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

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ABSTRACT

Rice pollination occurs when pollen grains fall by gravity from the anther onto the stigma and has been thought to be assured under suitable conditions. However, the position of the stigma relative to the anther pores may affect pollination. The inclination of panicle, that is quite usual in agricultural scene, may change the relative position of anther and stigma. We investigated the effect of panicle inclination on pollination stability. Pots of rice at flowering stage were tilted at one of the four inclinations (0° = control, 15°, 30°, and 45°). We assessed pollination (numbers of total and germinated pollen grains on the stigma) and morphology of dehisced anthers. Inclination significantly affected the number of total and germinated pollen grains on the stigma: as it increased from 0° to 45°, the percentage of florets with <20 total pollen grains on the stigma (TP20) increased from 36.6% to 59.9%, and that of florets with <10 germinated pollen grains on the stigma (GP10) increased from 26.6% to 58.9%. Both an increase in TP20 and a decrease in the rate of pollen germination were major causes of the increase in GP10. Inclination did not affect anther morphology. A panicle inclination of ≥30° could greatly reduce rice production because rice fertilization requires >10 germinated pollen grains on the stigma. Uprightness of the panicle at flowering should be an important objective in rice breeding and production.

Abbreviations: TP20: percentage of florets with <20 total pollen grains on the stigma; GP10: percentage of florets with <10 germinated pollen grains on the stigma

Introduction

Pollination is indispensable for seed set of flowering plants. Self-pollination is an adaptation to conditions where mates or pollinators are limited (Darwin, 1876; Kalisz et al., 2004; Opedal et al., 2016) and promotes reproduction in colonizing populations (Baker, 1955). The relationship between the 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 (Darwin, 1877; Muller, 1883; Webb & Lloyd, 1986), and the capacity for autonomous self-pollination is associated with differences in the anther–stigma
distance in several species (Brys & Jacquemyn, 2011; Kobayashi et al., 2009; Luo & Widmer, 2013; Toräng et al., 2017). 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 (Food and Agricultural Organization [FAO], 2013), 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 (Hoshikawa, 1993). Matsui and Kagata (2003) demonstrated that the stability of rice pollination depends on the size of the basal pore of the anther. 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; Dong et al., 2016) 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.

Materials and methods

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 7-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, P₂O₅-equivalent, and K₂O-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 (Satake, 1972). With this method, we got uniform and straight panicles. In our observation, panicles did not spread during the treatment.

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 the four inclinations (0° = control, 15°, 30°, or 45°) using wooden bases as shown in Figure 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 grassland. Every evening during the 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 a total of 9 days. After treatment, pots were collected and the plants were grown as before the treatment.
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.

Pollination and anther character observations

Samples were collected on 6 days from 10 to 18 September (avoiding rainy days on 12, 14, and 17 September) (Figure 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 (Matsui et al., 2001; Satake & Yoshida, 1978). 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, 16 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 3).

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 during 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.

Results

Microclimate conditions during treatment

The daily maximum air temperature during the observation period (10 to 18 September) ranged from 20.3°C to 29.9°C (Figure 2(a)). That on sampling days ranged from 25.5°C to 29.4°C. RH at the time of maximum temperature on sampling days ranged from 40% to 55% (Figure 2(b)). The average wind velocity around flowering time on sampling days ranged from 0.83 to 1.69 m s⁻¹ (Figure 2(c)).

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 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).
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 ($R^2 = 0.564$, $p < 0.01$; Figure 4(a)) and negatively correlated with TP20 ($R^2 = 0.657$, $p < 0.001$; Figure 4(b)) and GP10 ($R^2 = 0.730$, $p < 0.0001$; Figure 4(c)).
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\(^\circ\) and 45\(^\circ\) than at 0\(^\circ\) at a given TP20 by Dunnett’s test and increased with TP20 \( (R^2 = 0.951, p < 0.0001; \text{Figure 4}) \) (\text{Figure 5}).

Discussion

The inclination of pots significantly affected pollination stability (\text{Table 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 (Matsui et al., 2001; Satake & Yoshida, 1978), a panicle inclination of ≥30\(^\circ\) 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 on 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 (\text{Figure 6}) for the unstable pollination from the morphological aspect. The first factor is the 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 \( \omega \), where \( \omega \) is angle of inclination with respect to vertical) and perpendicular (mg sin \( \omega \)) components to the theca wall. Thus, force to move the pollen grains decrease to mg cos \( \omega \).

Moreover, the perpendicular force generates 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 (Matsui et al., 2005). Self-pollination of cultivars with a small basal pore is unstable because of the delayed and decreased pollen release from the basal pore (Matsui &

\text{Figure 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.

\text{Table 1.} Mean values of pollination parameters as affected by levels of panicle inclination.

| Treatment | Total pollen grains (no.) | Germinated pollen grains (no.) | Pollen germination (%) | TP20 | GP10 |
|-----------|---------------------------|-------------------------------|------------------------|------|------|
| 0\(^\circ\) (control) | 38.80 a | 19.24 a | 58.26 a | 36.61 b | 26.61 b |
| 15\(^\circ\) | 37.32 a | 18.56 a | 58.60 a | 37.21 b | 28.69 b |
| 30\(^\circ\) | 24.94 b | 12.16 b | 51.92 ab | 50.99 ab | 51.08 a |
| 45\(^\circ\) | 26.41 b | 11.71 b | 49.00 b | 59.89 a | 58.94 a |
| Date | | | | | |
| 10 September | 25.18 b | 13.01 b | 60.14 a | 56.00 a | 46.83 ab |
| 11 September | 49.05 a | 21.53 a | 52.75 ab | 27.69 b | 21.76 c |
| 13 September | 25.09 b | 12.58 b | 52.28 ab | 48.96 ab | 43.36 b |
| 15 September | 30.16 b | 14.40 b | 50.10 b | 38.70 ab | 38.19 b |
| 16 September | 30.61 b | 15.00 b | 56.45 ab | 59.21 a | 56.71 a |
| 18 September | 31.12 b | 15.98 ab | 54.93 ab | 46.48 ab | 41.11 b |
| Date (D) | | | | | |
| ** | ** | ** | ** | ** | *** |
| Treatment (T) | | | | | |
| ** | ** | ** | ** | ** | *** |
| D x 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.
Kagata, 2003). Thus, self-pollination of IR72 is originally unstable in comparison with other cultivars with long basal dehiscence (Matsui et al., 2005). 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 6). The change in relative position may reduce pollination by gravity. The third factor is rotational moment generated on the stamens (Factor 3). The 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 show gravi- and phototropic responses (Shimizu et al., 2005). If rice pistil and/or stamen have such response, the situation may be much more complex.

The significantly lower germination percentage of pollen grains with greater inclination (Table 1) suggests that germination was also hampered by inclination. The decreased germination percentage seemed, in turn, to increase GP10 at any given TP20. Song et al. (2001) reported that rice pollen germination decreases from 85% to 42.5% within 6 min after anther dehiscence. The decrease in pollen germination percentage may be explained by the delay of pollen release shown in the above hypothesis concerning factor 1.

Figure 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 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 (Matsui et al., 2019). Fægri and Van der Pijl (1979) also reported that shaking of flowers by wind facilitates autogamy in homogamous hermaphrodite flowers.

The effects of temperature on pollination and anther morphology were not significant. Very high temperatures at flowering (Kobayashi et al., 2011; Matsui et al., 1997,

| Treatment | Anther length (µm) | Apical dehiscence length (µm) | Apical dehiscence width (µm) | Basal dehiscence length (µm) | Basal dehiscence width (µm) |
|-----------|---------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 0° (control) | 1756.93 | 524.27 | 180.77 | 251.31 | 60.73 |
| 15° | 1748.11 | 527.26 | 179.34 | 255.32 | 61.93 |
| 30° | 1765.99 | 535.22 | 178.13 | 248.50 | 61.19 |
| 45° | 1743.47 | 531.04 | 177.18 | 250.80 | 61.13 |

Table 2. Mean values of anther characters as affected by levels of panicle inclination.

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.
and low temperatures at booting (Shimazaki et al., 1964) induce floret sterility through poor anther dehiscence and defective pollination. The maximum temperatures on the sampling dates ranged from 25.7°C to 29.4°C (Figure 2(a)) and the daily minimum temperature during the booting stage ranged from 16.2°C to 23.7°C (data not shown). As day/night temperatures above 26/16°C at booting have minimal effect on sterility (Shibata et al., 1970), 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 (Matsui et al., 1999). 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.
Figure 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 ω) in the inclined theca and occurrence of friction to 'mg sin ω', 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 increases the weight of anthers and the rotational moment. m: mass of pollen grain; g: acceleration by gravity; ω: angle of inclination with respect to vertical.

Figure 7. Factors that caused the increase in the 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.

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