Panicle Inclination Influences Pollination Stability and Floret Sterility in Rice (Oryza sativa L.)

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(イネの穂の傾斜が受粉の安定性と不稔発生に及ぼす影響)

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The United Graduate School of Agricultural Science, Gifu University

Science of Biological Environment

(Gifu University)

Aung Win

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Dissertation summary

Rice is a self-pollinated crop and its flowering and pollination normally occur for about one hour around 10:00 to 12:00. Rice anthers are located just above the stigma at the start of flowering and the anther dehisces and pollination is completed when the pollens fall due to gravity. For this reason, rice pollination has been considered to be stable unless it is under extreme environmental conditions. However, on the other hand, it is known that a slight difference in floral character affects the pollination stability of rice and the stress tolerance of pollination. In this study, it was clarified that a slight inclination of the panicle greatly affects the stability of rice pollination, and showed the importance of rice panicle being upright at the time of flowering.

In the first study, we investigated the effect of the difference in panicle angle on pollination under isolated conditions using pot-grown rice plants. Twenty uniform rice (IR72) seedlings at around the 5-leaf stage were transplanted in to 4-L pots in a circular pattern, and grown to heading stage in a flooded state. The pots of rice at the flowering stage were tilted one of four inclinations (0 ° =control, 15 °, 30 ° and 45 °). The florets were sampled after flowering, and total and germinated pollen grains deposited on the stigma were investigated. At 30 ° inclined treatment, the percentage of flowers with less than 20 total pollen grains on the stigma (TP20) and the percentage of flowers with less than 10 germinated pollen grains on the stigma (GP10) were significantly affected. The average value increased by 14% in TP20 and 24% in GP10. For fertilization of rice, more than 10 germinated pollens are required, and an increase in GP10 of 24% corresponds to a decrease in seed set of 24%. In addition, a strong positive correlation was observed between TP20 and GP10, indicating that the increase in GP10 was mainly due to the increase in TP20. From these results, it is considered that the inclination of 30 ° or more of rice florets could induce floret sterility and affect yield through unstable pollination. Based on the morphological aspect and the mechanism of pollination, the inclination of the floret is a factor that impairs the stability of

pollination. We proposed the three possible factors that impair the stability of pollination: 1) decrease in gravity force to move the pollen grain in the inclined theca and generation of frictional force between the pollen grain and the theca wall, 2) increase in horizontal distance between anther pores and center of sigma, and decrease in elevation angle, and 3) generation of the rotational moment on the stamen, and the increase of moment due to delaying pollen release concerning factor 1.

In the second study, we investigated the effects of panicle position relative to canopy and of panicle angle on pollination, and seed set, using pot-grown rice plants at heading stage in the paddy field. Twenty uniform rice (IR72) seedlings at around the 5-leaf stage were transplanted in 4-L pots in a circular pattern and grown to heading stage in a flooded state. Pots of rice at heading stage were inserted in the canopy and set on the wooden stands to position their panicles either upright (0°) or incline (30°), either at 25 cm above the canopy surface or at the canopy height in Experiment 1, and either beneath or at the canopy height in Experiment 2. Due to the 30 ° panicle inclination, TP20, GP10 and floret sterility were increased by 21%, 21% and 10% in Experiment 1, and 27%, 30% and 26% in Experiment 2, respectively. On the other hand, the effect of the panicle position in the canopy on the floret sterility was not significant. Floret sterility was positively correlated well with GP10 by covariate analysis with trial as independent variable. GP10 was well correlated with TP20. From these results, it was shown that even in the canopy condition, the panicle inclination of about 30 ° has a substantial adverse effect on rice seed set through unstable pollination.

From the above results, it was experimentally shown that a slight inclination of rice florets affects seed set through unstable pollination. There are varietal differences in the angle of culm that is directly linked to the inclination of rice florets. In recent years, high yielding by super large panicle has been promoted in China. The panicles incline even when the community structure deteriorates due to heavy fertilizer application. For these reasons, the uprightness of panicles is important as breeding objective and in rice production.

CHAPTER I

General introduction

1.1 Morphology of floral organs and autogamous capacity

Most plants are hermaphroditic, but possess traits that promote outcrossing such as self-incompatibility, and spatial and temporal separation of male and female function within flower (2). Spatial separation of anther and stigma promote out crossing and reduced autogamy among the population of harkogamy (3, 48, 49). The relationship between stability of self-pollination and morphology of floret organs has been studied mianly in entomophilous flowers. The distance between anther and stigma influenced the efficiency of autonomous polliantion in raddish (*Raphanus sativus* L.) (24), in *Centaurium* (4) and *Arabis alpine* (59). Moreover, orientation of anther dehiscent side relative to stigma has been associated with autonomous capacity in the genus *Leavenworthia* in the Brassicaceae (30, 50). Therefore, the distance between stigma and anther pores, and their relative orientation are relevant to a plant's autogamous capacity.

1.2 Rice floral morphology and its pollination

Rice has self-pollinated flower. The flower is enclosed in the lemma and palea. It consists of the pistil, stamens, and lodicules. The components of the pistil are the stigmas, styles, and ovary. The stigma is plumose, on to which pollen grains are shed. There are six well-developed stamens, composed of anther and filament. Two small, oval, thick, and fleshy bodies, called the lodicules, are situated at the base of the ovary. The lodicules become distended with water and assist in separating the lemma and palea at flowering.

Fowering and pollination normally occur for one hour around 10:00 to 12:00. At the beginning of flowering, tip portions of the lemma and palea start to open, filaments elongate, and anthers begin to exert from the lemma and palea. The filaments elongate further to bring the anthers out of the lemma and palea. The spikelet then closes leaving the anthers outside.

During anthesis, anther dehiscence occurs at basal and apical parts just before or when lemma and palea open (64). At that time, basal pores of erect anther were just above the stigma (43) and drop the pollen grains on to the stigma. Therefore, rice pollination has been thought sure under suitable conditions. However, even a small change in floral character can affect stability of pollination (33). Anther with small basal pore retained more residual pollens at the peak of anthesis and released them when anther drooped over the glume outside the floret (33). The size of basal dehiscence has been associated with stability of pollination (25, 34, 35, 58). Moreover, incorrectly positioned anther and stigma at dehiscing time seriously affected the pollination (29, 53). Therefore, very minute change in floral character and relative position between stigma and pollen release point may affect the stability of rice pollination. To ascertain the success of pollination and fertilization in rice, it is important to get the sufficient number of pollens onto the stigma (41, 53). Therefore, relative position between stigma and dehisce anther rice is critical to get the sufficient number of pollens onto the stigma.

Panicle angle should be relevant. Inclination of panicle may cause the floret inclination. It can change the relative position between stigma and anther pore and may affect gravity drop of the pollen grains onto the stigma. In recent years, high yield by rice varieties with extra heavy panicle type have been promoted. The heavy weight of panicle increased the bending movement of the culm (28, 32) and will increase the panicle angle. Panicle angle is a common in agriculture scene, which is amplified in long weak culms and large heavy panicles under the influence of rain or wind. Panicle inclination may, therefore, affect the stability of rice pollination and floret fertility by changing the relative position between the stigma and pollen release point.

1.3 Canopy microclimate on rice pollination

Rice pollination is anemophilous. It is very susceptible to meteorological factors such as temperature and wind speed. High temperature above 35°C and low temperature below 20°C result in poor pollination and loss of yield (16, 17). Poor anther dehiscence, decrease in number of pollen grains on the stigma, and poor germinated pollen grains on the stigma are principal cause of heat induced spikelet sterility under higher temperature (38, 41, 53). Wind helps rice pollination by physical vibration effect on floret (37). Windy conditions can mitigate the effect of high temperature with high vapour pressure deficit (VPD) condition on floret sterility by transpirational cooling effect on spikelets (36, 58). However, strong wind speed could blow away pollen grains to some extent from the stigma and cause unstable pollination under high temperatures with very high VPD condition (20, 36).

The microclimate conditions change dramatically with height particularly around the canopy surface (42, 65). Wind velocity of 1.57 m s^{-1} at 1.2 m above the canopy decreased to about 1.25 m s^{-1} at 30 cm above the canopy, to about 1.00 m s^{-1} at the surface of the canopy, and to about 0.45 m s^{-1} at 20 cm below the canopy surface (19). Drastic change in the wind velocity profile around the canopy surface affected the stability of rice pollination and following fertilization depending on the panicle position with respect to the canopy surface (37). Therefore, Panicle position may also influence the effect of panicle inclination on pollination stability and seed set under canopy condition.

1.4 Importance of pollination in rice production

Pollination is indispensable process for seed set in rice. Although rice is autogamous plant, its pollination is very susceptible to abiotic stresses. Nowadays, world rice production has been threatened by increasing frequency of extreme weather events related to climate change. Loss in rice production caused by these stresses has been reported in word rice producing regions (8, 5, 14, 21, 27). The primary cause of rice yield reduction by heat (25, 38, 40, 45, 62), cold (66) and drought (29) at flowering and even in normal condition related to panicle position (37) is mainly through poor pollination. As pollination is directly linked to floret fertility, its stability should be paid more attention for stable rice production under future global climate change.

1.5 Objectives

This research was conducted to clarify the effect of panicle angle on the stability of rice pollination by using isolated pot-grown rice experiment and to clarify the effect of panicle angle and panicle position related to canopy surface by conducting two model experiments under paddy field condition.

CHAPTER II

Panicle inclination influences pollination stability of rice (*Oryza sativa* L.)

2.1 Introduction

Pollination is indispensable for seed set of flowering plants. Self-pollination is an adaptation to conditions where mates or pollinators are limited (6, 22, 46) and promotes reproduction in colonizing populations (1). The relationship between stability of self-pollination and morphology of floret organs has been studied mainly in entomophilous flowers. The distance between stigma and anther pores and their relative orientation are relevant to a plant's ability to self-fertilize (7, 44, 61), and the capacity for autonomous self-pollination is associated with differences in the anther–stigma distance in several species (4, 24, 31, 59). Relative distance, orientation, and angle between stigma and pollen release points all affect the capacity for self-pollination.

Rice, the staple food for more than half of the world's population (11), is an autogamous plant. Its pollination is autonomous. The falling of pollen grains from the anther onto the stigma by gravity is thought to be sure under suitable conditions (18). Stability of rice pollination depends on the size of the basal pore of the anther (33). In rice with a small basal pore, more pollen grains remain in the anthers at the peak of anthesis and are released from the apical pore after the stamen bends down. This observation suggests that the position of the stigma relative to the pollen release point (i.e., distance and angle above) and the presence of obstacles (lemma and palea) can affect pollination stability, even under autonomous pollination. Panicle inclination may be relevant. Tiller angle (2.5° to 34.4° at heading stage; (9)) should affect panicle inclination, which is amplified in long weak culms and large heavy panicles under the influence of rain or wind. Panicle inclination may therefore affect the stability of pollination and floret fertility by changing the relative position between the stigma and pollen release points.

The objective of this study was to clarify the effect of panicle angle on the stability of rice pollination in an experiment with isolated pot-grown rice plants tilted at different inclinations during flowering.

2.2 Materials and methods

2.2.1 Experimental site and test materials

The experiment was conducted in a research field at Gifu University, Gifu Prefecture, Japan (35°27′N, 136°44′E, 13 m a.s.l.), in 2015. Seeds of IR72 were sown in a nursery bed three times at seven-day intervals from 25 March. Seedlings at around the five-leaf stage were transplanted into 4-L pots in a circular pattern at 20 seedlings per pot. Each pot contained the equivalent of 3.2 kg of air-dried sandy loam (pH 7.64). Each was supplied with a slow-release compound fertilizer containing 0.5 g N, P_2O_5-equivalent, and K_2O-equivalent as a basal dressing. The soil was puddled with water the day before transplanting. The plants were grown outdoors with the soil surface submerged until treatment. Tillers were removed as they appeared during the vegetative stage to get uniform panicles on the main culms (52). With this method, we got uniform and straight panicles. In our observation, panicles did not spread during the treatment.

2.2.2 Experimental procedures

The experiment was set up in the evening just before the first sampling day. Pots of plants at the 30% heading stage were tilted at one of four inclinations (0° = control, 15°, 30°, or 45°) using wooden bases as shown in Figure 2.1. Decreases in panicle height caused by inclination treatments were estimated from 0.71 cm to 16.6 cm from the control. Each pot on its base was set in a 30-L bucket to keep the soil submerged avoiding the effect of differences in soil water condition in each pot as well as between pots during treatment. The direction of the inclination was leeward of the forecast wind direction. Three pots were used for each treatment, laid out in a randomized complete block design with three blocks on shaved open grass land. Every evening during treatment period, inclined pots were rotated to avoid gravitropic recovery and to be stable the subjected degree of inclination. Three trials (Trial 1, 2 and 3) were performed for total of nine days. After treatment, pots were collected and the plants were grown as before the treatment.

2.2.3 Meteorological observations

The microclimate was measured at the centre of the site using multiple sensors (WXT520, Vaisala Inc., Helsinki, Finland), 240 cm above the soil surface. Measurements of air temperature, relative humidity (RH), wind velocity, and sun radiation were taken every 10 s, and 1-min averages were recorded on a data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA). To measure wind conditions around the panicles, we used hot-wire anemometers (Model 6541-21 probe, and model 6501-00 data logger, Kanomax Japan Inc., Suita, Japan), setting the probe at panicle height of one pot per treatment during sampling days.

2.2.4 Pollination and anther character observations

Samples were collected on six days from 10 to 18 September (avoiding rainy days on 12, 14, and 17 September) (Figure 2.2). Growth stages of pot-grown rice plants were nearly the same and all panicles flowered during the treatment period. Plants which had finished flowering and had not started flowering at treatments were left as they were. During the treatment period, 10 spikelets were randomly sampled from each pot promptly after floret closure and three averages of 10 spikelets from three pots were used as three replicates. To assess pollination, we detached stigmata from the florets and stained them with cotton blue solution. The solution was prepared by dissolving 100 mg of cotton blue in 100 ml of 1% acetic acid solution, and then, being diluted with 100 ml of glycerol and 100 ml of distilled water. We counted total and germinated pollen grains on the stigma at 100× magnification under an optical microscope (Model BX51, Olympus Corporation, Tokyo, Japan). In rice,

>10 germinated pollen grains are required for fertilization and for >10 germinated pollen grains, >20 total pollen grains are necessary (41, 53). Therefore, we calculated TP20 (percentage of florets having <20 total pollen grains on the stigma after anthesis) and GP10 (percentage of florets having <10 germinated pollen grains on the stigma) as indices of pollination stability. We collected all anthers from the 10 spikelets which we used for stigma sampling in each pot every day. And then, sixteen anthers were randomly selected and examined under a digital microscope (KH-7700, Hirox Co., Ltd., Tokyo, Japan) at 80× magnification, and measured the anther length and the width and length of dehiscence of the basal and apical pores (Figure 2.3).

2.2.5 Statistical analysis

We used a split-plot design for data analysis, using sampling date as the main plot factor, treatment as the subplot factor, with three blocks. We calculated the mean values of anther characters, numbers of germinated and total (germinated and ungerminated) pollen grains on the stigma after anthesis per floret, percentage of pollen germination, GP10, and TP20. ANOVA was conducted in Statistix v. 8.0 software (Analytical Software, Tallahassee, FL, USA). Treatment means were compared by Tukey's honestly significant difference (HSD) test at the 5% probability level. Analysis of covariance (ANCOVA) was performed to verify the effect of wind velocity around the flowering time which was based on our observation, on pollination, and Dunnett's test was used to compare the differences between treatment means and the control at 5%. As the percentage of pollen germination, TP20, and GP10 contained 0s, we used empirical logit transformation.

2.3 Results

2.3.1 Microclimate conditions during treatment

The daily maximum air temperature during the observation period (10 to 18 September) ranged from 20.3 to 29.9 °C (Figure 2.2a). That on sampling days ranged from

25.5 to 29.4 °C. RH at the time of maximum temperature on sampling days ranged from 40% to 55% (Figure 2.2b). The average wind velocity around flowering time on sampling days ranged from 0.83 to 1.69 m s^{-1} (Figure 2.2c).

2.3.2 Effects of panicle inclination treatments on pollination and anther characters

The effects of treatment (panicle inclination) and date on numbers of total and germinated pollen grains, percentage pollen germination, TP20, and GP10 were significant (Table 2.1), but their interactions were not. Numbers of total pollen grains ranged from 24.9 to 38.8 among treatments, and numbers of germinated pollen grains ranged from 11.7 to 19.2 among treatments. Numbers of total and germinated pollen grains at 0° (control) and 15° were significantly higher than those at 30° and 45°. The rate of pollen germination ranged from 49.0% to 58.6% among treatments. It was significantly lower at 45° than at 0° and 15°. TP20 ranged from 36.6% to 59.9% among treatments. It was significantly higher at 45° than at 0° and 15°. GP10 ranged from 26.6% to 58.9% among treatments. It was significantly higher at 45° and 30° than at 0° and 15°.

The effect of treatment on anther characters was not significant, but apical dehiscence length and width were significantly greater on 18 September than on 10 September (Table 2.2).

2.3.3 Effects of wind velocity on pollination

In the ANCOVA with wind velocity as a covariate and treatment as an independent variable, average wind velocity during flowering was positively correlated with the number of total pollen grains (R^2 = 0.5637, $p < 0.01$; Figure 2.4a) and negatively correlated with TP20 (R^2 = 0.6573, $p < 0.001$; Figure 2.4b) and GP10 (R^2 = 0.73, $p < 0.0001$; Figure 2.4c).

2.3.4 ANCOVA between the two pollination indices

ANCOVA of GP10 with TP20 as a covariate and treatment as an independent variable showed that the effects of TP20 ($p < 0.0001$, F (1, 19)) and treatment ($p < 0.05$, F (3, 19)) on GP10 were significant. GP10 was significantly higher at 30° and 45° than at 0° at a given TP20 by Dunnett's test and increased with TP20 ($R^2 = 0.9510$, $p < 0.0001$; Figure 2.5).

2.4 Discussion

Inclination of pots significantly affected pollination stability (Table 2.1). As inclination increased, TP20 increased from 36.6% to 59.9% and GP10 from 26.6% to 58.9%. Since rice pollination requires >20 total or >10 germinated pollen grains on the stigma (41, 53), a panicle inclination of ≥30° would give damage to rice production through unstable pollination.

Panicle inclination causes floret inclination. The direction of each floret was not exactly parallel to the panicle axis. However, it could be considered to be almost parallel to the axis in average. Although we cannot exclude the effects of physiological damage and change in meteorological condition by the inclination on pollination, we would like to propose three possible factors (Figure 2.6) for the unstable pollination from morphological aspect. The first factor is decrease in force to move the pollen grain in inclined theca (Factor 1). Gravity force for pollen grain (mg, where m is mass of pollen grain and g is acceleration by gravity) is divided into two components: parallel (mg cos ɵ, where ɵ is angle of inclination with respect to vertical) and perpendicular (mg sin ɵ) components to the theca wall. Thus force to move the pollen grains decrease to mg cos ɵ. Moreover, the perpendicular force generates the friction on the theca wall against the parallel force. The basal dehiscence length of IR72 was around 250 µm shorter than those of other cultivars as previously reported (34). Self-pollination of cultivars with a small basal pore is unstable because of the delayed and decreased pollen release from the basal pore (33). Thus self-pollination of IR72 is originally unstable in comparison with other cultivars with long basal dehiscence (34). Anther inclination may impede the pollen movement in the theca tube and thus aggravate the delay of pollen release to generate trouble in pollination. The second factor is the change in relative position between the anther pore and the stigma (Factor 2). In inclined florets, inclination extends the horizontal distance and decrease the vertical distance between anther pore and centre of stigma (Figure 2.6). The change in relative position may reduce pollination by gravity. The third factor is rotational moment generated on the stamens (Factor 3). Inclination of stamens increases the rotational moment on the filaments. It may encourage the bending down of anthers and accelerating their inclination. The delay of pollen release increases the anther weight and thus the rotational moment. These hypotheses suppose simple elongation of stamen and pistil, depending on our observation. In some entomophilous flower, however, stamen and pistil shows gravi- and photo- tropic responses (56). If rice pistil and/or stamen have such response, situation may be much more complex.

The significantly lower germination percentage of pollen grains with greater inclination (Table 2.1) suggests that germination was also hampered by inclination. The decreased germination percentage seemed, in turn, to increase GP10 at any given TP20. Rice pollen germination decreases from 85% to 42.5% within 6 min after anther dehiscence (57). The decrease in pollen germination percentage may be explained by the delay of pollen release shown in the above hypothesis concerning factor 1.

Figure 2.7 explains the direct primary cause of the increase in GP10 in the inclined treatment. At 0°, the ratio of GP10/TP20 is 0.73. If this is the same germination percentage in inclined florets, GP10 of other inclination should be plotted on Line 1 at any given TP20. From Line 1, we can predict that an increase in TP20 would explain 11% of the 24% increase in GP10 at 30° and 18% of the 32% increase at 45°. The remaining 13% at 30° and 14% at 45° may therefore be due to loss of the germination ability of pollen grains on the stigma probably through the delay of pollen release. Thus, both an increase in TP20 and a decrease in germination ability are important causes of the increase in GP10.

Wind (0.80–1.43 m s^{-1}) helped pollination (Figure 2.4). Wind velocity was, therefore, one of the factors in the significant effect of date on pollination parameters. This result is consistent with our recent report that high wind velocity assisted the pollination of rice in an open field through its physical vibration effect on pollen shedding (37). Shaking of flowers by wind facilitates autogamy in homogamous hermaphrodite flowers (10).

The effects of temperature on pollination and anther morphology were not significant. Very high temperatures at flowering (25, 38, 40, 45, 62) and low temperatures at booting (55) induce floret sterility through poor anther dehiscence and defective pollination. The maximum temperatures on the sampling dates ranged from 25.7 to 29.4 °C (Figure 2.2a) and the daily minimum temperature during the booting stage ranged from 16.2 to 23.7 °C (data not shown). As day/night temperatures above 26/16 °C at booting have minimal effect on sterility (54), our experiment may have coincided with normal temperatures.

The effect of date on apical dehiscence was significant, and RH during the maximum temperature was negatively correlated with apical dehiscence length ($R^2 = 0.29$, $p < 0.01$; data not shown). Widening of anther dehiscence is a desiccatory process (39). Thus, high vapor deficit may help the widening of apical dehiscence.

Since a panicle inclination of only 30 ° significantly reduced pollination stability, erect panicles should be a breeding objective. As the primary cause of sterility induced by heat at flowering and by cool weather at booting (as described above) is poor pollination, erect panicles may also improve tolerance to sterility caused by these extreme temperatures.

Table 2.1 Mean values of pollination parameters as affected by levels of panicle inclination.

	Total pollen grains (no.)	Germinated pollen grains (no.)	Pollen germination (%)	TP20 (%)	GP10 (%)
Treatment					
0° (control)	38.8 a	19.2 a	58.3 a	36.6 b	26.6 b
15°	37.3 a	18.6 a	58.6 a	37.2 b	28.7 b
30°	24.9 b	12.2 b	51.9 ab	51.0 ab	51.1 a
45°	26.4 b	11.7 b	49.0 b	59.9 a	58.9 a
Date					
10 September	25.2 b	13.0 b	60.1 a	56.0 a	46.8 ab
11 September.	49.1 a	21.5 a	52.8 ab	27.7 b	21.8 c
13 September	25.1 b	12.6 b	52.3 ab	49.0 ab	43.4 b
15 September	30.2 b	14.4 b	50.1 b	38.7 ab	38.2 b
16 September	30.6 b	15.0 b	56.5 ab	59.2 a	56.7 a
18 September	31.1 b	16.0 ab	54.9 ab	46.5 ab	41.1 b
ANOVA					
Date (D)	**	**	*	**	***
Treatment (T)	**	***	**	**	***
D × T	n.s.	n.s.	n.s.	n.s.	n.s.

TP20: percentage of florets with <20 total pollen grains on the stigma; GP10: percentage of florets with <10 germinated pollen grains on the stigma. Means followed by the same letter within a column are not significantly different by Tukey's HSD test ($p < 0.05$). Percentage of pollen germination, TP20, and GP10 were analysed after empirical logit transformation. ***: $p < 0.001$; **: $p < 0.01$; *: $p = 0.05$; n.s.: not significant.

Table 2.2 Mean values of anther characters as affected by levels of panicle inclination.

	Anther length (µm)	Apical dehiscence length (µm)	Apical dehiscence width (µm)	Basal dehiscence length (µm)	Basal dehiscence width (µm)
Treatment					
0° (control)	1756.9	524.3	180.8	251.3	60.7
15°	1748.1	527.3	179.3	255.3	61.9
30°	1766.0	535.2	178.1	248.5	61.2
45°	1743.5	531.0	177.2	250.8	61.1
Date					
10 September		510.4 b	176.9 abc		
11 September		524.2 ab	167.5 c		
13 September		532.4 ab	188.1 ab		
15 September		536.8 ab	172.1 bc		
16 September		526.5 ab	175.9 abc		
18 September		546.4 a	192.6 a		
ANOVA					
Date (D)	n.s.	*	*	n.s.	n.s.
Treatment (T)	n.s.	n.s.	n.s.	n.s.	n.s.
D × T	n.s.	n.s.	n.s.	n.s.	n.s.

Means followed by the same letter within a column are not significantly different by Tukey's HSD test ($p < 0.05$). *: $p < 0.05$; n.s.: not significant.

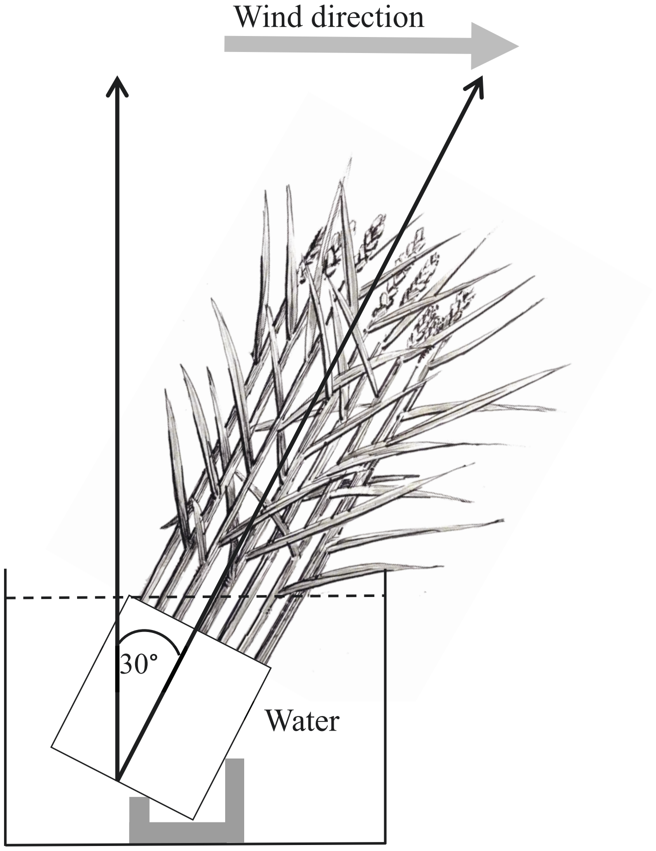

Figure 2.1 Inclination treatment of pot-grown rice plants.

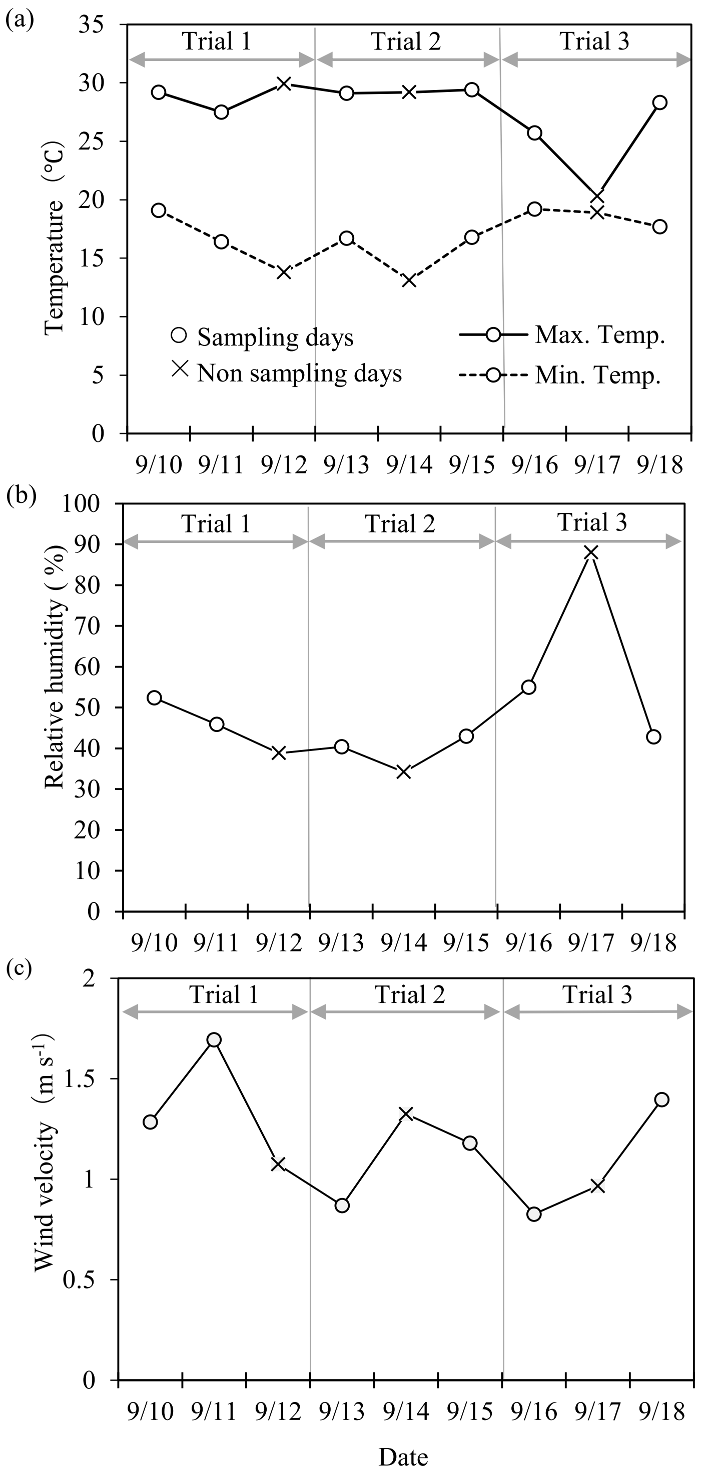

Figure 2.2 Weather conditions at the experimental site during the treatment periods: (a) daily maximum and minimum temperatures during observation period, (b) RH (%) at the time of maximum temperature, and (c) average wind velocity (m s^{-1}) around flowering time (10:00 h–12:00 h) during the observation period. ○ indicates sampling days and × indicates non-sampling days due to rain.

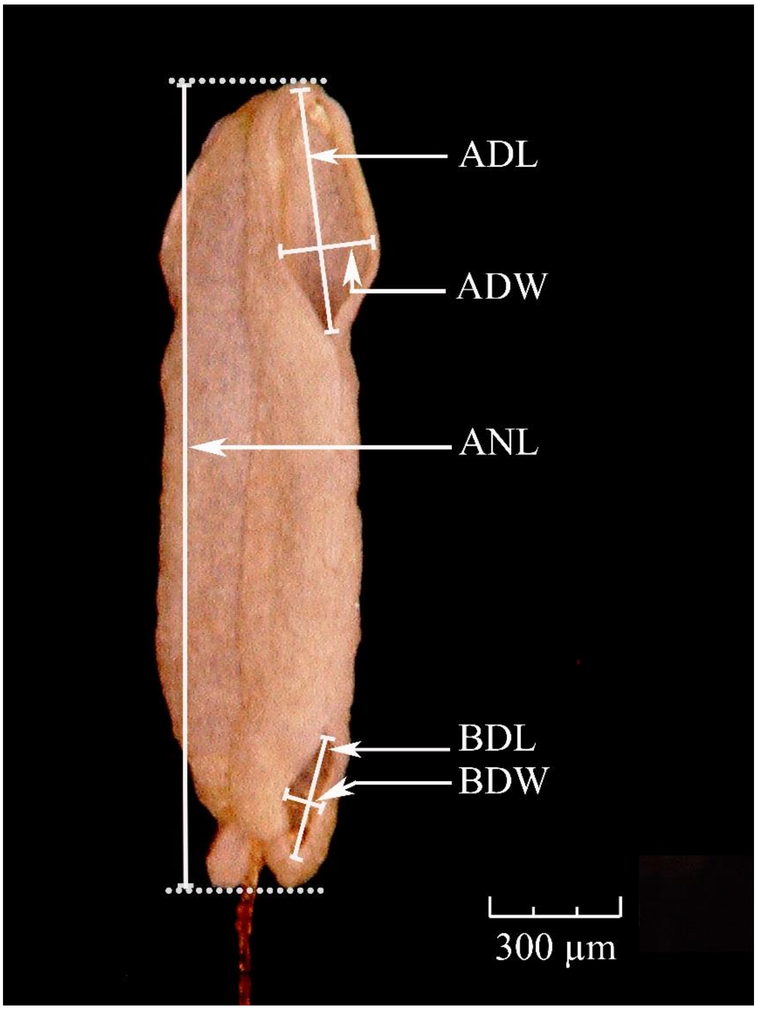

Figure 2.3 Exterior of dehisced anther. Anther characters were measured under a digital microscope. ADL: apical dehiscence length; ADW: apical dehiscence width; ANL: anther length; BDL: basal dehiscence length; BDW: basal dehiscence width.

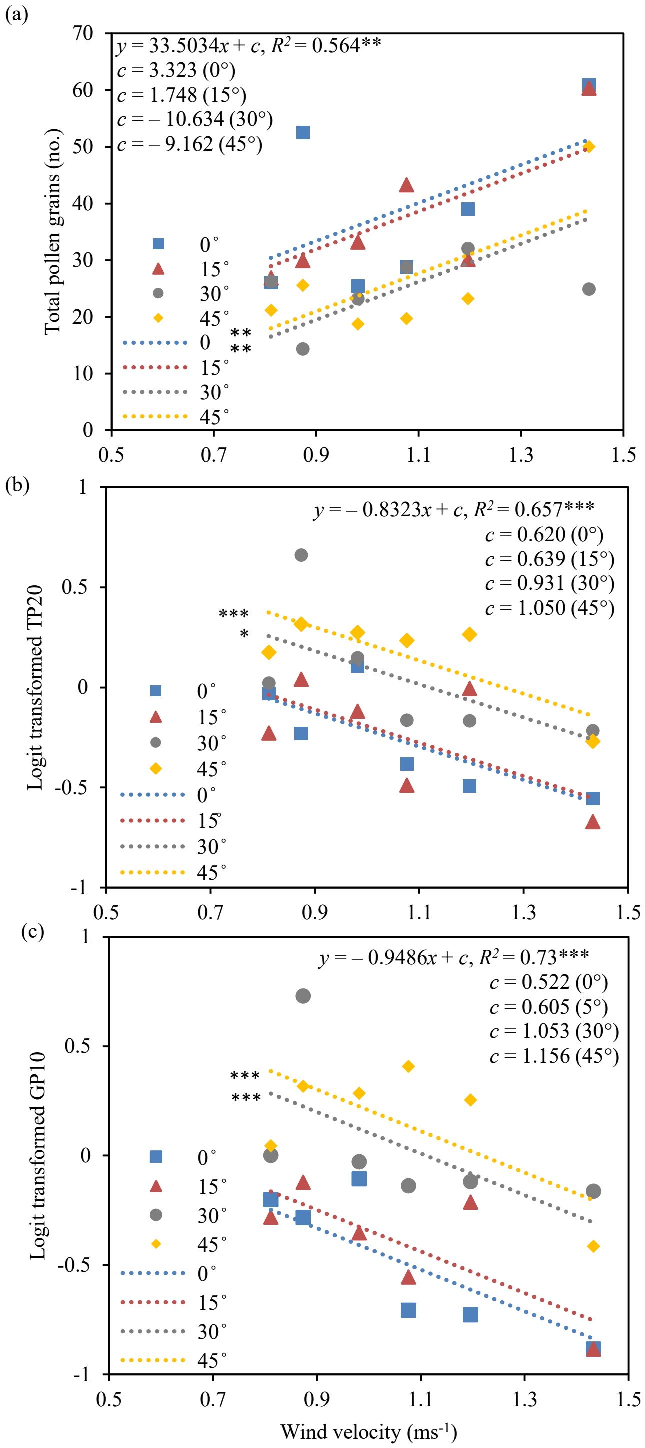

Figure 2.4 Relationships between average wind velocity during flowering time and (a) number of total pollen grains on the stigma, (b) logit-transformed percentage of florets with <20 total pollen grains on the stigma (TP20), and (c) logit-transformed percentage of florets with <10 germinated pollen grains on the stigma (GP10). Means are compared with the control (0°) by Dunnett's test. *, ** and *** on lines indicate significant differences from the control and those on equations indicate significances of correlation at 5%, 1% and 0.1% levels, respectively.

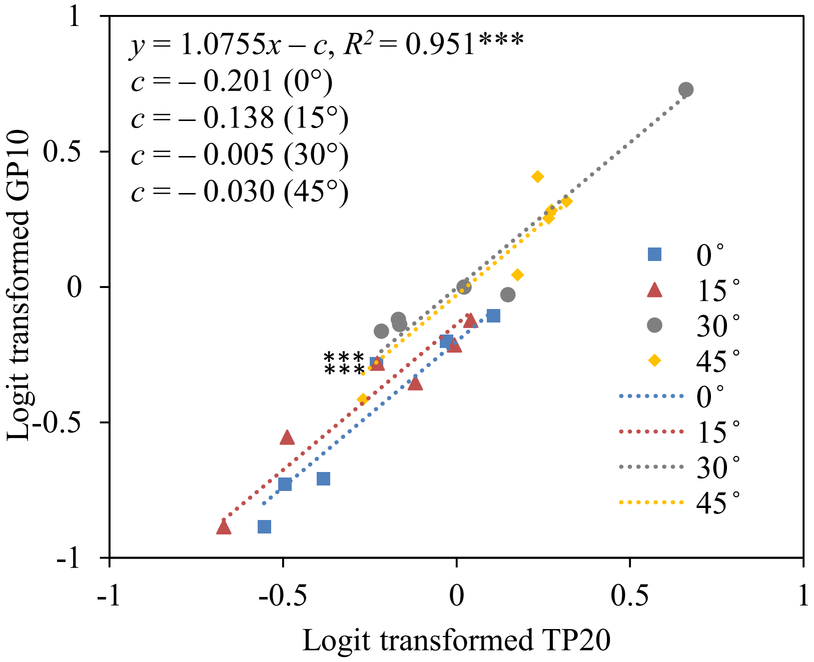

Figure 2.5 Relationship between logit-transformed percentages of florets with <20 total pollen grains on the stigma (TP20) and florets with <10 germinated pollen grains on the stigma (GP10). Means are compared with the control (0°) by Dunnett's test. *** on lines indicates significant difference from the control and that on equation indicates significance of correlation at 0.1% level.

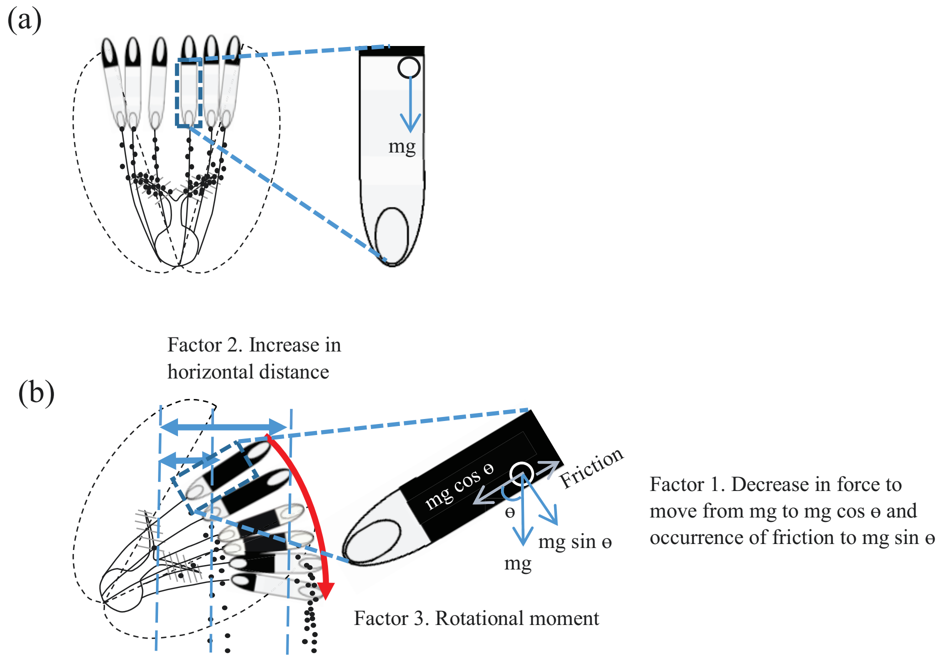

Figure 2.6 Schematic diagram showing possible mechanism in which the inclination reduces the pollination stability. In the erect floret (a), pollen grains easily drop out of the basal pore of the erect anther by force of gravity (mg), and filament elongation extends vertically favoring the gravity shedding of pollen grains onto stigma. In the inclined floret (b), pollination stability reduced possibly by three factors: Factor 1, decrease in force to move the pollen grains (mg cos ɵ) in the inclined theca and occurrence of friction to 'mg sin ɵ', which delay or obstruct the pollen release; Factor 2, increase in horizontal distance and decrease in vertical distance between anther pore and centre of stigma by elongation of filament, which reduce gravity shedding of pollens onto the stigma; and Factor 3, increasing rotational moment generated on the filament, which encourages the bending down of anthers and accelerating their inclination. Delayed pollen release increase the weight of anthers and the rotational moment. m: mass of pollen grain; g: acceleration by gravity; ɵ: angle of inclination with respect to vertical.

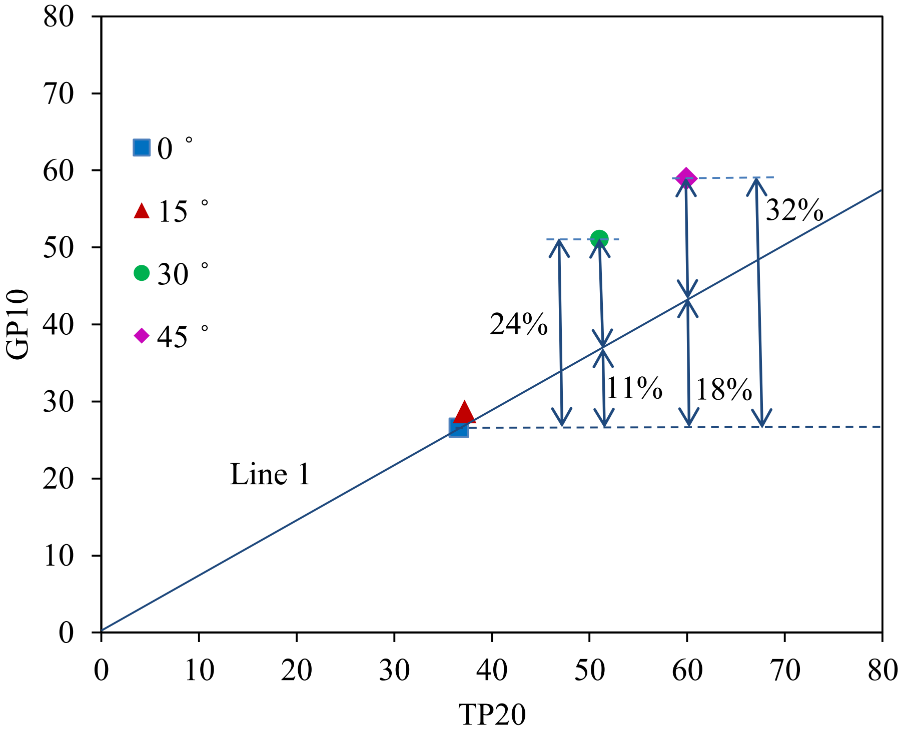

Figure 2.7 Factors that caused the increase in percentage of florets with <10 germinated pollen grains (GP10) on the stigma. TP20: percentage of florets with <20 total pollen grains on the stigma. At 0°, the ratio of GP10/TP20 is 0.73. If this is the germination percentage in inclined florets, GP10 should lie on Line 1 at any given TP20. From Line 1, we can predict that an increase in TP20 would explain 11% of the 24% increase in GP10 at 30° and 18% of the 32% increase at 45°. The remaining 13% at 30° and 14% at 45° may therefore be due to loss of the germination ability of pollen grains on the stigma.

CHAPTER III

How panicle angle and panicle position in the canopy determine pollination and seed set in rice (*Oryza sativa* L.)

3.1 Introduction

Rice is the primary staple food for more than half of the world's population (FAO, 2014). Its pollination is indispensable for seed set. Anthesis of rice occurs in late morning in many cultivars under normal condition. Each flower opens once for around one hour. At the start of the floret opening, anther dehisces right above the stigma and pollen grains drop from the anther dehiscence to the stigma (18). There is seemingly little risk of disruption of rice pollination by direct external factors.

However, we found that small panicle inclination reduced pollination stability in isolated pot-grown rice probably by changing the relative position between anther and stigma, and delaying pollen release (63). Panicle inclination of 30° increased percentage of florets having less than 10 germinated pollen grains on the stigma after flowering by 24%. Since rice fertilization requires 10 or more germinated pollen grains (53), increase in such insufficiently pollinated flowers may induce floret sterility. Panicle inclination can be observed in agricultural scene. Inclined tillers, long weak culms and huge panicles can cause the inclination of panicle at flowering. However, the effect of inclination of panicle on the seed set has not yet examined.

In rice production, the flowering occurs with canopy. The meteorological conditions such as wind speed and solar radiation change dramatically with height, particularly around the canopy surface (42, 65). It has been shown that shown that pollination stability and floret sterility of rice depend on the height of florets at flowering. Poorly pollinated florets and floret sterility was minimum around the surface of canopy and increased as increase in the distance from the surface to the floret height (37). The canopy may affect the relationship between the inclination of panicle and pollination stability in practical rice production.

The purpose of this study is to clarify if the small inclination of panicle really induces the floret sterility in rice, and if the canopy affects the relationship between inclination of panicle at flowering and pollination stability. We conducted model experiments using pot-grown rice plants set in the rice canopy at different heights and inclinations and examined pollination stability, pollen germination and seed set.

3.2 Materials and Methods

3.2.1 General concept

We conducted two experiments with pot grown-rice plants at the heading stage set in canopy with four treatments in each experiments: two steps of panicle height × two inclinations (upright and 30°). In Experiment 1, we set the panicle at 25 cm above the canopy surface and at the height of the canopy surface to detect the effect of canopy on the relationship between panicle angle, pollination and floret sterility. In Experiment 2, we set the panicle around 40 cm below the canopy surface and at the height of the canopy to know the effect of depth of the panicle in the canopy on that relationship.

3.2.2 Preparation of background canopy

The experiments were conducted in a research field at Gifu University, Gifu Prefecture, Japan (35°27′N, 136°44′E, 13 m a.s.l.). We used ‘Khao Nok’ to create a background canopy. It is a tall and late cultivar, and rather tolerant to lodging. Moreover, its leaves are kept nearly upright at narrow angle until the heading stage. Therefore, we chose ‘Khao Nok’ as background cultivar. The seeds were sown in nursery beds on 30 May 2017 and on 25 April 2018. Seedlings at around the five-leaf stage were transplanted at a spacing of 30 cm (east–west) × 15 cm (north–south) in the field. The field had already received a basal dressing of compound fertilizer at 80 kg/ha N, P2O5-equivalent and K2O-equivalent. The field soil was kept submerged until the ripening stage. The locations of the plots followed a randomized complete block design. The field had three bays as blocks, each 15 m (north–south) × 2.5 m (east–west). Each block was divided into four plots.

3.2.3 Pot preparation

We used rice variety ‘IR72’ as a tested variety. Its plant height is shorter than that of ‘Khao Nok’. With the combination of tall ‘Khao Nok’ and short ‘IR72’, we can easily adjust the different panicle heights from beneath to above the background canopy surface. Seeds were sown in nursery beds on 14, 21 and 28 April 2017, and on 6, 16 and 26 April 2018 for three trials in each experiment. Seedlings at around the five-leaf stage were transplanted into 4-L pots (15 cm diameter, 20 cm height) in a circular pattern at 20 seedlings per pot. The pot contained 2.5 kg equivalent of air-dried sandy loam (pH 7.64). Each pot received a basal dressing of slow-release compound fertilizer at 0.5 g N, P2O5-equivalent and K2O-equivalent and was puddled in water the day before transplanting. The plants were grown outdoors with the soil surface submerged until treatment. Tillers were removed as they appeared during the vegetative stage to achieve uniform (52), straight panicles on the main culms.

3.2.4 Canopy treatment in Experiment 1, 2017

Pots were set on wooden stands to position the panicles at or above the canopy surface in one of two inclinations: at the canopy height (Canopy) or at 25 cm above the canopy (Above); either upright (Upright) or inclined 30° from the vertical (Inclined). Panicle heights were set and background canopy heights were measured at the start of each trial. Leaf area index (LAI) was measured with an LAI 2000 plant canopy analyser positioned at the bottom of the canopy on the middle day of each trial. The background canopy height ranged from 0.95 to 1.20 m across the trials. LAI ranged from 3.2 to 4.0 across the trials.

3.2.5 Panicle depth treatment in Experiment 2, 2018

Pots were set to position the panicles at or under the canopy surface in one of the two inclinations as above: at the canopy height or under the canopy (Under); either upright or inclined 30°. The pots were placed either on wooden stands or on the soil surface.

The panicle height of the pot-grown plants and the background canopy height were measured at the start of each trial (Table 3.1). From each pot that was set in the canopy, we randomly selected four panicles at anthesis and measured their heights (from the ground to the tips of the panicles). From the hills surrounding each pot, we selected one hill randomly and measured the height of the highest tips of the leaves. LAI was measured at the bottom of the canopy and at flowering height (Table 3.2). The measurements were conducted on the middle day of each trial.

3.2.6 Common procedures and data collection in Experiments 1 and 2

Five pots were used in each plot. In the evening of the day before the start of data collection in each trial, pots with 40% to 50% of plants at heading were inserted into each plot on a north–south line at the centre of the bay, about 0.6 to 0.7 m apart, on wooden bases set to adjust the inclination and panicle height. Each pot was set in a bucket (32 cm deep, 22 cm wide) to keep the soil submerged in water. Every evening during the treatment period, inclined pots were rotated to avoid gravitropic recovery and to maintain the inclination. Three trials of 5 to 7 days were performed, totalling 16 days in Experiment 1 and 20 days in Experiment 2. After the treatment periods, pots were collected from the field and grown as before.

The site's microclimate was measured at its centre with multiple sensors (WXT520, Vaisala Inc., Helsinki, Finland) set at 230 cm above the soil surface. Air temperature, relative humidity (RH), and wind speed were measured every 10 s, and 1-min averages were recorded on a data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA). Plants flowered earlier in Experiment 2 (09:00 h to 12:00 h) than in Experiment 1 (10:00 h to 12:00 h), probably because of the higher temperature in Experiment 2 (Fig. 1a).

One of the five pots in each plot was used to collect floret samples for observation of anther characters and the other four were used for examination of pollination and fertility. To examine the fertilization of the florets, we randomly selected and tagged three panicles on which florets started anthesis on the day before the start of data collection in each trial, in each of the latter four pots. Their fertilities were examined at maturity by manual inspection of ovarian development.

To observe pollination, we randomly sampled ten florets from the four pots in each plot every day during the treatment period, except on rainy days. We sampled the florets from the dispersed panicles 1 h after anthesis without regard to the location of the florets on the panicles. We did not get samples from the tagged panicles and off-type plants. Stigmata were detached from the florets and stained with cotton blue solution. The numbers of total and germinated pollen grains on each stigma were counted at 100× magnification under an optical microscope (Model BX51, Olympus Corporation, Tokyo, Japan). Fertilization in rice requires at least 10 germinated pollen grains or at least a total of 20 pollen grains on the stigma after anthesis (53, 41). We calculated “TP20” (percentage of florets having <20 total [germinated and ungerminated] pollen grains on the stigma after anthesis) and “GP10” (percentage of florets having <10 germinated pollen grains on the stigma after anthesis) as indices of pollination stability.

To observe the anther dehiscence characters, we sampled seven florets from the designated pot in each plot at anthesis and used three randomly selected florets. From each floret, we randomly selected four anthers to observe at 80× magnification under a digital microscope (KH-7700, Hirox Co., Ltd., Tokyo, Japan). We measured the lengths of dehiscence formed at basal and apical parts of anther (63).

3.2.7 Statistical analysis

We used a split plot design (combined with the randomized block design) with two sub-factors without replication in data analysis, in which trial and (randomized) block were the main effects, and panicle position and inclination were the sub-plot effects for all parameters. We calculated the mean values of anther characters, numbers of total and germinated pollen grains, GP10, TP20 and percentage of floret sterility. Analysis of variance (ANOVA) was conducted to test the statistical differences among the treatment effects. Treatment means were compared using Tukey's honestly significant difference (HSD) test at the 5% probability level. Analysis of covariance (ANCOVA) was performed in TIBCO Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA) to verify the effect of GP10 on floret sterility. Floret sterility, TP20 and GP10 were analysed after logit transformation. Since TP20 and GP10 included 0 values, we used empirical logit transformation.

3.3 Results

3.3.1 Experiment 1

3.3.1.1 Microclimate conditions during treatment

In Experiment 1, the daily maximum temperature varied from 24.9°C to 35.5°C during the treatment period (Figure 3.1a) and from 27.1°C to 33.5°C on the sampling days. The RH at the time of maximum temperature on the sampling days varied from 45.1% to 76.3% (Figure 3.1b). Wind speed around flowering time (10:00–12:00 h) on the sampling days varied from 0.54 to 1.56 m s^{-1} (Figure 3.1c).

3.3.1.2 Effect of treatment on sterility and pollination

The effects of trial and inclination on floret sterility were significant but that of position was not (Table 3.3). Sterility was significantly higher in the inclined treatment (69.5%) than in the upright treatment (59.8%). It was significantly higher in Trial 1 than in

Trial 3. The position × inclination interaction was significant. The difference in increment of sterility by the inclined treatment between panicle positions was small (8.8% above the canopy, 10.5% at the canopy surface). The other interactions were not significant.

The effects of trial and position on pollination parameters (numbers of total and germinated pollen grains, TP20 and GP10) were not significant (Table 3.3). The effect of inclination on all pollination parameters was significant: numbers of total and germinated pollen grains were significantly higher and TP20 and GP10 were significantly lower in the upright treatment than in the inclined treatment. The effect of the position × inclination interaction on all pollination parameters was significant: the differences in TP20 and GP10 between inclined and upright treatments were larger above the canopy than at the canopy surface. The other interactions were not significant.

3.3.1.3 Effect of treatment on anther dehiscence

The effects of panicle position and inclination treatments on anther dehiscence were not significant (Table 3.4).

3.3.1.4 Relationship between sterility and pollination

In the ANCOVA of percentage of sterility with GP10 as a covariate ($P < 0.001$, $F\ (1, 8)$) and trial as an independent variable ($P < 0.0001$, $F\ (2, 8)$), sterility increased with GP10 ($R^2 = 0.940$, $P < 0.0001$; Figure 3.2). GP10 was, in turn, strongly correlated with TP20 ($R^2 = 0.786$, $P < 0.001$; Figure 3.3).

3.3.2 Experiment 2

3.3.2.1 Microclimate conditions during treatment

In Experiment 2, the daily maximum temperature varied from 28.5°C to 38.3°C during the treatment period (Fig. 1a) and from 29.5°C to 38.3°C on the sampling days. The RH at the time of maximum temperature on the sampling days varied from 30.8% to 46.4% (Fig. 1b). Wind speed around flowering time (09:00–12:00 h) on the sampling days varied from 0.98 to 1.97 m s^{-1} (Fig. 1c).

3.3.2.2 Effect of treatment on sterility and pollination

The effects of trial and inclination on floret sterility were significant but that of position was not (Table 3.5). Sterility was the highest (significantly) in Trial 3. It was significantly higher in the inclined treatment (65.5%) than in the upright treatment (39.5%). The position × inclination and trial × inclination interactions were significant. The difference in sterility between inclined and upright treatments was larger at the canopy surface (29.9%) than under the surface (22.0%). The other two interactions were not significant.

The effect of trial on number of germinated pollen grains was significant (Table 3.5); the number was highest in Trial 1. The effects of position and inclination on all pollination parameters were significant. Numbers of total and germinated pollen grains were significantly higher and TP20 and GP10 were significantly lower in the at-canopy and upright treatments than in the under-canopy and inclined treatments. The effects of position × inclination and trial × inclination interactions on numbers of total and germinated pollen grains, TP20 and GP10 were significant. The differences in TP20 and GP10 between inclined and upright treatments were larger at the canopy surface than under the surface. The other two interactions were not significant.

3.3.2.3 Effect of treatment on anther dehiscence

The effects of panicle position and inclination treatments on anther dehiscence were not significant (Table 3.6).

3.3.2.4 Relationship between sterility and pollination

In the ANCOVA of percentage of sterility with GP10 as a covariate ($P < 0.01$, F (1, 8)) and trial as an independent variable ($P = 0.162$, F (2, 8)), sterility increased with GP10 ($R^2 = 0.772$, $P < 0.01$; Figure 3.4). GP10 was, in turn, strongly correlated with TP20 ($R^2 = 0.827$, $P < 0.001$; Figure 3.5).

3.4 Discussion

The temperature was hotter and the wind speed was stronger during Experiment 2 than during Experiment 1 (Figure 3.1). The maximum temperature reached 38.3 °C in Experiment 2 and 35.5 °C in Experiment 1. The average value during the treatment period of average wind speed around flowering time was low, 0.99 m s^{-1} and 1.18 m s^{-1} in Experiment 1 and 2, respectively. A building 20 m high on the northern side of our field might block southerly winds. The low wind speed might be one of the causes of the low stability of pollination and seed set (Tables 3.3, 3.5), especially in Experiment 2 under high temperature (58).

Under these conditions, our results confirmed that panicle inclination significantly increased floret sterility and poor pollination even under the canopy. Inclination increased floret sterility by 10% in Experiment 1 (Table 3.3) and by 26% in Experiment 2 (Table 3.5). Panicle inclination, therefore, reduces paddy rice production. GP10 and TP20 were also significantly higher in inclined plants than in upright plants (Tables 3.3, 3.5), and floret sterility was well correlated with GP10 (Figures 3.2, 3.4), which was in turn well correlated with TP20 (Figures 3.3, 3.5). These results suggest that the differences in floret sterility between inclination treatments were caused mainly by the differences in pollination stability.

The effect of position on pollination stability parameters (i.e. TP20 and GP10) was significant in Experiment 2 (Table 3.5) but not in Experiment 1 (Table 3.3). It has been shown that the optimal position for pollination and floret fertility was around the canopy surface, and that pollination stability and seed set became worse as the distance of the floret from the optimal position increased (37). The distance from the canopy surface was somewhat greater in Experiment 2, so the significance might reflect the distance. In contrast, the effect of position on floret sterility was not significant in Experiment 2 (Table 3.5). It has been shown that floret fertility was greater at deeper position within the canopy than shallower position at the same GP10, and suggested that the shallower position reduced floret fertility through processes after pollen germination (37). Our data agree with this hypothesis.

Anther dehiscence characters are important that determine the stability of rice pollination (33). In the present study, we did not detect the effect of treatment on anther dehiscence characters (Tables 3.4 and 3.6). Therefore, anther dehiscence characters did not affect the stability of rice pollination.

Present study demonstrated that panicle inclination of 30° significantly increased floret sterility under the canopy condition. We sometimes observe inclination of panicle at the flowering stage in agricultural scenes. Huge panicle in high yielding variety recently developed in China causes panicle inclination at flowering because of long rachis of the panicle and of long flowering period. Excessive application of fertilizer for tall race such as hybrid rice sometimes induces panicle incline. Tall cultivar shows inclination of panicle under windy condition. The results show the importance of an erect panicle in rice breeding and growing. Since the primary cause of sterility induced by heat (25, 38, 40, 45, 62) and drought (29) at flowering and by cold at booting (55) and flowering (66) is poor pollination, erect panicles may improve the tolerance to floret sterility caused by these stresses through improving stable pollination. We could not collect the data under high wind conditions, which affect the stability of pollination (37) and thus might affect the interaction between panicle inclination and panicle height in canopy on the pollination stability and the floret sterility. Further studies are needed for understanding the interactions between panicle inclination and panicle depth under strong wind conditions.

Table 3.1 Background canopy height and panicle height of pot-grown rice in Experiment 2.

Treatment		Trial 1		Trial 2		Trial 3	
Position	Inclination	Canopy height (m)	Panicle height (m)	Canopy height (m)	Panicle height (m)	Canopy height (m)	Panicle height (m)
Canopy	Inclined	1.20	1.06	1.26	1.14	1.32	1.20
Canopy	Upright	1.19	1.08	1.27	1.18	1.33	1.21
Under	Inclined	1.16	0.81	1.23	0.85	1.29	0.85
Under	Upright	1.19	0.82	1.22	0.82	1.29	0.85

Canopy: Panicles in pots were set at the height of canopy surface. Under: Panicles in pots were set beneath the height of the canopy surface. Inclined: Pots were inclined at 30° from the vertical. Upright: Pots were set upright. Measurements were taken at the start of each trial.

Table 3.2 Leaf area index (LAI) and cumulative LAI from the surface of the canopy to the height of the florets at the flowering stage in Experiment 2.

Treatment		Trial 1		Trial 2		Trial 3	
Position	Inclination	LAI	Cumulative LAI	LAI	Cumulative LAI	LAI	Cumulative LAI
Canopy	Inclined	4.0	0.7	3.6	0.7	4.4	0.7
Canopy	Upright	4.1	0.4	3. 8	0.8	4.4	0.6
Under	Inclined	3.5	1.3	3.4	1.5	4.4	1.8
Under	Upright	3.5	1.4	3.5	1.6	4.0	2.2

Canopy: Panicles in pots were set at the height of canopy surface. Under: Panicles in pots were set beneath the height of the canopy surface. Inclined: Pots were inclined at 30° from the vertical. Upright: Pots were set upright. LAI was measured at the base of the canopy. Cumulative LAI was measured at the height of the flowering florets. Measurements were taken on the middle day of each trial by using LAI 2000 crop canopy analyser.

Table 3.3 Summary of analysis of variance (ANOVA) and Tukey's HSD test for mean values of floret sterility, numbers of total and germinated pollen grains, TP20 and GP10 as affected by panicle position and inclination in Experiment 1.

	Sterility (%)	Pollen (no.)	Germinated pollen (no.)	TP20 (%)	GP10 (%)
Trial (T)					
1	75.0 a	23.7 a	8.6 a	57.7 a	69.1 a
2	66.4 ab	20.2 a	8.6 a	67.3 a	70.6 a
3	52.6 b	22.9 a	10.0 a	64.5 a	66.2 a
Position (P)					
Above	64.0 a	21.1 a	8.9 a	64.1 a	68.5 a
Canopy	65.3 a	23.5 a	9.2 a	62.3 a	68.7 a
Inclination (I)					
Inclined	69.5 a	17.7 b	6.8 b	73.7 a	79.1 a
Upright	59.8 b	26.9 a	11.3 a	52.6 b	58.1 b
P×I					
Above Inclined	68.4 ab	15.0 b	6.1 b	76.3 a	81.5 a
Above Upright	59.6 b	27.1 a	11.7 a	51.8 b	55.6 b
Canopy Inclined	70.5 a	20.3 ab	7.4 b	71.1 a	76.8 a
Canopy Upright	60.1 b	26.6 a	11.0 a	53.5 b	60.7 b
ANOVA					
Trial (T)	*	n.s.	n.s.	n.s.	n.s.
Position (P)	n.s.	n.s.	n.s.	n.s.	n.s.
Inclination (I)	**	***	***	***	***
P×I	**	***	***	***	***
T×P	n.s.	n.s.	n.s.	n.s.	n.s.
T×I	n.s.	n.s.	n.s.	n.s.	n.s.
T×P×I	n.s.	n.s.	n.s.	n.s.	n.s.

TP20: Percentage of florets with <20 total pollen grains on the stigma after anthesis. GP10: Percentage of florets with <10 germinated pollen grains on the stigma after anthesis. Above: Panicles in pots were set 25 cm above the canopy surface. Canopy: Panicles in pots were set at the height of canopy surface. Inclined: Pots were inclined at 30° from the vertical. Upright: Pots were set upright. Means followed by the same letter in each column are not significantly different by Tukey's HSD test ($P < 0.05$). Percentage of sterility, TP20 and GP10 were logit-transformed for analysis. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s: not significant.

Table 3.4 Summary of analysis of variance (ANOVA) and Tukey's HSD test for mean values of anther dehiscence characters as affected by panicle position and inclination in experiment 1.

	Apical Dehiscence Length (μm)	Basal Dehiscence Length (μm)
Trial (T)		
1	561.3 a	294.8 a
2	548.6 ab	285.0 a
3	540.6 b	293.5 a
Position (P)		
Above	549.2 a	290.4 a
Canopy	551.1 a	291.8 a
Inclination (I)		
Inclined	543.7 a	292.5 a
Upright	556.6 a	289.7 a
P×I		
Above Inclined	544.7 a	294.2 a
Above Upright	553.7 a	286.6 a
Canopy Inclined	542.7 a	290.8 a
Canopy Upright	559.6 a	292.8 a
ANOVA		
Trial (T)	*	n.s
Position (P)	n.s	n.s
Inclination (I)	n.s	n.s
P×I	n.s	n.s
T×P	n.s	n.s
T×I	n.s	n.s
T×P×I	n.s	**

Above: Panicles in pots were set 25 cm above the canopy surface. Canopy: Panicles in pots were set at the height of canopy surface. Inclined: Pots were inclined at 30° from the vertical. Upright: Pots were set upright. Means followed by the same letter in each column are not significantly different in Tukey's HSD tests ($P < 0.05$). **: $p < 0.01$; *: $p < 0.05$; n.s.: not significant.

Table 3.5 Summary of analysis of variance (ANOVA) and Tukey's HSD test for mean values of floret sterility, numbers of total and germinated pollen grains, TP20 and GP10 as affected by panicle position and inclination in Experiment 2.

	Sterility (%)	Total pollen (no.)	Germinated pollen (no.)	TP20 (%)	GP10 (%)
Trial (T)					
1	46.5 b	26.2 a	10.3 a	58.1 a	65.6 a
2	49.7 b	28.9 a	7.6 b	55.1 a	72.9 a
3	61.4 a	21.3 a	7.2 b	65.8 a	76.1 a
Position (P)					
Canopy	50.8 a	27.6 a	9.6 a	55.2 b	67.1 b
Under	54.3 a	23.3 b	7.1 b	64.1 a	76.1 a
Inclination (I)					
Inclined	65.5 a	17.7 b	5.3 b	73.3 a	86.5 a
Upright	39.5 b	33.2 a	11.4 a	46.0 b	56.7 b
P×I					
Canopy Inclined	65.7 a	18.6 c	5.6 c	70.3 a	83.3 a
Canopy Upright	35.8 b	36.7 a	13.4 a	40.1 b	50.8 b
Under Inclined	65.3 a	16.9 c	4.8 c	76.3 a	89.6 a
Under Upright	43.3 b	29.8 b	9.5 b	51.8 b	62.5 b
ANOVA					
Trial (T)	**	n.s.	*	n.s.	n.s.
Position (P)	n.s.	*	**	**	**
Inclination (I)	***	***	***	***	***
P×I	***	***	***	***	***
T×P	n.s.	n.s.	n.s.	n.s.	n.s.
T×I	**	*	*	*	*
T×P×I	n.s.	n.s.	n.s.	n.s.	n.s.

TP20: Percentage of florets with <20 total pollen grains on the stigma after anthesis. GP10: Percentage of florets with <10 germinated pollen grains on the stigma after anthesis. Canopy: Panicles in pots were set at the height of canopy surface. Under: Panicles in pots were set beneath the height of panicles in the canopy. Inclined: Pots were inclined at 30° from the vertical. Upright: Pots were set upright. Means followed by the same letter in each column are not significantly different by Tukey's HSD test ($P < 0.05$). Percentage of sterility, TP20 and GP10 were logit-transformed for analysis. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s: not significant.

Table 3.6 Summary of analysis of variance (ANOVA) and Tukey's HSD test for mean values of anther dehiscence characters as affected by panicle position and inclination in experiment 2

	Apical Dehiscence Length (μm)	Basal Dehiscence Length (μm)
Trial (T)		
1	494.2	248.2
2	505.6	248.5
3	517.8	221.7
Position (P)		
Canopy	511.2	235.4
Under	500.5	243.6
Inclination (I)		
Inclined	509.2	237.9
Upright	502.6	241.1
P×I		
Canopy Inclined	518.3	232.1
Canopy Upright	504.2	238.6
Under Inclined	500.0	243.6
Under Upright	501.0	243.5
ANOVA		
Trial (T)	n.s	n.s
Position (P)	n.s	n.s
Inclination (I)	n.s	n.s
P×I	*	n.s
T×P	n.s	n.s
T×I	n.s	n.s
T×P×I	n.s	n.s

Canopy: Panicles in pots were set at the height of canopy surface. Under: Panicles in pots were set beneath the height of panicles in the canopy. Inclined: Pots were inclined at 30° from the vertical. Upright: Pots were set upright. *: $p < 0.05$; n.s.: not significant.

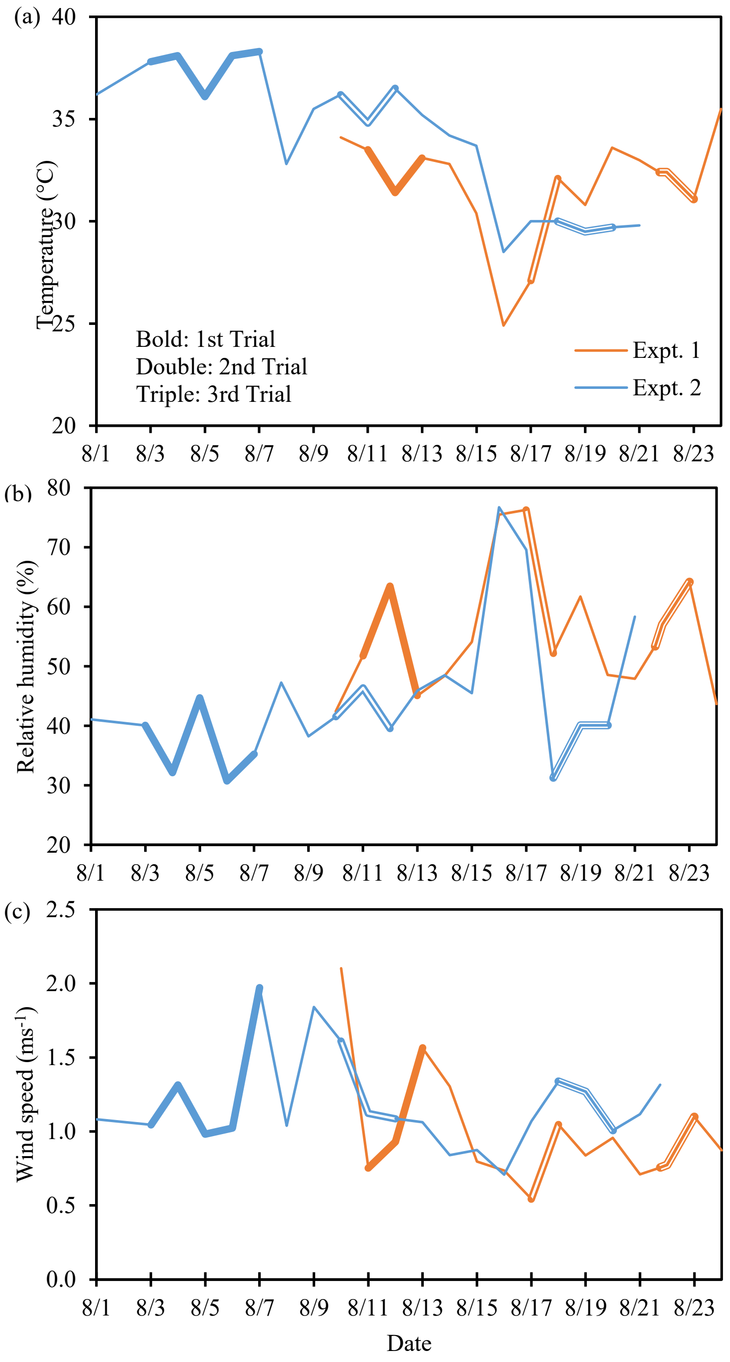

Figure 3.1 Weather conditions at the experimental paddy field during the treatment periods of Experiments 1 (2017) and 2 (2018): (a) daily maximum temperature, (b) relative humidity at the time of maximum temperature, and (c) average wind speed above the canopy (230 cm above the ground) around flowering time (10:00–12:00 h in Experiment 1, 09:00–12:00 h in Experiment 2). Bold, double and triple lines indicate sampling days in first, second, and third trial, respectively.

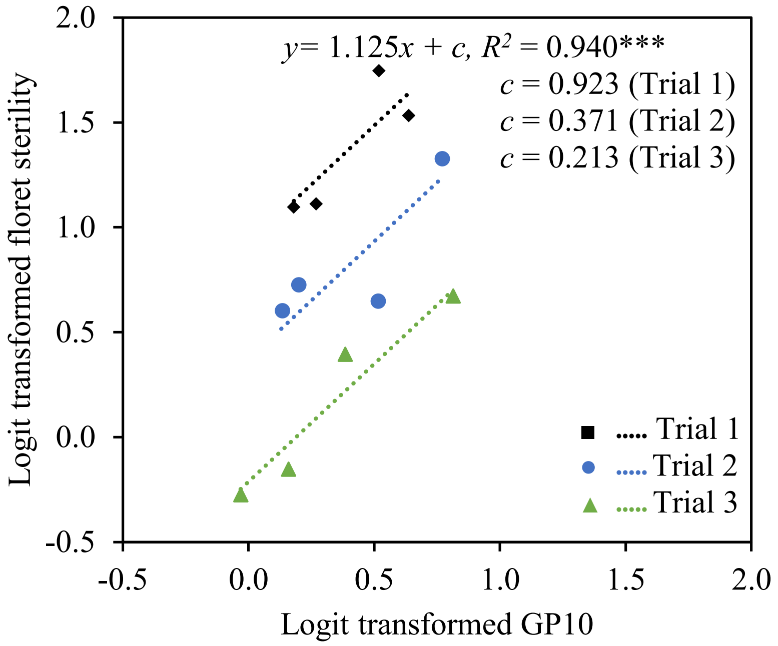

Figure 3.2 Relationship between logit-transformed percentage of florets with <10 germinated pollen grains (GP10) on the stigma and logit-transformed percentage of floret sterility in Experiment 1. In ANCOVA with GP10 as a covariate and trial as an independent variable, the effects of both GP10 and trial on sterility were significant at the 0.1% level (***).

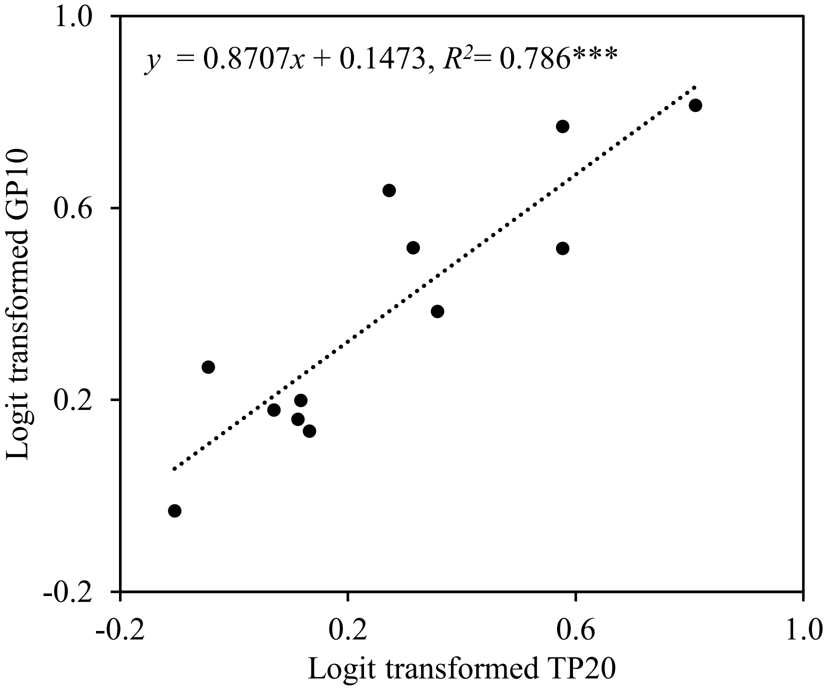

Figure 3.3 Relationship between logit-transformed percentage of florets with <20 total pollen grains on the stigma (TP20) and logit-transformed percentage of florets with <10 germinated pollen grains on the stigma (GP10) in Experiment 1. ***Significant at the 0.1% level.

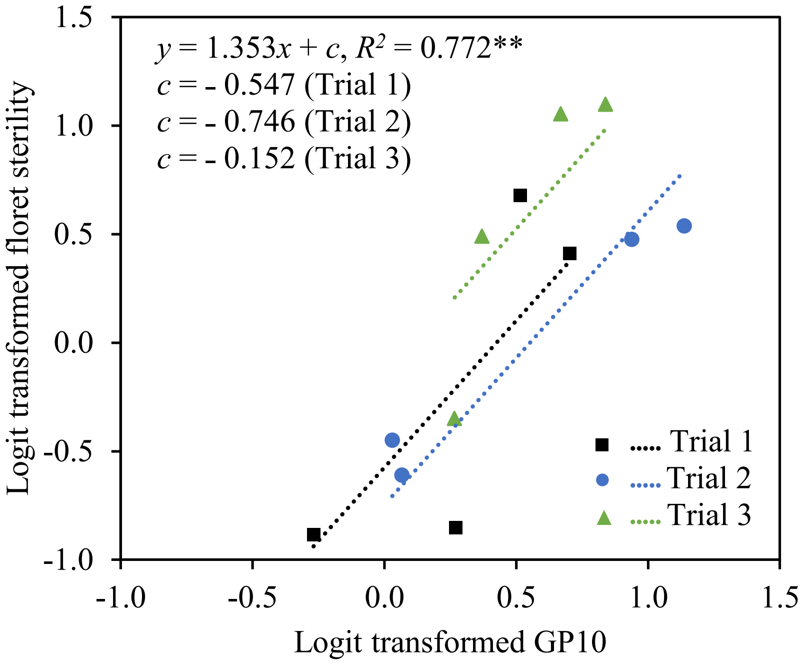

Figure 3.4 Relationship between logit-transformed percentage of florets with <10 germinated pollen grains on the stigma (GP10) and logit-transformed percentage of floret sterility in Experiment 2. In ANCOVA with GP10 as a covariate and trial as an independent variable, the effect of GP10 on sterility was significant at the 1% level (**), but the effect of trial was not.

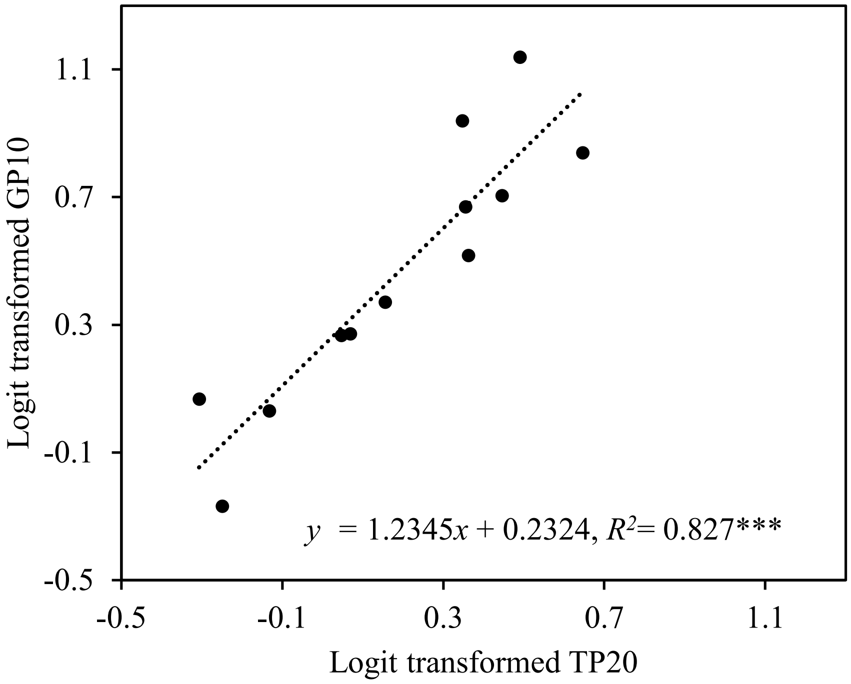

Figure 3.5 Relationship between logit-transformed percentage of florets with <20 total pollen grains on the stigma (TP20) and logit-transformed percentage of florets with <10 germinated pollen grains on the stigma (GP10) in Experiment 2. ***Significant at the 0.1% level.

CHAPTER IV

General Discussions

Global food demand is expected to grow significantly in upcoming decades and it will increase by 59–98% between 2005 and 2050 (60). Scientists have been tried to maximize crop yield to fulfill the increasing demand of world's food supply by various ways and means. Ideotype approach have been used to improve the rice plant type for high yield. China super hybrid rice have been achieved with high yield by emphasizing the top three leaves and lowering panicle height within the canopy to exploit the radiation use efficiency during the grain filling period (47). On the other hand, rice production has been threatened by various abiotic stress generated by global climate change. Rice production has decreased by various abiotic stresses at flowering mainly through the poor pollination. Pollination stability is, therefore, important for sustainable rice production under future global climate change. The present study examined the effect of panicle inclination on pollination stability and floret sterility in rice.

In the first study, we examined the effect of panicle angle on pollination stability in rice with isolated pot-grown rice. We confirmed that panicle inclination seriously affected the stability of pollination probably through the change in relative position between anther and stigma and delay of pollen release. Panicle inclination is common in agricultural scenes. It ranges from 0° -40° at heading stage in Myanmar rice varieties as our field observation (unpublish data). Moreover, floret inclination could be affected by panicle type. Even in erect panicle type, if its structure is loose, the secondary branched will bent outwards and may cause the floret inclination. Huge panicle in high yield rice variety causes panicle inclination at flowering because of long rachis of the panicle and of long flowering period. Excessive application of fertilizer for tall races in both hybrid and landraces sometimes induces panicle incline. Tall cultivar shows inclination of panicle under windy condition. Therefore, our study stresses that panicle architecture is one of the important factors in the stability of rice pollination. In order to maximize the pollination, dehiscent anther should be erect and located directly above the stigma. Panicle inclination changed this optimal condition for gravity pollination. The decrease in stability of rice pollination through change in relative position between dehisced anther and stigma has been reported elsewhere (29, 33, 53). Thus, right time and right place of anther dehiscence is very important in the success of pollination.

We also observed that panicle inclination also hampered pollen germination probably through delay of pollen release. Delaying pollination might cause in pollen dehydration and loss of pollen viability. Very thin pollen wall rich in exinous microchannels (13) causes rapid loss of water from rice pollen and subsequent leads to a sharp drop in viability within a short time after anther dehiscence (23; 57). Even under typical environment, rice pollen grains are short-lived and lose their viability within 5 minutes (26). Thus, rapid germination and tube growth are necessary for success of fertilization in rice (15).

In the second study, we examined the effect of canopy on relationship between panicle angle, pollination and floret sterility with two model experiments. Our results showed that floret sterility was seriously affected by inclination through poor pollination. Therefore, panicle inclination will give damage to rice production.

Nowadays, abiotic stresses are bottlenecks of world rice production. Heat stress induced yield losses occurred in many rice producing regions: China (27), Japan (14) and in other parts of tropical and subtropical regions (21). On the other hand, low temperatures comprise a major climatic problem for rice growing in 25 countries, including Korea and Japan (5). Similarly drought is a major threat to rianfed rice production, with over 25 million ha affected in Asia alone (8). All these stresses at flowering seriously affected seed set mainly through poor pollination. Therefore, our study provides valuable information that erectness of panicle could improve tolerance to against these abiotic stresses though stable pollination and seed set.

We also detected negative effect of depth of canopy on stability of pollination. Although lower panicle height relative to canopy is an important character for high yield in rice (51), we need to consider the panicle position in the context of pollination stability.

In conclusion, our study confirmed that panicle inclination seriously affected seed set through unstable pollination even under canopy condition. It will be better to extend our observation to naturally variation in panicle angle at heading stage among rice varieties to examine their effect on pollination stability and seed set under normal and stress conditions.

Academic papers relating the dissertation

1. Win, A., Tanaka, T. S. T., and Matsui, T. (2020). Panicle inclination influences pollination stability of rice (*Oryza sativa* L.). Plant production Science, 23, 60-68.
2. Win, A., Tanaka, T. S. T., and Matsui, T. (2020). How panicle angle and panicle position in the canopy determine pollination and seed set in rice (*Oryza sativa* L.). Plant production Science, DOI:10.1080/1343943X.2020.1730702.

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