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Rapid Swelling of Pollen Grains in Response to Floret Opening Unfolds Anther Locules in Rice (*Oryza sativa* L.)

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Abstract : The changes in pollen grain diameter were examined in relation to the floret opening and anther dehiscence in rice (*Oryza sativa* L.). In the first experiment, the florets were artificially opened by excising the top of the glumes at various times before the expected natural flowering time. Pollen diameter increased rapidly in the artificially opened florets, but slowly in the controls in which the glumes were left intact. The time of anther dehiscence coincided well with the time when pollen grains reached their maximum diameter. In addition, the pollen grain diameter was significantly correlated with the percentage of dehisced anthers in the florets artificially opened at various times. These results indicate that floret opening induces the swelling of pollen grains and that the swelling is an important event for anther dehiscence. In the second experiment, anther segments with pollen left remaining intact and those with pollen removed were immersed in water. The septa in the anther segments without pollen failed to rupture in water, whereas 70% of the septa in the anther segments with pollen left remaining ruptured, indicating that the septa can be ruptured by the swelling pressure of pollen grains resulting in unfolding of anther locules. From these findings, we infer that the rapid swelling of pollen grains in response to floret opening is a driving force to rupture the septum and unfold the locules.

Key words : Anther dehiscence, Floret opening, Locule unfolding, *Oryza sativa* L., Pollen grain swelling, Rice.

The anticipated future global warming may increase the instability of rice (*Oryza sativa* L.) yield even in temperate regions, mainly through the increased probability of high temperature-induced floret sterility at flowering (Horie et al. 1996). The main cause of this kind of floret sterility is poor anther dehiscence (Satake and Yoshida, 1978 ; Matsui et al., 1997). To develop cultivars resistant to high-temperature damage, we must understand the anther dehiscence mechanism. In rice, as in other anemophilous plants, dehydration of the anther wall is considered responsible for the unfolding of the anther locule (Hoshikawa, 1993). However, this article presents another explanation for the mechanism of anther dehiscence. We found that pollen grains rapidly swell at the time of floret opening and that the swelling of the pollen grains causes the locules to unfold. We concluded that floret opening triggers rapid swelling of pollen grains, which works as a driving force to unfold the anther locules.

Materials and Methods

1. Plant materials

Twenty rice seeds (*Oriza sativa* L., cv. Akihikari) were sown in a circular pattern each in 4-liter pots. The plants were grown in a temperature-controlled greenhouse at 25°C until flowering. The day length was kept at 14 hours by supplementary lighting (white fluorescent lamp) after natural daylight. All tillers were removed as they appeared to obtain uniform panicles on the main culm (Satake, 1972). From 20 days before heading, the plants were grown under a submerged soil condition until the end of the experiment. At the heading stage, the plants were acclimated to the conditions shown in Figure 1 for over 3 days until the start of the experiments. The florets expected to open next were marked at 17 : 00 on the day before the experiment. The flowering order of the florets was judged from their position on the panicle. Since the marked florets on the plants started to open at 12 : 30±10 on the next day under the conditions shown in figure 1, all marked florets detached from the plants without further treatment before the natural opening were assumed to open at 12 : 30±10 (natural flowering time).

2. Experimental procedure

Experiment 1

The marked florets were detached from rice plants by cutting the rachis-branch at 210, 150, 90 and 30 minutes before their expected natural flowering time (12 : 30) for measurement of pollen grain diameter, and at 270, 210, 150, 90, 30 and 0 minutes before the time of natural flowering for investigation of percentage of dehisced thecae (pollen sac). Immediately after the detachment, glumes of the florets were artificially opened by cutting off the apical 2 mm of the glumes in one group, and the florets in the other group (control) were not opened. The florets in both groups were maintained under the conditions shown in Figure 1. After the detachment, the

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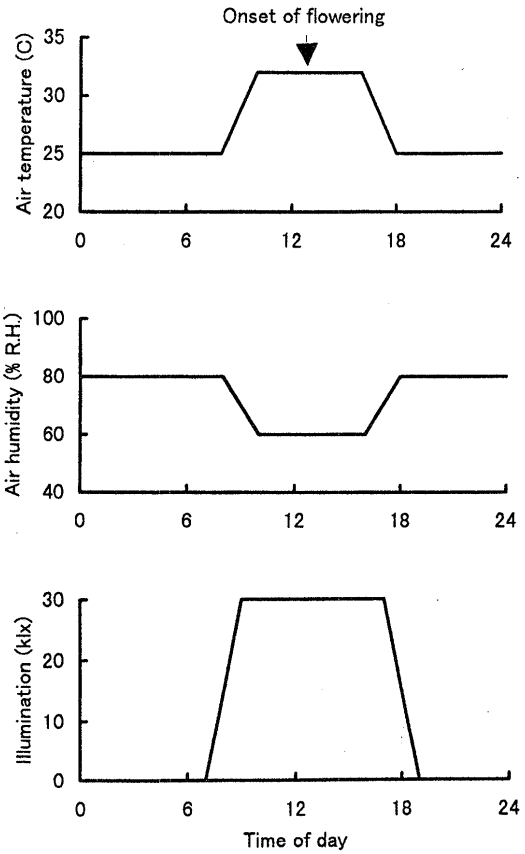

Fig. 1. Diurnal pattern of air temperature, air humidity, and irradiation in an Environment Simulator, a facility for simultaneous control of air temperature, humidity, wind velocity and irradiation (white fluorescent lamp), for the experiments 1 and 2. Wind velocity was constant at 0.5 m s^{-1}.

diameter of pollen grains was measured every two minutes for 30 minutes. Nine pollen grains from 3 anthers were used for each measurement. The percentage of dehisced thecae was determined 5 hours after detachment. Thirty anthers from 5 florets per treatment were used for the investigation. This experiment was repeated 4 times.

Experiment 2

Anthers were detached from florets of rice plants in the afternoon on the day before flowering of the floret. Both ends of each anther were cut off leaving the middle part about 0.3 mm in a 10% sucrose solution. The cut piece was again transversely cut into halves each with four locule tubes (about 0.15 mm in length, see Fig. 7*b*), and all pollen grains in one half were removed from the locule tubes with a small brush in this solution. The other half was left with the pollen grains remaining intact. After this treatment, both anther segments with and without pollen grains were immersed in water. Thirty minutes later, septa rupture was observed (cf. Fig. 7*b*, *c*). Five to ten minutes was required for anther cutting and pollen removal. This experiment was repeated 10 times.

Results and Discussion

In florets detached from the plant and artificially opened 30 minutes before the expected natural flowering

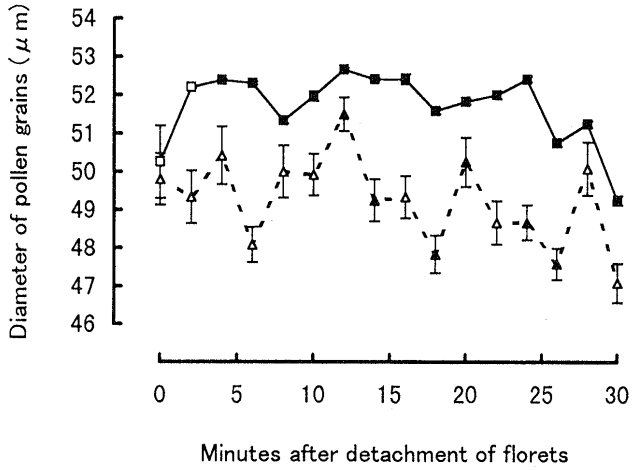

Fig. 2. Effect of artificial floret opening on change in diameter of pollen grains after detaching the florets from the plant at 30 minutes before their expected flowering time. Unopened florets (control), ---- ▲; Artificially opened florets, —— ■. Open symbols indicate that observed anthers contained no dehisced thecae. Bars indicate ±standard errors (n=9).

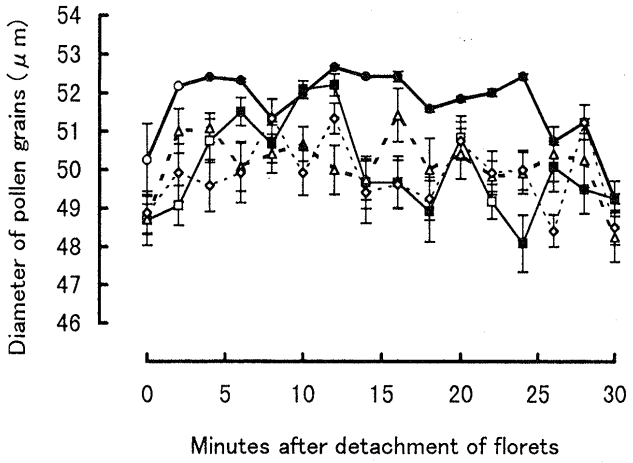

Fig. 3. Diameter change in pollen grains in the florets artificially opened after detachment from the plant at 30 (——, ●), 90 (——, ■), 150 (----, ▲) and 210 minutes (-----, ◆) before the natural flowering time. Open symbols indicate that observed anthers contained no dehisced thecae. Bars indicate ± standard errors (n=9).

time, pollen grains increased in diameter to reach the maximum within two minutes after the detachment and maintained the maximum level for about 20 minutes (Fig. 2). The diameter of the pollen grains in control florets detached at the same time without artificial opening fluctuated with the passage of time, but were always smaller than that in the artificially opened florets. We suppose that the artificial opening of florets triggered the rapid swelling of pollen grains.

Pollen grains in the florets artificially opened at 210, 150 and 90 minutes before the natural flowering time also repeated swelling and contraction after the detachment, but the degree of swelling was higher, the later the detachment (Fig. 3). This shows that the ability of pollen grains to swell rapidly in response to the artificial opening of florets increases from 210 to 30 minutes before the natural flowering time.

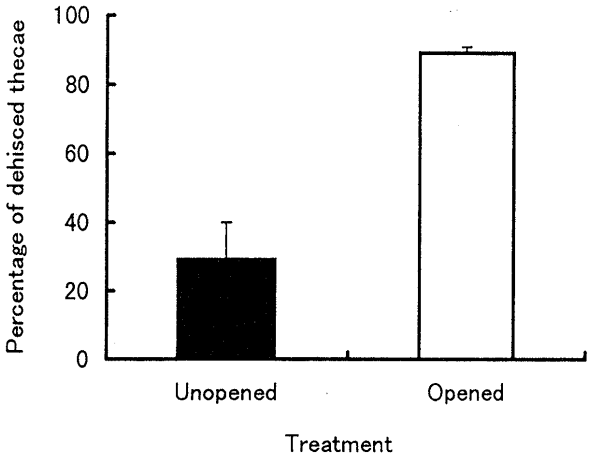

Fig. 4. Percentage of dehisced thecae in the unopened (black) and the artificially opened (white) florets detached from plants just before natural flowering. Bars indicate standard errors (n=4). The percentage in the opened florets was significantly larger than in the unopened florets ($P<0.01$).

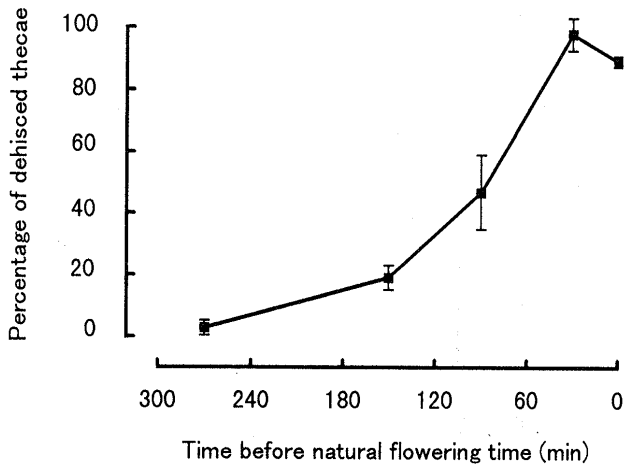

Fig. 5. Relationship between the percentage of dehisced thecae in the artificially opened florets and the time of detachment (artificial opening). Values were determined 5 hours after detachment. Bars indicate ±standard errors (n=4).

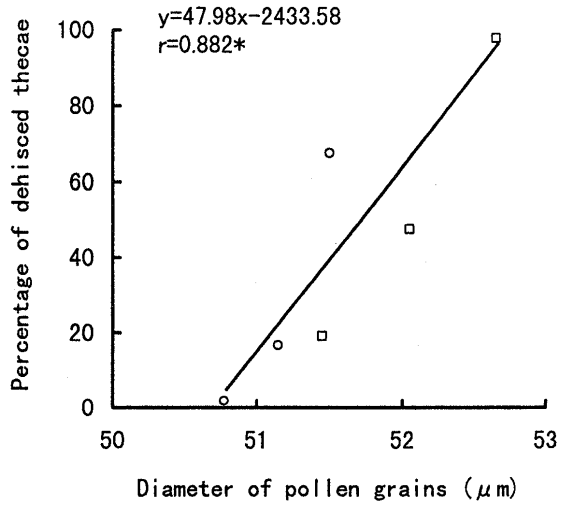

Fig. 6. Relationship between the maximum value of pollen grain diameter and the percentage of dehisced thecae in the florets detached at 150 to 30 minutes before the natural flowering time. Symbols: unopened florets (○), artificially opened florets (□). The asterisk indicates significance at the 5% level.

The percentage of dehisced anthers in the florets artificially opened just before the natural flowering time and examined five hours later was almost 90% (Fig. 4). In contrast, this percentage in the control florets detached from the plant at the same time was below 30%. These results indicate that the artificial floret opening promoted anther dehiscence. Under natural conditions, anther dehiscence synchronizes with floret opening suggesting that natural floret opening also promote anther dehiscence.

From 150 minutes before the time of natural flowering, the percentage of dehisced anthers (determined five hours after the detachment) in artificially opened florets sharply increased with the delay of the time of artificial floret opening (Fig. 5), indicating that the ability of the anther to dehisce increases in parallel with the increase in the ability of pollen grains to swell. Our results coincide with Tsuboi's (1961) observation that the rubbing of rice panicles by hand hastened floret opening, but the anthers in the florets thus opened 3 hours earlier did not dehisce. His rubbing treatment at 3 hours before flowering only hastened spikelet opening, but failed to cause anther dehiscence presumably because pollen grains had not yet acquired the ability to swell.

Several facts mentioned above support the hypothesis that pollen grain swelling is an important event for anther dehiscence. First, the time of anther dehiscence coincided well with the time when pollen grains reached their maximum diameter (Figs. 2, 3). Second, the increase in the ability of the anther to dehisce synchronized with the ability of pollen grains to swell in response to floret opening (Figs. 3, 5). Third, the pollen grain diameter was significantly correlated with the percentage of dehisced anthers in both artificially opened and unopened florets 150 to 30 minutes before the natural flowering time (Fig. 6).

We immersed the anther segments with pollen grains left remaining intact or removed into water. In the anther segments with locules holding pollen grains, 70% of the septa between locule tubes ruptured in water, but no septa ruptured in the anther segments holding no pollen grains (Fig. 7). This suggests that pollen pressure is a driving force for rupturing the septum and unfolding the locules.

The process of anther dehiscence can be described as follows. (1) From several hours before the time of natural flowering, the ability of pollen grains to swell in response to floret opening increases with time; (2) floret opening triggers rapid pollen-grain swelling; (3) the increase in the swelling pressure of pollen grains causes the locule to unfold. Although it is still uncertain whether pollen grain swelling per se causes the stomium (small epidermal cells covering dehiscence cavity, cf. Fig. 7*a*) to open, our data show that pollen grain swelling is an indispensable event for anther dehiscence.

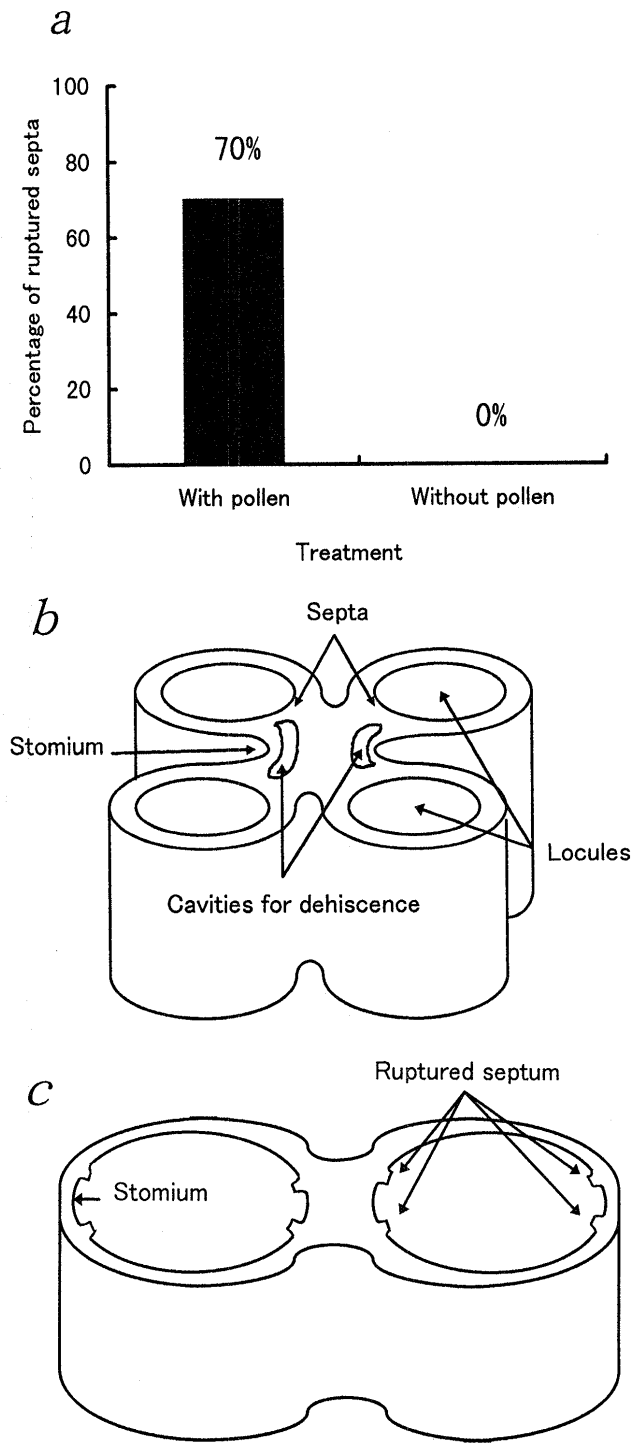

Fig. 7. Effect of immersion in water on the rupturing of septum in anther segments (transverse segments). *a*, percentage of ruptured septa in the anther segments immersed in water with or without pollen. *b*, diagram of an anther segment with unruptured septa. *c*, diagram of an anther segment with ruptured septa.

The question remaining is how the pollen grain absorbs water. Obermeyer and Blatt (1995) clarified the K^+ influx to pollen grain of lily before pollen germination. Although such an inward-directed K^+ flow has been regarded as one event in lily pollen germination (Weisenseel et al., 1975 ; Obermeyer and Blatt, 1995), such ion flow may also cause a reduction in osmotic potential and hence swelling of rice pollen grains as in guard cells. Koike and Satake (1987) demonstrated that the digestion of starch in rice pollen grain started 3 hours before flowering. Such slow digestion of starch may not directly cause the rapid swelling of pollen grains. However, the time of this digestion coincides with the time at which pollen grains acquired the ability to swell in our experiment. This coincidence suggests that such digestion plays a role in pollen grain swelling and subsequent germination ; reduction in base osmotic potential, and the production of sugar as energy or malic acid as an ion for the regulation of electrical potential.

Decrease of pollen volume and of accumulated starch in pollen grains have been observed in poorly dehiscent anthers in the florets damaged by a high temperature at the flowering stage (Sato et al., 1973). Insufficient pressure of pollen at the time of floret opening may be a cause of floret sterility observed under high-temperature conditions.

Acknowledgments

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References

Horie, T., Matsui, T., Nakagawa, H. and Omasa, K. 1996. Effect of elevated CO_2 and global climate change on rice yield in Japan. In K. Omasa, K. Kai, H. Taoda, Z. Uchijima and M. Yoshino eds. Climate Change and Plants in East Asia. Springer-Verlag, Tokyo. 39—56.

Hoshikawa, K. 1993. Anthesis. In M. Shimizu, K. Hoshikawa, E. Maeda and K. Yamazaki eds. Science of the Rice Plant Vol. 1. Rural Culture Association, Tokyo. 341—345.

Koike, S. and Satake, T. 1987. Sterility caused by cooling treatment at the flowering stage in rice plant. II. The abnormal digesting of starch in pollen grain and metabolic change in anther following cool treatment. Jpn. J. Crop Sci. 56 : 666—672.

Matsui, T., Omasa, K. and Horie, T. 1997. High temperature-induced spikelet sterility of japonica rice at flowering in relation to air temperature, humidity and wind velocity conditions. Jpn. J. Crop Sci. 66 : 449—455.

Obermeyer, G. and Blatt, M.R. 1995. Electrical properties of intact pollen grains of Lilium longiflorum : characteristics of the non-germinating pollen grains. J. Exp. Bot. 46 : 803—813.

Satake, T. 1972. Circular dense-culture of rice plants in pots, the purpose of obtaining many uniform panicles of main stems. Proc. Crop Sci. Soc. Jpn. 41 : 361—362*.

Satake, T. and Yoshida, S. 1978. High temperature-induced sterility in indica rices at flowering. Jpn. J. Crop Sci. 47 : 6—17.

Sato, K., Inaba, K. and Tozawa, M. 1973. High temperature injury of ripening in rice plant. 1. The effect of high temperature treatments at different stages of panicle development on the ripening. Proc. Crop Sci. Soc. Jpn. 42 : 207—213*.

Tsuboi, Y. 1961. Ecological studies on rice plants with regard to damages caused by wind. Bull. Natl. Inst. Agric. Sci. A8 : 1—156*.

Weisenseel, M.H., Nuccitelli, R. and Jaffe, L.F. 1975. Large electrical currents traverse grown pollen tubes. J. Cell Biol. 66 : 556—567.

*In Japanese with English summary.