypes. FD closely correlated with PD.

PA varied widely (12.2° to 27.0°) in high-panicle cultivars but varied little (9.3° to 11.1°) in the low-panicle cultivars. PA values for genotypes V7, V9, V10, and V11 with low panicle height, and V3 were not significantly different to that of the genotype with the lowest PA (i.e., V8) (corresponding to Gu154B).

All the plant posture characteristics examined were significantly correlated with each other; however, the correlation coefficients for PA vs. LH, PA vs. PH, and PA vs. FH were less than 0.5 (Table 3).

3.5. Flowering time

Effects of genotype and flowering date on FT were significant

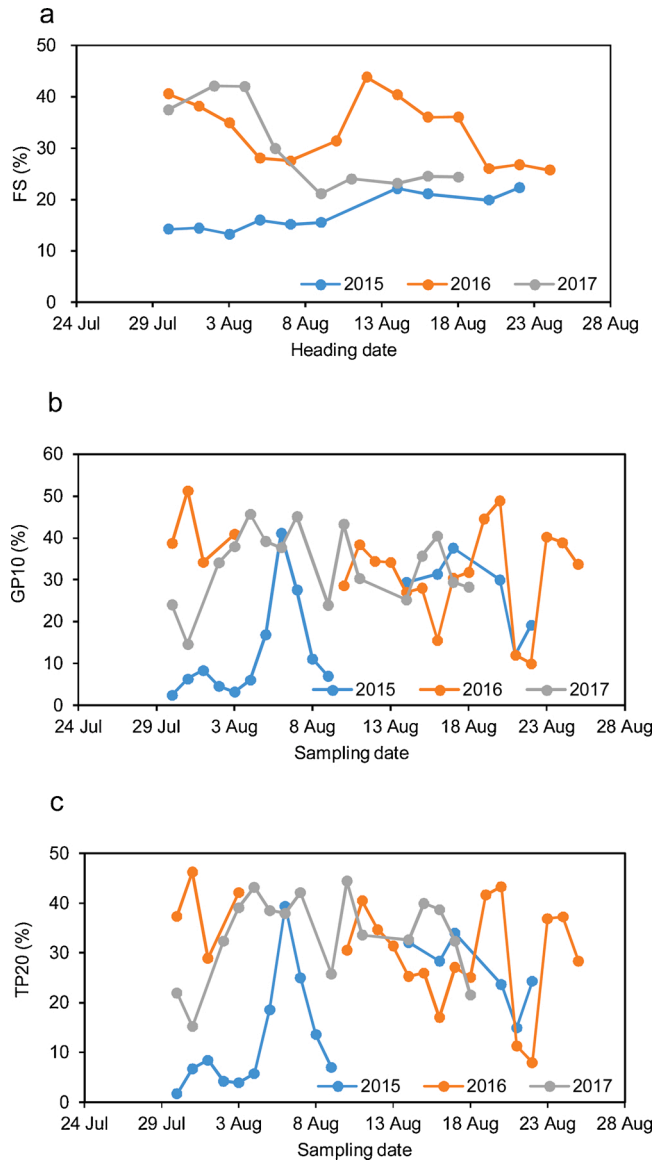

Fig. 3. Time courses of floret sterility (FS) on panicles (a), percentage of florets with fewer than 10 germinated pollen grains on the stigma after anthesis (GP10) (b), and percentage of florets with fewer than 20 pollen grains in total on the stigma after anthesis (GP10) (c).

according to ANOVA (Table 2). FT ranged from 10:02 h to 11:14 h across genotypes and from 9:47 h to 11:20 h across dates (data not shown). FT was significantly earlier and later for the V12 and V11 genotypes, respectively, than for all other genotypes, and difference in means of FTs of genotypes was within 17 min except for V12 and V11.

3.6. Correlation analysis for sterility and pollination variables

Logit(FS) showed a quadratic curve in response to T_{max} for three days after the FC date; the axis was at T_{max} =32.11 °C (Fig. 4).

For further regression analysis, we omitted samples for which one or more of the pollination measurements was missing in the three days on and after the FC date. Because we did not harvest samples for pollination or anther variables on rainy days, this meant that the regression datasets were limited to T_{max} over 31 °C, and in many cases greater than 32 °C.

In these datasets, logit(FS) was strongly correlated with logit (LT10GP) ($R = 0.61$, Table 4). Logit(FS) was also correlated with many rice traits and meteorological parameters: T_{max}, T_{mean}, T_{10-12}, logit(ID), and PA showed particularly high correlation coefficients (all $R > 0.45$, Table 4). Logit(LT10GP), in turn, was closely correlated with logit (LT20TP) ($R = 0.98$, Table 4). Logit(LT10GP) and logit(LT20TP) were strongly positively correlated with LH, PH, FH, and PA (all $R > 0.4$) and negatively correlated with BDL, W_{10-12}, $W_{flowering}$, and FT (all $R < -0.4$) (Table 4).

3.7. Multiple regression analysis for path analysis

FS was well explained with the following multiple regression equation containing meteorological variables, rice traits, and pollination variables:

$$Y = 0.2022X_1 + 0.0011X_2 + 0.1770X_3 + 0.4736X_4 + 0.6201X_5 + 0.0106X_6 - 0.46301X_7 - 14.1548 (R^2 = 0.7150,\ P < 0.0001,\ N = 134) \quad (1)$$

where Y is logit(FS), and X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are logit(LT10GP), AL (μm), T_{max} (°C), logit(ID), FT (hours from 0:00 h), FH (cm), and W_{10-12} (m s^{-1}), respectively (Table 5).

LT10GP was, in turn, well explained by LT20TP, ADL, and T_{max} as follows:

$$Y = 0.96229X_1 - 0.00083X_2 + 0.04841X_3 - 1.13224\ (R^2 = 0.964,\ P < 0.0001,\ N = 134) \quad (2)$$

where Y is logit(LT10GP), and X_1, X_2, and X_3 are logit(LT20TP), ADL (μm), and T_{max} (°C), respectively.

LT20TP was explained by meteorological variables and rice traits as follows:

$$Y = -0.5521X_1 - 0.0071X_2 - 0.9116X_3 + 0.0269X_4 + 0.0091X_5 + 0.0017X_6 - 0.0006X_7 + 7.3819 (R^2 = 0.795,\ P < 0.0001,\ N = 134) \quad (3)$$

where Y is logit(LT20TP), and X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are FT (hours from 0:00 h), BDL (μm), W_{10-12} (m s^{-1}), PA (°), FH (cm), ADL (μm), and AL (μm), respectively.

3.8. Path from exogenous and endogenous variables to sterility

We then performed path analysis based on the multiple regression data in Table 5. The results are illustrated in Fig. 5. In this analysis, the cumulative effects of the explanatory variables on logit(FS) (Table 6) were well consistent with (within 15 % of) the correlation coefficients between logit(FS) and the explanatory variables (Table 4, data repeated in Table 6); the one exception was that the magnitude of the cumulative effect of ADL was 43 % smaller than that of the correlation coefficient. T_{max}, W_{10-12}, AL, FH, and logit(ID) had great direct effects, and small indirect and correlative effects, while BDL, ADL, and PA showed no significant direct effects and large correlative effects on logit(FS) (Table 6). FT showed a great positive direct effect on logit(FS) but the negative correlative effect was even larger and seemed to surmount the positive direct effect of FT on logit(FS) such that the cumulative effect was negative.

The positive direct effect of FT on logit(FS) in path analysis is an estimate of the effect of early flowering at equivalent T_{max} and W_{10-12}, and therefore likely reflects the genetic component of the effect of early time flowering. In the present dataset, $T_{flowering}$ was approximated well by a combination of T_{max} and FT by the following multiple regression equation:

$$Y = 0.8634X_1 + 0.8500X_2 - 7.100\ (R^2 = 0.8090,\ P < 0.0001) \quad (4)$$

where Y is $T_{flowering}$ (°C), X_1 is T_{max}, and X_2 is FT (hours from 0:00). This equation means that at given T_{max}, the temperature around flowering increased by 0.850 °C per hour in FT.

3.9. Multiple regression analysis to estimate the effect of meteorological variables and rice traits on heat tolerance

The path coefficient between logit(LT10GP) and logit(FS) was low

Table 2
Effects on anther characteristics after flowering and flowering time: Summary of ANOVA examining the effects of genotype and date (sampling date) on these characteristics, and Tukey's HSD test of the effects of individual genotypes on these characteristics.

		Anther Length (μm)	Length of Apical dehiscence (μm)	Length of Basal dehiscence (μm)	Longitudinally dehisced thecae (%)	Indehisced Thecae (%)	Flowering Time (hrs)
ANOVA F value (DF)	Factor						
	Genotype	213.19 *** (11,11)	168.00 *** (11,11)	90.68 *** (11,11)	96.07 *** (11,11)	5.86 *** (11,11)	138.40 *** (11, 11)
	Date	3.09 *** (55, 1072)	7.18 *** (55, 1072)	3.66 *** (55, 1072)	3.50 *** (55, 1072)	2.96 *** (55, 1072)	26.96 *** (55, 1065)
Tukey's HSD test	Genotype						
	V1	1852.6 ef	662.7 abc	381.0 cd	8.0 c	1.1 bc	10:29 def
	V2	1902.4 de	666.9 ab	446.1 ab	20.7 a	1.0 bc	10:24 ef
	V3	1895.6 de	626.7 c	411.3 bc	8.3 bc	0.9 bc	10:33 bcd
	V4	1850.1 ef	398.5 f	264.7 g	1.5 e	1.7 ab	10:31 cde
	V5	2328.9 b	484.4 e	308.3 f	1.5 e	1.8 ab	10:02 f
	V6	1939.3 d	646.3 bc	392.2 cd	6.0 c	1.0 bc	10:22 def
	V7	1897.6 de	695.7 a	308.6 f	12.7 b	2.2 a	10:28 bc
	V8	2040.8 c	655.0 bc	364.1 de	2.4 de	1.2 abc	10:37 bcd
	V9	1843.2 ef	568.0 d	342.9 fg	2.4 de	1.1 abc	10:34 bc
	V10	2483.8 a	665.2 abc	462.7 a	2.1 de	0.8 c	10:37 b
	V11	1816.2 f	474.4 e	438.2 ab	3.4 d	0.9 bc	10:39 a
	V12	2049.5 c	534.6 d	332.4 ef	1.8 e	1.4 abc	11:14 g

For LD and ID, tests were conducted after logit transformation, but the means of the raw data are presented here.
Tukey's HSD tests: within each column, values labeled with the same letters were not significantly different from each other.
ANOVA:
*** $P < 0.001$.

(Table 5 and Fig. 5). Sampling error of LT10GP, which was probably in part generated from uncertainty of pollination in the field and a limited number of samples, may have diluted the path coefficient values. Underestimation of this path coefficient may cause underestimation of the effects of variables on logit(FS) via logit(LT10GP). To clarify the traits responsible for occurrence of sterility avoiding the effect of underestimating the path coefficient between logit(LT10GP) and logit(FS), we conducted a multiple regression analysis with meteorological and rice traits, without pollination parameters. FS was well explained without variables relating to pollination stability by the following equation:

$$Y = 0.2239X_1 - 0.5883X_2 + 0.0080X_3 + 0.0010X_4 - 0.0027X_5 + 0.0238X_6 + 0.3469X_7 - 13.7310 \ (R^2 = 0.640, P < 0.0001) \quad (5)$$

where Y is logit(FS) and X_1, X_2, X_3, X_4, X_5, X_6, and X_7 are T_{max} (°C), W_{10-12} (m s^{-1}), FH (cm), AL (μm), BDL (μm), PA (°), and FT (hours from 0:00 h), respectively (Table 7). T_{max} and W_{10-12} showed large standardized partial correlation coefficients (β), and β of W_{10-12} was equal to or greater than that of T_{max}. Thus, the effect of wind on logit(FS) seemed to be equal to or greater than that of temperature (Tables 7 and 8). FH, PA, AL, and BDL showed significant *P*-values and high β values (Table 7). Thus, we can conclude that these traits could be used effectively to lessen the occurrence of HIFS.

4. Discussion

4.1. Fitting of the multiple regression model in path analysis

In the present path analysis, FS, LT10GP, and LT20TP were well explained by a multiple regression model with the adopted explanatory variables with F_{in} and F_{out} of 5.0 at $R^2 > 0.71$ (Table 5). The cumulative effects of all adopted explanatory variables from path coefficients, except ADL, fell within the range approximately ± 15 % of the correlation coefficient obtained by single regression analysis (Table 6). Considering the uncertainty associated with pollination and sampling errors in field experiments, the agreement between the cumulative effects of variables adopted in the path analysis and the correlation coefficient with FS is good. The agreement means that our regression model well covers the exogenous and endogenous variables and paths involved in FS and pollination stability under the conditions we examined. The path coefficient between logit(LT10GP) and logit(FS) was, however, lower than we expected from our former study (Matsui and Hasegawa, 2019). Sampling error of LT10GP, which was probably in part generated from uncertainty of pollination in the field and a limited number of samples, may have diluted the path coefficient. Underestimation of this path coefficient, in turn, would cause underestimation of the effects of variables on logit(FS) via logit(LT10GP). The large difference between path coefficients in path analyses and β values of the multiple regression analysis when these analyses were conducted without the pollination parameters in BDL and PA that showed only indirect effects in the path analysis (Table 7), supports the notion that the path coefficient between logit(LT10GP) and logit(FS) was underestimated due to sampling errors of LT10GP and FS.

4.2. Meteorological factors inducing floret sterility

In controlled experiments, atmospheric temperatures over 35 °C have been shown to induce HIFS in rice (Satake and Yoshida, 1978; Matsui et al., 2001; Jagadish et al., 2007; Weerakoon et al., 2008). Here, we found that occurrence of high FS (>40 %) (Fig. 3a) coincided with T_{max} over 35 °C (Fig. 2a), and that logit(FS) was significantly correlated with T_{max} (Tables 5, 6, 8; Fig. 4), indicating that the moderately high atmospheric temperatures (32–36.5 °C) in our field experiment were sufficient to cause HIFS.

On the basis of controlled experiments, the primary direct cause of HIFS has been considered to be a decrease in the number of germinated pollen grains on the stigma (Satake and Yoshida, 1978; Matsui et al., 2001; Shi et al., 2018; Matsui et al., 2020): e.g., FS correlated with LT10GP under a fixed day temperature of 38 °C in Satake and Yoshida (1978). Our path analysis, however, highlights that, under the present experimental field conditions, T_{max} directly affected FS (Fig. 5), rather than just having indirect effects through pollination and pollen germination. This finding implies that high temperature affected a process after pollination and pollen germination.

In contrast to the controlled experiments, which adopted fixed day temperatures, the temperature in field conditions increases around FT in the morning until reaching the T_{max} in the afternoon. The high

Table 3
Effects on plant posture variables: Summary of ANOVA examining the effects of genotype and season on these variables, Tukey's HSD test of the effects of individual genotypes and individual seasons on these variables, and pair-wise correlation coefficients between these variables.

		LH		PH		FH		PD		FD		PA	
ANOVA F value (DF)	Factor												
	Season	11.15 (11,11)	***	10.86 (11,11)	***	9.62 (11,11)	***	1.88 (11,11)	ns	2.28 (11,11)	ns	14.91 (11,11)	***
	Genotype	115.88 (11,185)	***	266.03 (11,185)	***	294.69 (11,185)	***	44.48 (11,185)	***	52.34 (11,185)	***	11.89 (11,185)	***
Tukey's HSD test	Genotype												
	V1	135.7	cd	141.8	cd	132.8	d	−6.1	cd	2.9	ef	22.5	abc
	V2	128.1	de	133.6	d	124.1	e	−5.6	cd	4.0	de	18.5	bcd
	V3	138.7	c	142.4	c	131.4	d	−3.6	cd	7.4	d	12.2	de
	V4	148.4	b	153.4	b	146.0	b	−5.0	cd	2.4	ef	27.0	a
	V5	158.1	a	167.6	a	158.1	a	−9.5	d	0.1	f	20.0	bc
	V6	147.5	b	149.5	bc	140.5	bc	−2.0	c	7.0	d	24.4	ab
	V7	94.0	g	86.8	g	75.8	h	7.2	b	18.2	bc	9.7	f
	V8	102.3	fg	91.6	fg	80.6	gh	10.6	ab	21.7	b	9.3	f
	V9	111.0	f	95.8	f	84.2	g	15.2	a	26.8	a	10.3	f
	V10	120.5	e	110.5	e	99.6	f	10.0	ab	20.9	b	11.1	ef
	V11	100.0	g	92.7	fg	83.5	g	7.3	b	16.5	c	10.2	f
	V12	137.2	c	144.0	c	133.5	cd	−6.8	cd	3.7	de	17.1	cde
Tukey's HSD test	Season												
	2015 – 1	117.3	e	118.3	e	109.9	e	−1.0	cd	7.4	d	15.8	abcd
	2015 – 2	127.3	bc	126.6	cd	116.3	bcde	0.7	bcd	11.0	bcd	11.9	bcd
	2015 – 3	133.0	b	131.4	abc	121.1	ab	1.4	bcd	11.9	abcd	10.5	cd
	2015 – 4	130.9	bc	134.7	ab	123.6	ab	−3.8	d	7.28	d	9.6	d
	2016 – 1	119.4	de	120.5	de	111.3	de	−1.1	cd	8.1	cd	21.7	a
	2016 – 2	128.1	bc	128.6	bc	118.4	bcd	−0.5	cd	9.7	bcd	21.7	a
	2016 – 3	126.6	bcd	121.1	de	111.9	cde	5.5	ab	14.7	ab	17.3	abc
	2016 – 4	133.8	b	126.5	cd	116.7	bcde	7.2	a	17.1	a	9.1	d
	2017 – 1	128.7	bc	129.3	bc	118.6	bc	−0.5	cd	10.1	bcd	17.8	ab
	2017 – 2	129.4	bc	127.4	bcd	116.4	bcde	2.1	abc	13.0	abc	15.4	abcd
	2017 – 3	122.7	bd	121.9	de	111.9	cde	0.9	bcd	10.8	bcd	19.4	ab
	2017 – 4	143.7	a	137.6	a	126.5	a	6.1	ab	17.1	a	17.9	ab
Pearson correlation analysis *R*-value													
	Variable	LH		PH		FH		PD		FD		PA	
	LH			0.9643	***	0.9600	***	−0.5893	***	−0.5931	***	0.3755	***
	PH	0.9552	***			0.9974	***	−0.7823	***	−0.7780	***	0.4660	***
	FH	0.9488	***	0.9920	***			−0.7844	***	−0.7949	***	0.4996	***
	PD	−0.5443	***	−0.7683	***	−0.7593	***			0.9781	***	−0.5381	***
	FD	−0.5434	***	−0.7617	***	−0.7637	***	0.9833	***			−0.6226	***
	PA	0.4614	***	0.5567	***	0.5830	***	−0.5798	***	−0.6462	***		

LH, leaf height (cm); PH, panicle height (cm); FH, height of florets in anthesis (cm), PD, panicle depth in canopy (cm); FD, depth of florets in anthesis in canopy (cm); and PA, vertical angle of panicle (°).
Flowering Period, Seeds of each cultivars were sown 4times in each year for successive flowering in hot periods in the summers.
Tukey's HSD tests: For each column, values labeled with the same letters are not significantly different from each other.
Pearson correlation analysis: *R* values in the upper half are between data in each season in each year (N = 108) and those in the lower left are between the 3-day means (N = 134) that we used in the multiple regression analysis.
ANOVA: $^{*}P < 0.05$.
$^{***}P < 0.001$.

temperature after FT may disturb the process after pollen germination, thereby increasing the number of pollen grains necessary for fertilization. Moreover, the fixed day temperatures used in the above mentioned controlled experiments (37.0, 37.5, and 38 °C) are higher than the T_{max} values (26.0–36.8 °C) in the present field experiment. The $T_{flowering}$ values in the present experiment (27.5–34.8 °C) may not be enough to induce poor pollination. The low effect of high temperature on LT10GP and the direct effect of high temperature on FS may also decrease the path coefficient between logit(LT10GP) and logit(FS) and contribute to this coefficient being lower than expected.

W_{10-12} and T_{max} had negative and positive direct effects on logit(FS), respectively; the magnitude of the effect of W_{10-12} was similar to or greater than that of T_{max} (Table 6, 8). W_{10-12} also negatively and strongly affected logit(LT20TP) (Table 6, Fig. 5) consistent with our recent report with a simple model experiment in the field that wind at flowering makes pollination stable (Matsui et al., 2020). Because the estimate of the effect of logit(LT10GP) on logit(FS) was low, and W_{10-12} positively affected FT (Fig. 5), the direct effect of W_{10-12} on logit(FS) was estimated to be much greater than the indirect effect on logit(FS) (Table 6). Wind decreases the panicle temperature and the canopy temperature by transpirational cooling (Matsui et al., 2014, Yoshimoto et al., 2011) and wind has been shown to decrease the occurrence of HIFS via transpirational cooling under extremely hot conditions (>40 °C) in a flood-irrigated field in Australia (Matsui et al., 2014). Transpirational cooling may be one means by which wind mitigated HIFS in the present study.

4.3. Anther traits affecting occurrence of FS

Length of the anther and anther dehiscence (i.e., AL, ADL, and BDL) affected pollination stability (i.e., logit(LT10GP) and logit(LT20TP)) and logit(FS) (Fig. 5, Tables 6 and 7). Our path analysis showed that BDL

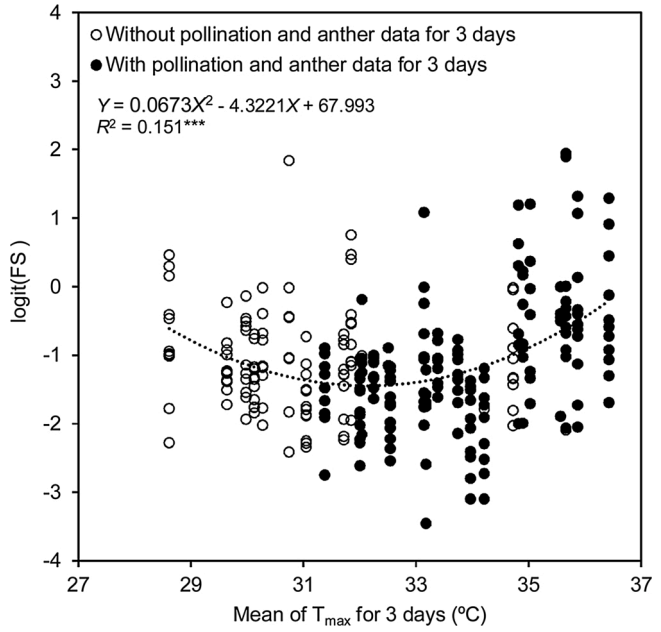

Fig. 4. Relationship between mean daily maximum temperature (T_{max}) for three days (heading date and following two days) in the main flowering period of panicles and logit-transformed floret sterility (logit(FS)). The relationship between T_{max} and FS was a quadratic curve with axis at 32.1 °C. Only the FS data of panicles for which pollination and anther data for three days during full flowering were available (closed circles) were used for multiple regression analysis.

strongly and negatively affected logit(LT20TP) but did not directly affect logit(FS) or logit(LT10GP), whereas ADL positively affected logit (LT20TP) and negatively affected logit(LT10GP). By using chromosome segment substitution lines with various values for ADL and BDL, Matsui and Hasegawa (2019) showed that the effect of BDL on LT20TP and FS was greater than that of ADL. When the anther dehisces cylindrically, pollen grains drop from the apical and/or basal dehiscence (Matsui and Kagata, 2003). Because the distance from the stigma is smaller for basal dehiscence than for apical dehiscence, arrival of pollen grains from the basal dehiscence is more stable.

The slope of −0.0071 for the relationship between BDL and logit (LT20TP) in Eq. (3), and the slope of −0.0027 for the relationship between BDL and logit(FS) in Eq. (5) show that elongation of BDL by 100 μm would improve LT20TP by around 15 % when LT20TP is around 50 %, and would improve FS by around 6.74 % when FS is around 50 %. These estimations agree well with previous reports that seed set increased by 6.7 % (Zhao et al., 2010) and 15.5 % (Tian et al., 2010) as BDL increased by 100 μm.

AL negatively affected logit(LT20TP) but positively affected logit(FS) directly in each multiple regression analysis (Fig. 5, Table 5) and, totally, positively affected logit(FS) in the path analysis (Table 6, direct + indirect effect). Therefore, a short anther may be a practical trait to improve heat tolerance at the flowering stage. Because AL correlates with number of pollen grains in the anther (Nishiyama, 1981; Suzuki, 1982), a long anther is considered advantageous for stable fertilization under cool conditions (Nishiyama, 1981; Suzuki, 1982). Although negative correlation between AL and logit(LT20TP) agree with the results of former studies that found that the number of pollen grains in a rice anther is proportional to the anther length, the mechanism by which short anther is advantageous for fertility is unclear. Here, AL was negatively correlated with logit(LD) ($R = -0.43$, $P < 0.0001$, data not shown) probably because of an increase in the proportion of dehiscence to AL ((ADL + BDL)/AL, 3.3 Anther morphology). We consider that LD is advantageous for quick pollen release and contributes to the process after pollen germination. Time of transit of pollen grains to the stigma

Table 4
Correlation coefficients for logit(FS) and pollination variables (logit(LT10GP) and logit(LT20TP)) versus exogenous and endogenous variables.

	Logit (FS)	(n = 134)	Logit (LT10GP)	(n = 134)	Logit (LT20TP)	(n = 134)
Pollination						
Logit (LT10GP)	0.6133	***				
Logit (LT20TP)	0.5530	***	0.9763	***		
Meteorological variables						
T_{max}	0.4953	***	0.2895	***	0.2293	**
T_{mean}	0.4891	***	0.1966	*	0.1161	ns
T_{10-12}	0.4810	***	0.2089	*	0.1264	ns
$T_{Flowering}$	0.4203	***	0.0673	ns	−0.0254	ns
W_{10-12}	−0.4401	***	−0.5307	***	−0.5137	***
$W_{Flowering}$	−0.4074	***	−0.5198	***	−0.5133	***
Rice traits						
AL	0.3573	***	0.0180	ns	0.0162	ns
ADL	−0.3725	***	−0.3144	***	−0.2388	***
BDL	−0.3216	***	−0.5680	***	−0.5858	***
Logit(LD)	−0.2939	***	−0.2044	*	−0.1857	*
Logit(ID)	0.4660	***	0.3931	***	0.3436	***
LH	0.3874	***	0.4499	***	0.4291	***
PH	0.4003	***	0.4591	***	0.4487	***
FH	0.4048	***	0.4530	***	0.4403	***
PD	−0.2895	***	−0.3204	***	−0.3353	***
FD	−0.3170	***	−0.3225	***	−0.3314	***
PA	0.4680	***	0.4756	***	0.4770	***
FT	−0.3139	***	−0.5541	***	−0.5905	***

Logit(FS), logit-transformed percentage of floret sterility, logit(LT10GP), logit-transformed percentage of florets with fewer than 10 germinated pollen grains on the stigma after anthesis; logit(LT20TP), logit-transformed percentage of florets with fewer than 20 pollen grains in total on the stigma after anthesis. T_{max}, daily maximum temperature (°C); T_{mean}, daily mean temperature (°C); T_{10-12}, mean temperature from 10:00 h to 12:00 h (°C); $T_{Flowering}$, mean temperature for 1 h from 30 min before to 30 min after flowering time (°C); W_{10-12}, mean wind speed from 10:00 h to 12:00 h (m s^{-1}); $W_{Flowering}$, mean wind speed for 1 h from 30 min before to 30 min after flowering time (m s^{-1}); AL, anther length (μm); ADL, apical anther length (μm); BDL, basal dehiscence length (μm); logit(LD), logit-transformed percentage of longitudinally dehisced thecae; logit (ID), logit-transformed percentage of indehisced thecae; LH, leaf height (cm); PH, panicle height (cm); FH, height of florets in anthesis (cm); PD, panicle depth in canopy (cm); FD, depth of florets in anthesis in canopy (cm); PA, vertical angle of panicle (°).
* $P < 0.05$;
** $P < 0.01$;
*** $P < 0.001$; ns, not significant.

may be more important under high than cool temperatures. The slope of 0.0010 for the relationship between AL and logit(FS) in Eq. (5) shows that shortening of AL by 500 μm would improve the FS by 12.4 % when the FS is around 50 %.

Quantitative trait loci for AL and BDL have been detected, and their effects on AL and BDL have been estimated (Tazib et al., 2015; Zhao et al., 2016a, 2016b). For hybrid rice, inheritance of anther dehiscence from restorers and cytoplasmic male-sterile lines to the F_1 generation has been clarified. It is possible to improve the tolerance of rice to sterility depending on these results.

4.4. Flowering time

The effects of FT on FS seem complex but agree with preceding studies, as described below. FT had a positive direct effect on logit(FS) in the path analysis. However, the simple correlation coefficient between FT and logit(FS) was negative. Variables such as T_{max}, FH, and logit(ID) were negatively correlated with FT and positively correlated with logit (FS). Thus, FT showed a negative correlation with logit(FS) through its relationship with these factors despite having a positive direct effect on logit(FS). High temperatures hasten flowering time (Kobayasi et al.,

Table 5
Summary of multiple regression analysis of logit(FS), pollination variables (logit(LT10GP) and logit(LT20TP)), PA, and FT used for path analysis (Table 7).

Equation	Response variable	Explanatory variable	Regression coefficients	β†	Partial correlation coefficient	*P* value	(Number of datasets, coefficient of determination, *P*-value for equation)
1	Logit(FS)	Logit (LT10GP)	0.2022	0.2439	0.2515	0.004185	(134, 0.715, < 0.000001)
		AL	0.0011	0.2731	0.4277	<0.000001	
		T_{max}	0.1770	0.2717	0.4077	0.000002	
		Logit(ID)	0.4736	0.3417	0.4626	<0.000001	
		FT	0.6201	0.2661	0.3660	0.000021	
		FH	0.0106	0.3302	0.4210	<0.000001	
		W_{10-12}	−0.4630	−0.2815	−0.3504	0.000050	
		Intercept	−14.1548			<0.000001	
2	Logit (LT10GP)	Logit (LT20TP)	0.96229	0.9430	0.9782	<0.000001	(134, 0.964, < 0.000001)
		ADL	−0.00083	−0.0802	−0.3772	0.000008	
		T_{max}	0.04841	0.0616	0.2991	0.000493	
		Intercept	−1.13224			0.021842	
3	Logit (LT20TP)	FT	−0.5521	−0.2004	−0.3370	0.000100	(134, 0.795, < 0.000001)
		BDL	−0.0071	−0.4594	−0.6503	<0.000001	
		W_{10-12}	−0.9116	−0.4690	−0.6848	<0.000001	
		PA	0.0269	0.2167	0.3493	0.000053	
		FH	0.0091	0.2399	0.3668	0.000021	
		ADL	0.0017	0.1702	0.2833	0.001193	
		AL	−0.0006	−0.1179	−0.2397	0.006419	
		Intercept	7.3819			0.000013	
	FT	W_{10-12}	0.0070	0.2420	0.2633	0.002196	(134, 0.262, <0.000001)
		FH	−0.0002	−0.3340	−0.3621	0.000018	
		T_{max}	−0.0030	−0.2570	−0.2781	0.001193	
		Intercept	0.5512			<0.000001	

Logit(LT10GP), logit-transformed percentage of florets with fewer than 10 germinated pollen grains on the stigma after anthesis; logit(LT20TP), logit-transformed percentage of florets with fewer than 20 pollen grains in total on the stigma after anthesis.
AL, anther length (μm); T_{max}, daily maximum temperature (°C); logit(ID), logit transformed percentage of indehisced thecae; FT, flowering time (hours from 0:00 h); FH, height of florets in anthesis (cm); W_{10-12}, average wind speed from 10:00 h to 12:00 h (m s^{-1}); ADL, apical dehiscence length of thecae (μm), BDL, basal dehiscence length of thecae (μm); PA, vertical angle of panicle (°).
† Standardized partial regression coefficient.

2009; Julia and Dingkuhn, 2012), thereby avoiding some of the deleterious effects of high temperature on florets (Julia and Dingkuhn, 2012; van Oort et al., 2015a, 2015b). The positive correlation between T_{max} and FT may reflect such a shift of FT with temperature, i.e., resultant escape from high temperature effects.

We consider that the positive direct effect of FT on logit(FS) in path analysis shows the genetic effect of early flowering on FS. In the present experiment, $T_{flowering}$ increased at 0.85 °C per hour of FT (Eq. (4)). In previous controlled experiments, an increase in temperature 1 °C around flowering increased HIFS by around 25 % (Satake and Yoshida, 1978), 20 % (Weerakoon et al., 2008), or 15 % (Matsui et al., 2010). The slope of 0.62 for the relationship between FT and logit(FS) in Eq. (1) shows that FS would be predicted to increase by about 15 % per hour of FT when FS is about 50 %. Thus, a major part of the positive direct effect of FT on logit(FS) may be due to increase in temperature at flowering time as the flowering time becomes later. These results support the theory of Nishiyama and Blanco (1980) that flowers could avoid high temperatures by early morning flowering and agree with studies that showed the advantage of early morning flowering under high-temperature conditions (Ishimaru et al., 2012).

In path analysis, FT showed a negative effect on logit(LT20TP) (path coefficient, −0.200) (Fig. 5, Table 5). The mechanism behind this negative effect is unclear. FT is an important factor in HIFS, and thus clarification of the mechanism by which it contributes is required.

A QTL for early morning flowering has been detected (Hirabayashi et al., 2015) and the positive effect of early morning flowering on floret fertility has been shown using a near isogenic line containing the QTL for early morning flowering in the dry season in the tropics with a large diurnal temperature range (Bheemanahallia et al., 2017). The present study showed the effect with much higher genetic diversity under humid conditions with a small diurnal temperature range. Early morning flowering is therefore a practical breeding objective for rice grown under various meteorological conditions.

4.5. Plant posture for pollination

The environment changes drastically around the canopy surface compared with under or above the canopy, and thus the vertical position where anthesis occurs (i.e., FD and PD) is an important factor that affects the stability of pollination and floret fertility (Matsui et al., 2020). In the current experiment, FH was chosen from among plant posture variables relating to flowering position in the canopy (i.e., LH, FH, FD, PH, and PD) by forward-backward stepwise selection for inclusion in the multiple regression model of logit(FS) and of logit(LT20TP); FH showed the highest F-in value for logit(FS) and logit(LT20TP). However, when interpreting the results of the present experiment, FH should be regarded as representative of the above group of four plant posture parameters, since they were all highly correlated with each across the genotypes examined here (Table 3). We note that the area of each plot was 6 m^2 (2 by 3 m), which may not be enough to generate the effect of canopy on pollination and fertilization; this may have contributed to the lower magnitude of the correlation between FD and logit(FS) compared with FH and logit(FS). The positive effect of FH on LT20TP and FS agrees with the results of Matsui et al. (2020) that high florets are disadvantageous for pollination and seed set if the florets are higher than the canopy.

Panicle angle also showed positive indirect effect on logit(FS) via logit(LT20TP) in the path analysis (Table 6, Fig. 5), and on logit(FS) in multiple regression analysis without pollination parameters (Table 7).

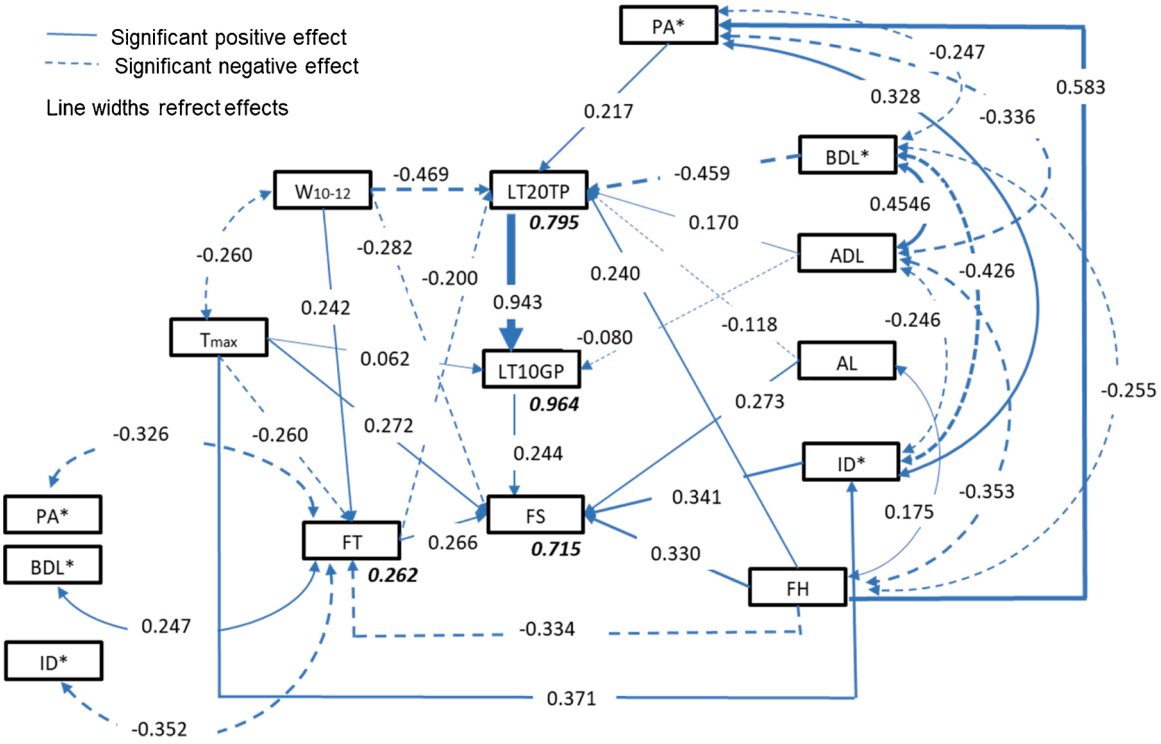

Fig. 5. Path diagram of the effects of various variables on floret sterility (FS) based on the results of multiple regression analyses in Table 6 for the response variables FS, percentage of florets with fewer than 20 total pollen grains on the stigma after anthesis (LT20TP), percentage of florets with fewer than 10 germinated pollen grains on the stigma after anthesis (LT10GP), and flowering time (FT) in rice in a hot, humid, and low-wind paddy field in Jianghan Basin, China. Straight arrows show directions of effects in paths. Bidirectional curved arrows represent the significant correlations that were not adopted in regression analysis. Values in plain type adjacent to straight arrows are standardized partial regression coefficients shown in Table 6 and adjacent to bidirectional curved lines are regression coefficients; values in bold italics are coefficients of determination by multiple regression shown in Table 6. Values shown in this figure are statistically significant ($P < 0.05$). *To aid clarity, factors marked with asterisks are described in two locations in this diagram.

Table 6
Effects of exogenous[†] and endogenous[††] factors on floret sterility (FS) in path analysis: Effects were calculated based on the results of the multiple regression analysis for response variables, FS, LT10GP, LT20TP, and FT in Table 5.

Factors		Direct effect	Indirect effect[†††]	Direct + indirect effect	Correlative effect[††††]	Cumulative effect[†††††]	Correlation coefficient with FS[††††††]
Exogenous factors							
Meteorology							
	T_{max}	0.2717	0.0999	0.3716	0.0873	0.4589	0.4953
	W_{10-12}	−0.2815	−0.0545	−0.3360	−0.1135	−0.4495	−0.4401
Morphology							
	AL	0.2731	−0.0271	0.2460	0.0636	0.3096	0.3573
	ADL	–	0.0196	0.0196	−0.2318	−0.2122	−0.3725
	BDL	–	−0.1057	−0.1057	−0.2354	−0.3411	−0.3216
	FH	0.3302	0.0099	0.3401	0.0630	0.4031	0.4048
Endogenous factors							
	ID	0.3417	–	0.3417	0.1655	0.5073	0.4660
	PA	–	0.0498	0.0498	0.3807	0.4306	0.4680
	FT	0.2661	−0.0460	0.2200	−0.5356	−0.3156	−0.3139

T_{max}, daily maximum temperature (°C); W_{10-12}, average wind speed from 10:00 to 12:00 h (m s^{-1}); AL, anther length (μm); ADL, apical dehiscence length (μm); BDL, basal dehiscence length (μm); FH, flowering height (cm from the ground surface); ID, percentage of indehisced thecae; PA, panicle angle (°); FT, flowering time (hours from 0:00 h).

ID was logit transformed prior to path analysis.

[†] of which value is determined by the state of other variables in the system.

[††] of which value is independent from other variables in the system.

[†††] Effect of factor on FS via endogenous factors such as LT10GP, LT20TP, and FT (the product of standardized partial regression coefficients on the path from the factor to FS).

[††††] Effect of factor on FS via exogenous factors correlated with the factor under investigation (the sum of products of correlation coefficient between the variables under investigation and the exogenous variables and sum of direct and indirect effects of the exogenous factors to FS).

[†††††] Total of direct, indirect, and correlative effe cts.

[††††††] Correlation coefficients displayed in Table 5.

Table 7
Summary of multiple regression analysis without pollination variables for estimating the effects of various traits on FS.

Explanatory variables	Regression coefficients	β†	Partial correlation coefficient	*P*-value
Meteorology				
T_{max}	0.2239	0.3435	0.4539	<0.000001
W_{10-12}	−0.5883	−0.3577	−0.4806	<0.000001
Rice trait				
FH	0.0080	0.2476	0.2956	0.000707
AL	0.0010	0.2439	0.3589	0.000032
BDL	−0.0027	−0.2050	−0.3047	0.000470
PA	0.0238	0.2266	0.2770	0.001548
FT	0.3469	0.1488	0.2043	0.020699
Intercept	−13.7310			<0.000001

$n = 134$, $R^2 = 0.640$, $P < 0.000001$.
T_{max}, daily maximum temperature (°C); W_{10-12}, average wind speed from 10:00 to 12:00 h (m s^{-1}); FH, height of florets in anthesis (cm); AL, anther length (μm); BDL, basal dehiscence length of thecae (μm); PA, vertical angle of panicle (°); FT, flowering time (hours from 0:00 h).
† Standardized partial correlation coefficient.

These results are in agreement with the finding of Win et al. (2019) who showed that, in a simple model experiment using inclined pots set in a field, inclination of panicles increased FS through instable pollination. The indirect effect of PA on FS through pollination variables was small (Table 6) but may be underestimated because of the small path coefficient between LT10GP and FS.

The slopes of 0.0080 for FH and 0.0238 for PA in Eq. (5) show that decreasing FH by 50 cm and decreasing PA angle by 10° would improve the FS by 10 % and 5.7 %, respectively. Both low florets and erect panicles at flowering seem practical and effective traits for tolerance to high temperature at flowering.

In practical breeding programs, PH, which is highly correlated with FH, is a much more convenient marker of FH than FH itself. That is because PH can be easily estimated even at harvest time. The trait of low panicle height in the canopy, which means that the panicle does not interrupt the sun radiation to the upper leaves, and the trait of large panicle, which enables a greater sink for high yield, have already been incorporated in the "super hybrid rice" breeding program in China and partially in the International Rice Research Institute's "New Plant Type" breeding program (Peng et al., 2008). However, extremely large panicle size, which induces inclination of the panicle even at flowering stage, should be reviewed for rice production under high temperature conditions.

5. Conclusion

The mechanism responsible for the differences in occurrence of HIFS between cultivars was not simple; rather there was a complex combination of many factors such as flowering time, anther morphology, plant posture and pollination that significantly influenced HIFS. We consider that research into and screening of such visible traits would be more feasible in comparison with physiological traits in breeding programs.

The present study showed that low FH, PA, and AL decreased the occurrence of HIFS; the magnitudes of these impacts are equivalent to those of known heat tolerance traits, such as BDL and FT, under practical paddy field conditions. This information will be available for breeding of rice for advantageous plant shape under global warming.

The study showed that moderately high temperatures (32–36.5 °C) induce FS in combination with low wind. The study also demonstrated that high temperatures increase the occurrence of HIFS through processes after pollen germination, contradicting the previous theory that high temperatures affect HIFS mainly through pollination, although the stable pollination still mitigated the occurrence of HIFS. The heat tolerance of the processes after pollen germination will be an important research subject to mitigate the effects of global warming on rice production.

Author contributions

Tsutomu Matsui: Conceptualization, Formal analysis, Investigation, Writing–original draft, Funding acquisition, Kazuhiro Kobayasi: Investigation, Writing–review & editing. Mayumi Yoshimoto: Investigation, Writing–review & editing. Toshihiro Hasegawa: Writing–review & editing. Xiaohai Tian: Investigation, Project administration, Resources, Writing–review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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