Mechanism of Anther Dehiscence in Rice (*Oryza sativa* L.)

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This paper presents a new explanation of the mechanism of anther dehiscence in rice during the period from floret opening to pollen dispersal. The theca dehisced on the stomium in the apical part and the anther wall in the basal part of the large locule. Comparison of the anther dehiscence process under various air humidity conditions showed that the process, until the splitting at the apical and basal parts, was a moisture-requiring process whereas the widening of the splits in both parts was a desiccatory process. Observation of the anther transverse section, revealed the marked development of the U-shaped thick cell wall in the endothecium adjacent to these two splits. From these observations, the anther dehiscence mechanism may be explained as follows. At the time of anthesis, pollen grains swell rapidly in response to the floret opening and cause the theca to bulge, rupturing the septum. The pollen pressure combined with the inward bending of the locule walls adjacent to the stomium causes splitting of the stomium in the apical part of the theca. At the same time, the septum rupture extends to the bottom of the large locule supported by the pollen pressure. After these processes, the locule walls adjacent to both splits straighten probably due to their water loss. This straightening widens the splits and the swollen pollen grains overflow from the widened splits.

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Key words: Anther dehiscence, *Oryza sativa* L., pollen grain swelling, rice, septum, stomium, theca.

INTRODUCTION

Pollination is an indispensable process for the fructification of grain crops. Thus, poor dehiscence of anthers causes a decreased yield of grain crops. In Asian rice, (*Oryza sativa* L.) which is a major staple food in East Asia, cool weather ($< 20 °C$) during the booting stage and high temperature ($> 35 °C$) at flowering lowers the yield (Horie, Yajima and Nakagawa, 1992; Horie *et al.*, 1996), mainly through poor anther dehiscence (Shimazaki *et al.*, 1964; Ito *et al.*, 1970; Satake and Yoshida, 1978; Matsui *et al.*, 1997*a*; Matsui, Omasa and Horie, 1997*b*). Anther dehiscence is the most susceptible event to temperature stresses in rice production.

Despite the importance of the anther dehiscence in rice, little attention has been paid to this process. Although, as in other anemophilous plants, it has generally been believed that the rice anthers open as a result of the outward bending of the locule walls caused by their desiccation (Hoshikawa, 1993), there are several lines of evidence against this theory. Tsuboi (1961) found that floret opening was hastened in rice by rubbing the panicle with ones hands and that the anthers in the florets thus opened at 3 h before the expected flowering time and could not dehisce. This poor dehiscence indicates that the anther has a low ability to dehisce until 3 h before flowering time, thus suggesting that the mechanism of anther dehiscence is not as simple as the above theory.

Moreover, as evaporation from the anther is increased at a high temperature and the desiccation of the locule wall is accelerated, the disturbance of anther dehiscence by high temperature at the time of flowering observed by Satake and Yoshida (1978), means that the desiccation of the locule wall may not be the main driving force to open the anthers as shown in Liliaceae (Keijzer, 1987, 1999).

In connection with the above issue, Matsui, Omasa and Horie (1999) found a rapid swelling of pollen grains in response to floret opening in rice, and pointed out its importance in anther dehiscence. The purpose of the present article is to clarify the mechanism of anther dehiscence during the period from floret opening to pollen shedding. For this purpose, we observed the structure of the rice anther and the process of anther dehiscence, and examined the effect of air humidity on this process.

MATERIALS AND METHODS

Transverse section and process of anther dehiscence

Paddy rice (*Oryza sativa* L. 'Nipponbare') plants were grown in submerged 4 l pots under outdoor conditions. Intact anthers were collected and fixed in FAA on the day before flowering (the day of flowering was judged from the position on the panicles). The fixed anthers were dehydrated with a methanol-butanol series, and embedded in paraffin. Ten-micron-thick transverse sections were cut and stained with toluidine blue-O.
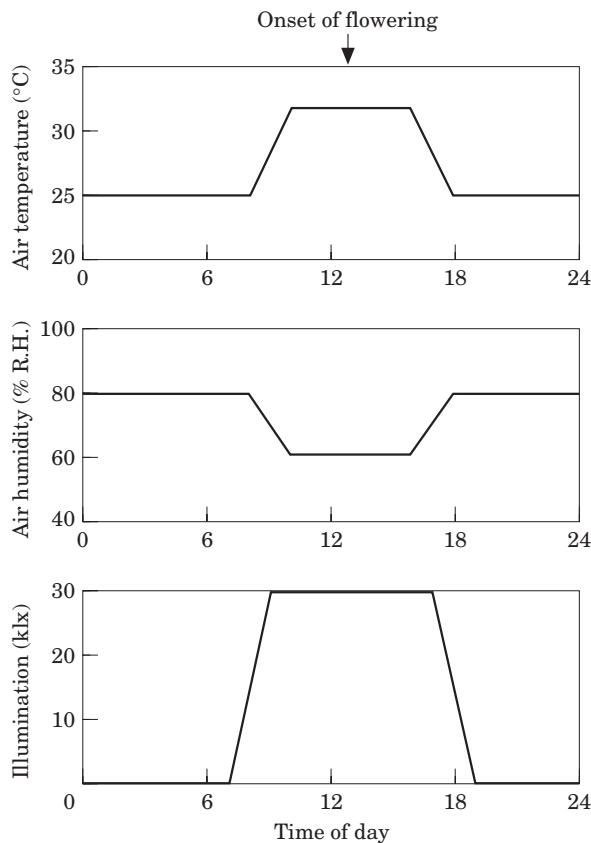
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Fig. 1. The phytotron conditions. Wind velocity was fixed at 0·5 ms^{-1}.

Apart from this, intact florets of the paddy rice ('Akitakomachi') grown under the same condition were collected just before flowering time to observe the anther dehiscence process. After removal of the lemma, the process of anther dehiscence was observed under a stereo microscope. Observation began within 30 sec from the sampling of the anthers.

Effect of air humidity on anther dehiscence

Oryza sativa L. ('Akihikari') plants were grown in a greenhouse (25 °C, 14-h day length supplemented with artificial light) in the submerged 4 l pots, and acclimatized to the conditions shown in Fig. 1 for more than 3 d at the flowering stage. The florets expected to flower on a given day (the day of flowering was judged from the position on the panicle) were sampled at noon (about 1 h before maximum flowering) and artificially opened by removal of lemma. The opened florets were immediately subjected to various air humidity conditions (100%, 60% and 20% R.H.) at 24 °C. After 30 min, the percentages of dehisced stomiums and septums were calculated. The anthers in the florets subjected to 100% R.H. were then exposed to open air (60% R.H., 24 °C) and the process of anther dehiscence was observed. Five florets were used for each treatment and the experiment was repeated twice.

RESULTS

Structure of the anther

The rice anther consists of two thecae, bound together with connective tissue (Figs 2 and 3). Each theca has a long (longer at the base) and a short locule. These two locules are linked with small epidermal cells (stomium) and septum (Fig. 3 A, B, C). Although a cavity for the dehiscence forms in the centre of the septum, some of the septum cells still persist (Fig. 3 C), keeping the locules closed, supported by the deteriorated tapeta. The U-shaped cell-wall thickening in the endothecium develops better in the apical part especially adjacent to the stomium, and in the basal part of the locule than in the other parts (Fig. 3 A, B, D).

Process of anther dehiscence

After removal of the lemma, the process of anther dehiscence began immediately. The first detectable change relating to anther dehiscence was the rapid outward emergence of the whole stomium (Fig. 4 A, B). The already partly disappeared septum (Fig. 3 A, B, C), which had kept the two adjacent locules separated, became completely ruptured, thus dissociating the stomium from the connective tissue. At the same time, the locule wall at the base of the large locule ruptured along the extension line of the ruptured septum (Fig. 4 C). Subsequently, the stomium ruptured at the apical part of the theca (Fig. 4 D). Within a few minutes, the slits in the stomium and the locule wall began to widen and pollen grains overflowed from both widened slits (Fig. 4 E).

From the above observation, the process of anther dehiscence in rice can be divided into two steps: (1) rupture of the whole septum, and splitting of the locule wall at the basal part of the large locule (stomium) and splitting of the stomium at the apical part of thecae (step 1); (2) the widening of the slits at the apical part of the theca and the
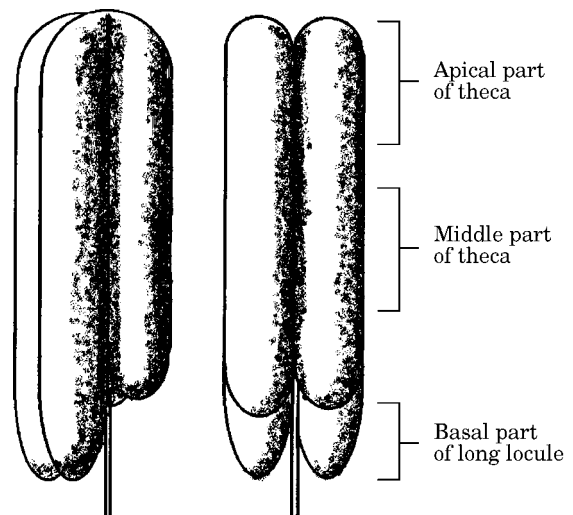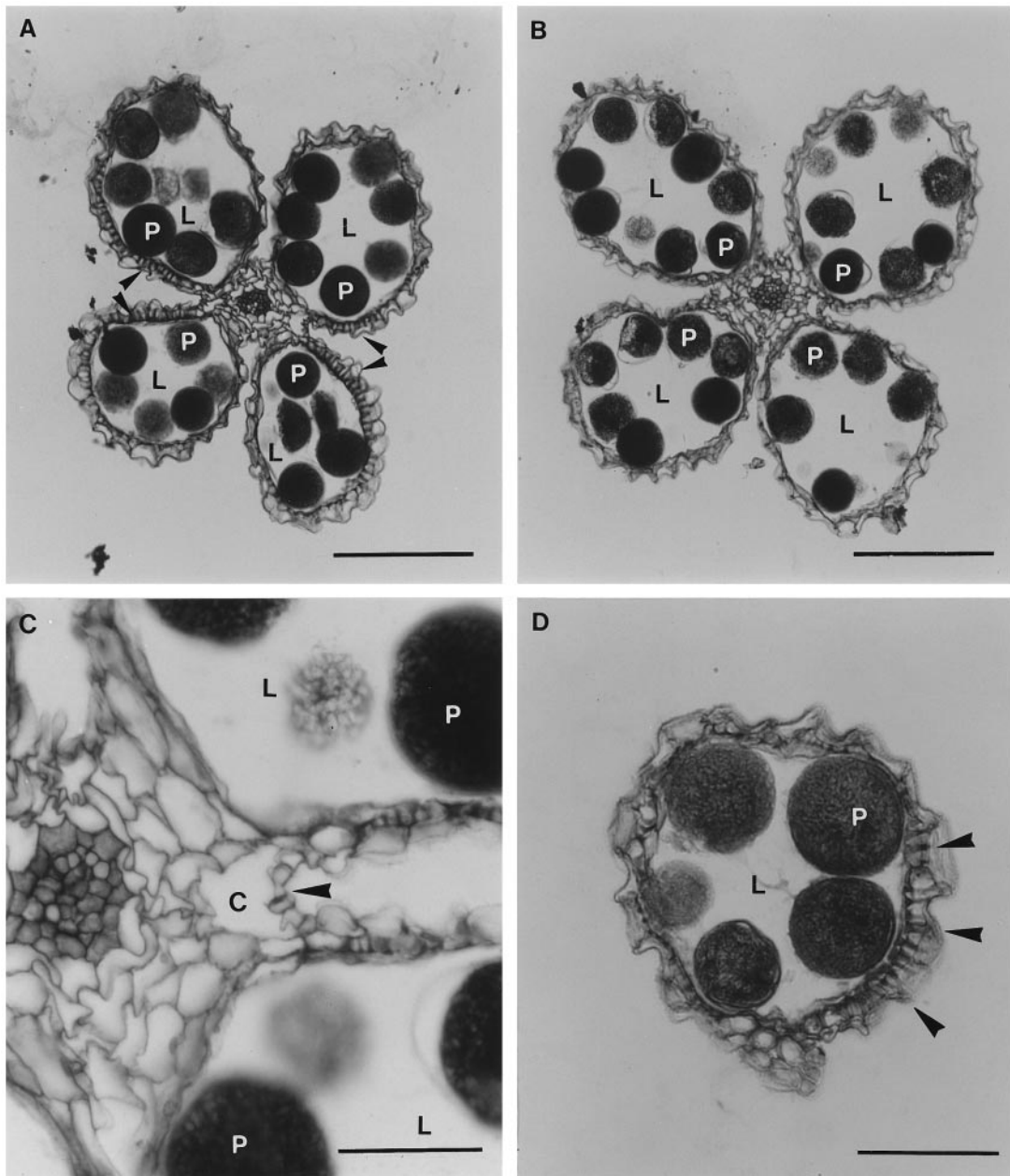

Fig. 2. Exterior of a rice anther.

FIG. 3. The transverse sections of rice anther. (A) apical part of anther (L, locule; P, pollen grains; arrowheads, well developed cell wall thickening in endothecium; bar = 100 μm); (B) middle part of anther (L, locule; P, pollen grains; bar = 100 μm); (C) magnification of cavity for dehiscence in middle part of anther (L, locule; P, pollen grains; (C) cavity for dehiscence; arrowhead, stomium; bar = 25 μm); (D) Basal part of long locule (L, locule; P, pollen grains; arrowheads, well developed cell wall thickening in endothecium; bar = 50 μm).

basal part of the large locule, and the overflow of the pollen grains from both of the widened slits (step 2).

Figure 5 shows schematically the shape of the anther walls during both steps. At step 1, the whole locule wall (AB) became bulged at the middle part of the theca. At the apical part of the theca, the part of the locule wall which faced the other locule (BC) remained bent inward while the other parts of the locule wall (AB) became bulged. At the basal part of the large locule, the same part of the locule wall (BC) remained bent inward while the other parts (AB) became bulged. At step 2, the parts of the locule wall which had remained bent inward at step 1 (BC), at the apical and the

basal parts, straightened. The shape of the locule wall at the middle part remained unchanged at this step. The parts of the locule wall that were bent inward at step 1 and straightened at step 2 (BCs), coincided with the parts of the locule wall having the well-developed U-shaped cell wall thickening.

Effect of air humidity on the process of anther dehiscence

Only 46% of the septa were ruptured at a R.H. of 20%, and the percentage of ruptured septa increased with the

FIG. 4. Photographs showing the process of anther dehiscence. (A) just after removal of the lemma; (B) rising of stomium (arrowhead); (C) split of locule wall at the basal part (arrowhead); (D) split of stomium at the apical part (arrowhead); (E) overflow of pollen grains from both the widened slits at the apical and basal parts (arrowheads).

increase in air humidity (Table 1). At a R.H. of 100%, all septa were ruptured. Moreover, 80% of stomiums were split at the apical part of the thecae and 100% of the locule walls at the basal part of the large locules were split along the extension lines of the ruptured septa. However, the slits ruptured at a R.H. of 100% were narrow and most of the pollen grains were held in the thecae. After subsequent desiccation in the open air (60% R.H.), the remaining unruptured stomiums split, and both of the slits on stomiums at the apical and basal parts widened (data not shown). Stomiums on the thecae with unruptured septa did not open under any humidity conditions.

DISCUSSION

Hoshikawa (1993) reported that the outward bending of locule walls in rice caused by desiccation, causes rupture of the septum and splits the stomium. In contrast, Cheng, Greyson and Walden (1979, 1986) suggested that in maize, rupture of the septum does not involve endothecial fibrous wall thickening which generates the outward force on the locule wall by desiccation, and assumed that the shortening of the outer epidermal wall, from microspore stage to the time of anther dehiscence, generates the tensile force responsible for the separation of the stomium from the underlying connective tissue. Our experimental data, however, suggested that desiccation is unnecessary for the rupture of septum; rather, the desiccation disturbs the rupture, in line with the findings of Keijzer (1987). Thus septum rupture is a moisture-requiring process.

On the other hand, Matsui *et al.* (1999) found that pollen grains swell rapidly in response to floret opening and that the swelling is indispensable for anther dehiscence. Moreover, they compared the rupture of the septum in the anther segments containing pollen with that in the anther segments lacking pollen under the submerged condition, and showed that the swelling of pollen in water causes the rupture of the septum. They infer from these data that the rapid swelling is the driving force of septum rupture in rice. The present results also indicate that septum rupture is a moisture-requiring process, in agreement with their hypothesis. Keijzer (1987) showed that enzymatic breakdown of cell-wall material and breaking tapetal membranes by the pollen pressure lead to the opening of the septum in *Gasteria verrucosa*. Moreover, Keijzer, Leferink-ten Klooster and Reinders (1996) reported that in maize, the formation of the dehiscent cavity is a lytical-enzymatical process preceding the dehiscence and that such an enzymatic cell wall breakdown is found even in the stomium. In the experiment of artificial water-induced septum rupture by Matsui *et al.* (1999), the septum which had not dehisced by the artificial opening of the floret, probably because of the low ability of pollen grains to swell, dehisced by soaking in water. Their data indicate that the rupture does not depend on the change in the physical character of the tissue but on the swelling ability of the pollen grains. The rapid swelling of pollen grains would be a driving force to rupture the septum, weakened by the lytical-enzymatical process.

The stomium split only in the apical part of the theca at step 1. At the middle part, the whole theca bulged and the
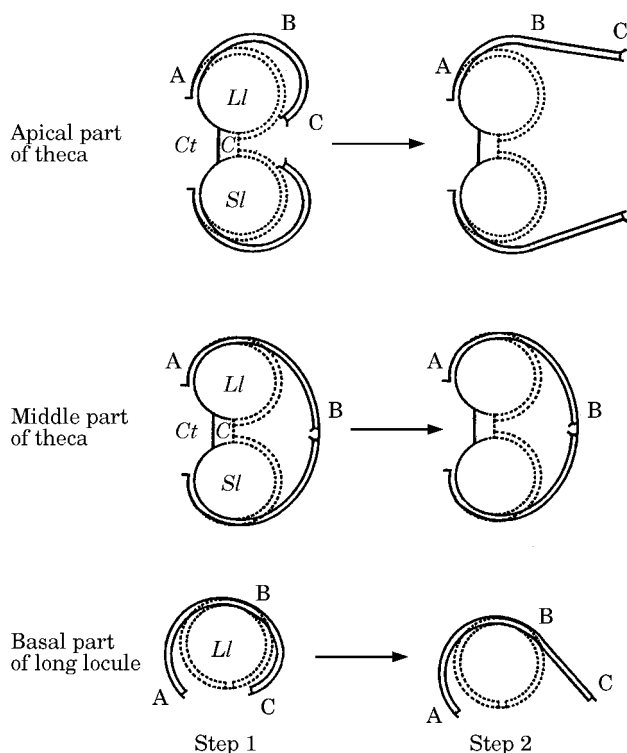
FIG. 5. The shape of locule walls in anther the dehiscence processes. Dotted lines indicate original position of locule walls. *Ll*, large locule; *Sl*, small locule; *C*, cavity for dehiscence; *Ct*, connective tissue.

Step 1: Rupture of whole septum, split of stomium in the apical part and split of locule wall in the basal part. Step 2: widening of the slits in the stomium in the apical part and in the locule wall in the basal part.

TABLE 1. *Effect of air humidity on theca opening process*

Relative humidity (%)	Ruptured septum (%)	Ruptured stomium in the apical part of thecae (%)	Ruptured locule wall in the basal part of large locule (%)
20	46·3[a]	46·3[a]	46·3[a]
60	74·1[a, b]	74·1[a]	74·1[a, b]
100	100·0[b]	80·0[a]	100·0[b]

Within columns, means with same letters are not significantly different ($P < 0.01$).

slit of the locule wall on the stomium stretched, but the stomium did not split. This indicates that the pollen pressure alone could not split the stomium. From observation of the locule wall at the split, we suppose that the bulge of the theca caused by pollen pressure combined with the remaining inward bending of the locule walls adjacent to the stomium, stretches the stomium more severely in the apical parts than in the middle part and causes it to split. In our observation of the transverse-section of the anther, the locule walls adjacent to the splitting stomium showed the marked U-shaped thickening in their endothecium. Since locule walls with such well developed U-shaped thickening

in their endothecium bend outward by desiccation and inward by water uptake (Keijzer, 1987), the above theory that the inward bending of the locule walls adjacent to the stomium helps the stomium to split, is in agreement with the present data indicating that the desiccation of the locule wall is unnecessary until the stomium splits. The remaining inward bending will be maintained by turgor in the locule wall. Keijzer (1987) found inward bending of the locule wall in *G. verrucosa* disrupts the stomium with the help of the epidermal cells adjacent to the stomium acting as a fulcrum. Such assistance of the epidermis was not found in our observation; the locule walls adjacent to the splitting septum remained separate.

At step 1, the locule wall in the basal part of the large locule splits along the extension line of the ruptured septum. This rupture was observed at a R.H. of 100%, indicating that it is not a desiccatory process. Since a theca consists of only one locule in its basal part, the locule wall would be ruptured by pollen pressure as in the case of the septum which kept the locules closed in the upper part.

At step 2, the locule walls adjacent to the longitudinal slits at both apical and basal parts became unbent and the slits widened. This is a desiccatory process because the locule walls remained bent under 100% R.H. even after rupture of the septum and straighten with subsequent desiccation. The desiccation will straighten the parts of the locule wall having well-developed U-shaped thickening in the endothecium by the combination of shrinkage of the tangential outer wall and fixed inside circumference as observed in other plants (Keijzer, 1987).

In conclusion, the swelling of pollen grains is the driving force for the final rupture of the whole septum and for the splitting of the stomium in the apical part and the locule wall in the basal part, and U-shaped cell wall thickening is the key structure for the splitting of the stomium in the apical part and for widening of splits both in the apical and basal parts. However, the outward force caused by desiccation of the locule wall is not sufficient to rupture the septum and stomium.

The process of anther dehiscence in rice can be described as follows: (1) at the beginning of anthesis, pollen grains swell rapidly in response to the floret opening and the increased pollen pressure causes the theca to bulge, rupturing the septum; (2) at this stage, pollen pressure, combined with the inward bending of the locule walls adjacent to stomium, causes the stomium to split at the apical part of the theca; (3) at the same time, the septum split runs to the bottom of the large locule along the extension line of the septum supported by the pollen pressure; (4) after these processes, the locule walls adjacent to the splits in the basal part of the large locule and the apical part of the thecae straighten due to their water loss; and (5) such straightening of the locule wall widens the splits in the apical and basal parts, and the swollen pollen grains overflow from both widened splits.

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