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Function of Long Basal Dehiscence of the Theca in Rice (*Oryza sativa* L.) Pollination under Hot and Humid Condition

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Summary

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Anticipated global warming is expected to increase floret sterility in rice (*Oryza sativa* L.). For selection of genotypes tolerant to high temperatures during the flowering period, it is important to identify the morphological traits controlling tolerance to temperature stress. We investigated the relationship between the length of dehiscence at the basal part of thecae and the viability of pollination in *japonica* type cultivars of rice subjected to a hot and humid condition (37/25 °C, day/night, >90% relative humidity) for three days at flowering. Control plants were left under the ambient conditions in a semi-cylindrical house covered with cheesecloth (30% shading; temperature range: 24-35 °C). The length of basal dehiscence of thecae and the number of pollen grains on the stigmata were examined with a light microscope after flowering. The percentage of florets with more than 20 pollen grains closely correlated with the length of basal dehiscence under the high-temperature treatment (r = 0.906, significant, $P < 0.0001$) and the percentage of florets with more than 80 pollen grains correlated with the length of the basal dehiscence of thecae under the control condition (r = 0.745, significant, $P < 0.01$). The length of basal dehiscence under the high-temperature treatment was highly correlated with that in the control (r = 0.911, significant, $P < 0.0001$). We concluded that the long basal dehiscence of the theca helps the pollen grains to fall from the theca onto the stigmata and increases the stability of the pollination under hot and humid conditions as well as normal conditions. We also concluded that we can estimate the tolerance of the variety to high temperature by measuring the basal dehiscence under a normal condition.

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Introduction

An increase in the concentration of gases with a greenhouse effect, such as carbon dioxide, in the atmosphere may cause the global warming (HANSEN & al. 1984). The increase in carbon dioxide concentration and temperature may have a significant impact on crop production. Crop scientists have attempted to assess the effects of increasing temperature and of high carbon dioxide in the atmosphere on rice yield, using crop simulation models (JIN & al. 1995, HORIE & al. 1996, MATTHEWS & al. 1997) or controlled environments (COLLINS & al. 1995, HORIE & al. 1995, KIM & al. 1996a, b, MATSUI & al. 1997, ZISKA & al. 1997). Some show that an anticipated high temperature would induce floret sterility and increase the instability of rice yield even in temperate regions (KIM & al. 1996b, HORIE & al. 1996). Differences in the tolerance of florets to high temperatures at flowering among varieties has been reported (SATAKE & YOSHIDA 1978, MATSUSHIMA & al. 1982, MATSUI & al. 2001b) and HORIE & al. 1996 showed, using a crop simulation model, that adoption of cultivars tolerant to a high temperature at flowering is one of the most effective countermeasures to maintain high productivity and stability of rice under the anticipated climate change in temperate regions.

The difference in the high temperature-tolerance among the varieties suggests the possibility of genetic improvement of tolerance (MACKILL & al. 1982). To produce high-temperature-tolerant cultivars, it is important to clarify the traits that determine tolerance. Ecophysiological analysis of high-temperature-sterility showed that the direct cause of the sterility is poor pollen shedding induced by high temperatures at flowering, including indehiscence of the thecae, and decrease in the number of germinated pollen grains deposited on the stigmata (SATAKE & YOSHIDA 1978, MATSUI & al. 2000, 2001b). MATSUI & OMASA 2002 showed that some modern Japanese cultivars tolerant to a high temperature have well-developed lacunae for anther dehiscence between the septum and the stomium of the theca. The well-developed lacunae may weaken the septum and help theca dehiscence, and thus increase the tolerance of anther dehiscence to the high temperature (MATSUI & al. 2001a, MATSUI & OMASA 2002). The well-developed lacuna is, however, not a useful marker for exploration of genetic resources and breeding for high-temperature tolerant cultivars because the lacuna is not externally visible, requiring time consuming dissection for mesurement.

On the other hand, MATSUI & KAGATA 2003 reported that long basal dehiscence of the theca for pollen dispersal increases the stability and probability of pollen transport to the stigmata under normal conditions. Increase of the stability and the sureness of pollination may help fertilization under high-temperature treatments. It is easy to measure the size of the dehiscence. The purpose of this article is to examine whether long basal dehiscence increases tolerance of pollination to a high temperature. We examined the effectiveness of the long basal dehiscence in pollination under a hot and humid condition. To exclude the effect of water deficit on the plant, we exposed the plants to a high temperature treatment under a humid condition.

Material and Methods

Plant materials and treatment

Twelve Japanese rice varieties (*japonica*) were used (Table 1). The most of seeds were obtained from the Gene Bank of the National Institute of Agrobiological Sciences (Japan). Seeds were sown on May 3. Seedlings at the 5th-leaf stage were transplanted in a circular pattern into four-liter pots, 20 seedlings per pot, and grown in waterlogged soil. The soil was obtained from paddy field in the Experimental Farm of Kyoto University (Osaka, Japan). To avoid severe wind, we grew the plants in a semi-cylindrical house covered with cheesecloth (30% shading) set up outdoors throughout the experimental period. Plants in each pot were supplied with 0.6g P_2O_5 as basal-dressing, and 0.1g N and 0.1g K_2O as top dressing on June 24 and on July 9 to maintain a healthy leaf color. Tillers were removed during the vegetative stage when they appeared. The ears emerged from late-July to early September.

A growth chamber was used for high-temperature treatment. Three pots of each cultivar at the middle heading time were exposed to an air temperature of 37.0 °C for six hours (10:00 – 16:00) for three consecutive days. From 18:00 to 8:30, the air temperature was 25.0 °C. From 8:00 to 10:00 and from 16:00 to 18:00, the air temperature was changed stepwise. Air humidity was over 90 % R.H. all day. During the day time (6:00 to 18:00) plants were exposed to light from fluorescent lamps (230 μmol m^{-2} s^{-1} photosynthetic photon flux density). Another three pots were left in the semi-cylindrical house covered with cheesecloth as the control (temperature, 25 to 36 °C). Flowering time was 11:00 to 14:00 under the high-temperature treatment and was 10:00 to 13:00 under the control condition.

Experimental procedure

In the plants exposed to a high temperature, florets that finished flowering were sampled every day at 16:00 during the three-day high-temperature treatment. In the control, florets were sampled about three hours after the start of floret opening every day for more than three days during the flowering period. Seventeen florets on the primary branches in each variety were sampled on each day. The number of indehisced thecae was counted, and in the dehisced thecae, the length of the dehiscence at basal parts of the thecae was measured. Anthers from five florets randomly selected from the 17 florets were used for this measurement. The means of the percentage of indehisced thecae and of length of the basal dehiscence was calculated each day. The stigmata from all 17 florets were stained with cotton blue and pollen grains on the stigmata were counted. The percentage of florets with more than 80 pollen grains deposited on the stigmata in the control and that with more than 20 pollen grains in both conditions were calculated each day for each variety. Since most of the florets with more than 20 pollen grains deposited on the stigmata will fertilize even under a high-temperature condition (SATAKE & YOSHIDA 1978, MATSUI & al. 2001b), the percentage of florets with more than 20 pollen grains indicates viability of pollination of the cultivar under the condition. On the other hand, the percentage of florets with more than 80 pollen grains is strongly correlated with the number of pollen grains deposited on the stigmata and its coefficient of variation under a normal condition (MATSUI & KAGATA 2003). It is an index showing reliability of the pollination under the normal condition.

Data analysis

The means of the values (the percentages of indehisced thecae, the length of the basal dehiscence, and the percentages of the florets with more than 80 pollen grains and with more than 20 pollen grains) on the measurment days for each variety were calculated as the scores of the variety. One-way ANOVA was conducted to determine the significance of effect of cultivars on the values, considering different mesurement days to be replications. LSDs were calculated for the means, when the effect of cultivars was significant at $P > 0.05$. The means were also used for regression analysis.

Results

The percentages of florets with more than 20 pollen grains and 80 pollen grains on the stigmata in the control varied from 94% to 100% and 63% to 90%, respectively (Table 1), and the effect of cultivars on the percentages were not significant among the varieties (data not shown). In contrast, the percentage of florets with more than 20 polen grains widly ranged from 24% to 88%. Effect of cultivars on the percentage of florets was significant at $P < 0.001$ (data not shown) and the percentage of Kamiji2, Homura3, Kinmaze, and Koshihikari were significantly higher than that of Kokuryomiyako, Somewake, Husakushirazu, Takanari, Ginbouzu. The percentage of florets with more than 20 pollen grains under the high-temperature treatment correlated with the percentage of florets with more than 80 pollen grains under the control condition ($r = 0.7905$, significant, $P < 0.01$).

Table 1. Percentage of florets with more than 80 pollen grains on the stigmata after anthesis under the control condition and that of florets with more than 20 pollen grains under high-temperature treatment. Each value is the mean ± standard error of sampling days ($n \geq 3$). *Seeds were obtained from the Gene Bank of the National Institute of Agrobiological Science (Japan). ns denotes insignificance of the effect of cultivars on the % of florets at $P = 0.05$ in one-way ANOVA.

Cultivar	Control		High temperature
	% of > 20-pollen-grain florets	% of > 80-pollen-grain florets	% of > 20-pollen-grain florets
Somewake*	94.1 ±4.2	63.2 ±13.5	27.5 ±13.7
Homura3*	100.0 ±0.0	84.3 ±9.8	84.3 ±7.1
Koshihikari	100.0 ±0.0	79.2 ±8.8	74.5 ±7.8
Nipponbare	98.5 ±1.5	78.8 ±7.6	62.7 ±20.5
Kokuryomiyako*	98.5 ±1.5	64.7 ±6.4	43.1 ±2.0
Ginbouzu*	94.1 ±3.4	70.6 ±6.4	23.5 ±12.2
Husakushirazu*	100.0 ±0.0	80.0 ±10.5	27.5 ±12.9
Takenari	100.0 ±0.0	70.6 ±11.3	27.5 ±8.5
Tairaippon*	98.5 ±1.5	76.5 ±5.4	62.7 ±5.2
Magatama*	100.0 ±0.0	86.8 ±5.0	70.6 ±3.4
Kameji2*	100.0 ±0.0	89.7 ±3.7	88.2 ±5.9
Kinmaze	100.0 ±0.0	83.8 ±6.5	78.4 ±5.2
LSD (0.05)	ns	ns	29.3

The length of basal dehiscence varied with the cultivar from about 350 to 620 μm under the high-temperature treatment and from 300 to 600 μm in the control (Table 2). The percentage of florets with more than 20 pollen grains correlated closely with the length of basal dehiscence under the high-temperature treatment ($r = 0.906$, significant, $P < 0.0001$) and the percentage of florets with more than 80 pollen grains correlated with the length of the basal dehiscence of thecae in the control ($r = 0.745$, significant, $P < 0.01$). The length of basal dehiscence under the high-temperature treatment correlated highly with that under the control condition ($r = 0.911$, significant, $P < 0.0001$). The percentage of indehisced thecae under the high-temperature treatment was below 23% (Table 2). The correlation between the percentage of indehisced thecae and the percentage of florets with more than 20 pollen grains was not significant (r = -0.5481, not significant at P = 0.05) under the high-temperature treatment.

Table 2. Characteristics of theca after anthesis. Each value is the mean ± standard error of sampling days (n ≥ 3).

Cultivar	Indehisced thecae under the high-temperature treatment (%)		Length of basal dehiscence (μm)			
			Control		High temperature	
Somewake	1.7	±1.7	0.38	±0.01	0.43	±0.01
Homura3	2.8	±2.0	0.49	±0.02	0.56	±0.03
Koshihikari	0.0	±0.0	0.54	±0.02	0.60	±0.01
Nipponbare	1.8	±1.0	0.49	±0.01	0.48	±0.01
Kokuryomiyako	5.5	±2.3	0.39	±0.01	0.43	±0.04
Ginbouzu	15.3	±2.2	0.31	±0.01	0.41	±0.07
Husakushirazu	23.0	±4.9	0.38	±0.02	0.36	±0.02
Takenari	1.4	±1.4	0.47	±0.01	0.47	±0.01
Tairaippon	7.4	±6.7	0.54	±0.03	0.53	±0.02
Magatama	0.3	±0.3	0.56	±0.02	0.58	±0.03
Kameji2	1.3	±1.3	0.58	±0.03	0.61	±0.04
Kinmaze	3.6	±3.6	0.60	±0.02	0.62	±0.00
LSD (0.05)	8.5		0.08		0.07	

Discussion

Since most of the florets with more than 20 pollen grains deposited on the stigmata fertilize soundly even under a high temperature condition (SATAKE & YOSHIDA 1978, MATSUI & al. 2001b), the percentage of florets with more than 20 pollen grains indicates viability of pollination of the cultivar under the condition. In the present experiment, the percentage of florets with more than 20 pollen grains on the stigmata was at least 94% in the vrieties in the control, suggesting almost the florets completed pollination soundly in the control. In contarast, the percentage of florets with more than 20 pollen grains ranged from 24% to 88%. This result indicates that the high-temperatrue treatment increased the percentage of florets with a insufficient number of pollen grains on the stigmata. The significant difference in the percentages of florets with more than 20 pollen grains under a high-temperature treatment among cultivars indicates genetic variation in tolerance of pollination to the high temperature.

The percentage of florets with more than 20 pollen grains under the high-temperature treatment was significantly correlated with that with more than 80 pollen grains under the control condition. Although the effect of cultivars on the pecentage of florets with more than 80 pollen grains in control was not significant, this correlation suggests that the pollen number deposited on the stigmata under normal conditions could be a good measure for evaluation of varieties in the tolerance of pollination to a high temperature.

MATSUI & KAGATA 2003 showed that the percentage of florets with more than 80 pollen grains and that of those with more than 40 pollen grains was highly correlated with the length of the basal dehiscence of thecae under a normal condition. They assumed that since basal dehiscence is located just above the stigmata and open at the floret opening, the pollen grains in thecae with a long basal dehiscence fall easily onto the stigmata because of the large passage and thus the pollination of cultivars with long basal dehiscence is sure (MATSUI & KAGATA 2003). The close correlation between the percentage of florets with more than 20 pollen grains and the length of basal dehiscence under the high-temperature treatment in our results suggests that the long basal dehiscence contributes to sure pollination even under the hot and humid condition.

Moreover, the length of basal dehiscence under the high-temperature treatment closely correlated with that under the control condition. This correlation shows that the character of long basal dehiscence is stable under both conditions. We can therefore estimate the tolerance of the variety to high temperature by measuring the basal dehiscence under normal conditions.

In the present experiment, the percentage of indehisced thecae under the high-temperature treatment was below 23% probably because of the high humidity. Moreover, the percentage of indehisced thecae did not correlate with the percentage of florets with more than 20 pollen grains under the high-temperature treatment. These results indicate that the primary cause of poor pollination under the high-temperature treatment is not indehiscence of thecae. This conclusion differs from that under drier and hotter conditions reported previously (MATSUI & al. 2000). The primary cause of the poor pollination may depend on both the humidity and temperature. In our observation, most thecae of the cultivars we used dehisced at the time of floret opening even under a hot and humid condition, but many pollen grains remained in the thecae (no data). The primary cause does not seem to be poor dehiscence of the thecae but seems to be the delay of pollen release. However, no pollen grains fall from the indehisced thecae. Long basal dehiscence would be effective to stabilize the pollination as long as the percentage of indehisced thecae is low.

In this study, we found that the greater the length of the split in the anthers allowing dehiscence at the basal part of the thecae the better pollination under hot and humid condition. The character of the long dehiscence and of the stable pollination were stable under both high- and normal-temperature at flowering. We can therefore estimate the tolerance of cultivars to high temperature at flowering from the stability of pollination and the length of basal dehisce under normal conditions.

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