Mechanism of Septum Opening in Anthers of Two-rowed Barley (*Hordeum vulgare* L.)

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To test the hypothesis that the rapid swelling of pollen grains driven by potassium movement opens the septum in anthers of poaceous plants, we studied (1) the behaviour of pollen grains during unfolding of the locule and (2) the distribution of potassium in the locule in two-rowed barley. In the first experiment, the unfolding of decapitated anthers was observed. The pollen grains paved the inner wall of the locule during the unfolding process, suggesting that the pollen grains bend the locule wall outward when they adhere to the wall. In the second experiment, the distribution of potassium in transverse sections of locules in dehisced and indehisced anthers was observed. In indehisced anthers, potassium was detected outside the pollen grains. In contrast, in dehisced anthers, potassium was detected inside pollen grains. This suggested potassium ions moved from the inter-pollen space (locular fluid) into the pollen grains in the locule at the time of pollen-grain swelling.   © 2000 Annals of Botany Company

Key words: *Hordeum vulgare* L., locule unfolding, pollen grain swelling, potassium ion, two-rowed barley.

INTRODUCTION

This article describes how pollen grains swell and the locule unfolds during the dehiscence of anthers in two-rowed barley. Historically, anther dehiscence was believed to be a simple desiccatory process of the tissue in general, probably because of the synchrony between dehiscence and floral opening. However, some scientists recognized the importance of pollen growth for anther dehiscence at the beginning of the 20th century (cited in Keijzer, 1987*a*). Keijzer (1987*a*) examined the anther dehiscence mechanism in liliaceous plants microscopically, and clarified that the growth of pollen mass ruptures the deteriorated tapeta that close the two adjacent locules after enzymatic rupture of septal cells. Thus, fusion of the locules is complete just before anthers dehisce. A similar process was described by Bonner and Dickinson (1989) in tomato (*Lycopersicon esculentum* Mill.).

In poaceous plants, Cheng *et al*. (1979, 1986) related the septum opening of maize (*Zea mays* L.) to a tangential tensile force generated by shrinking of the outer tangential wall of the epidermis. However, Keijzer *et al*. (1996) also showed enzymatic cell-wall lysis in the maize septum and concluded that this lysis results in the opening of the septum, and that the increase in pollen grain size in the 48 h prior to anthesis causes the septum to widen.

On the other hand, Matsui *et al*. (1999*a*, *b*) found that the pollen grains of rice (*Oryza sativa* L.) swell rapidly (within a few minutes) in response to floret opening. They concluded that the swelling pressure of the pollen forces the locules to unfold, rupturing the enzymatically-weakened septum and joining the two adjacent cylindrical locules into a single cylindrical cavity. They considered pressure to be the

driving force opening both the septum and stomium (Matsui *et al*., 1999*b*). Matsui *et al*. (2000) described a similar mechanism in cleistogamous two-rowed barley (*Hordeum vulgare* L.), in which the rapid swelling of pollen in response to the removal of the lemma opens the septum and stomium. Moreover, they detected potassium in the pollen grains sampled from dehisced antlers and proposed that this accumulation of potassium ions in the pollen grains causes the rapid swelling (Matsui *et al*., 2000). This theory can explain the synchrony between anther dehiscence and floret opening which occurs in some poaceous plants, e.g. rice, without considering the desiccation of the anther. However, some important questions still remain unanswered: (1) There is disparity between the rate of volume increase in the pollen grains and the theca during fusion of the two locules. Given the pollen grain diameter of barley measured by Matsui *et al*. (2000), the volume of pollen grains in dehisced anthers was 1·7-fold greater than that in indehisced anthers, but the theca volume increased two-fold during fusion of the two locules. How can pollen swelling force the joining of the locules and swelling of the theca? (2) What is the source of the potassium found in the pollen grains sampled from the dehisced anthers of barley? Does the potassium really migrate from outside the pollen grain into the pollen grain at the time of swelling?

In this paper we answer these questions, and confirm the theory that rapid swelling of pollen grains causes unfolding of the anther locules. First, we observed a decapitated anther during artificially-induced locule unfolding to clarify the behaviour of the pollen grains. Second, we observed the distribution of potassium in transverse sections of dehisced and indehisced anthers to examine the movement of potassium in the locule.

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MATERIALS AND METHODS

Plant material

Cleistogamous two-rowed barley (*Hordeum vulgare* L., 'Saikaikawa-24') was grown under field conditions (Experimental Farm of Kyoto University, Osaka, Japan) from November to May. Heading was from the end of March to mid-April.

Observation of the unfolding process of decapitated anther locules and the interior of thecae before and after unfolding

Florets with anthers that could dehisce in response to the removal of the lemma (hereafter referred to as mature anthers) were sampled prior to pollination. Such florets were selected by observing the sequential pollination of florets on a panicle. In the same panicle, pollination occurs from the middle towards both apical and basal ends (Hoshikawa, 1980). This sequence of pollination corresponds to the order of the anther maturity: anthers in florets that pollinate earlier have a greater ability to dehisce than those in florets that pollinate later (Matsui *et al.*, 2000). Anther maturity was determined as follows. A floret in which the anthers had not dehisced was detached from the panicle, the lemma of the floret was removed and the anthers that dehisced within 5 min were regarded as mature anthers. Immediately after sampling, the lemma was removed and the anthers were decapitated. The unfolding process of the locules was observed under a stereomicroscope at 17°C and 90 % relative humidity (RH).
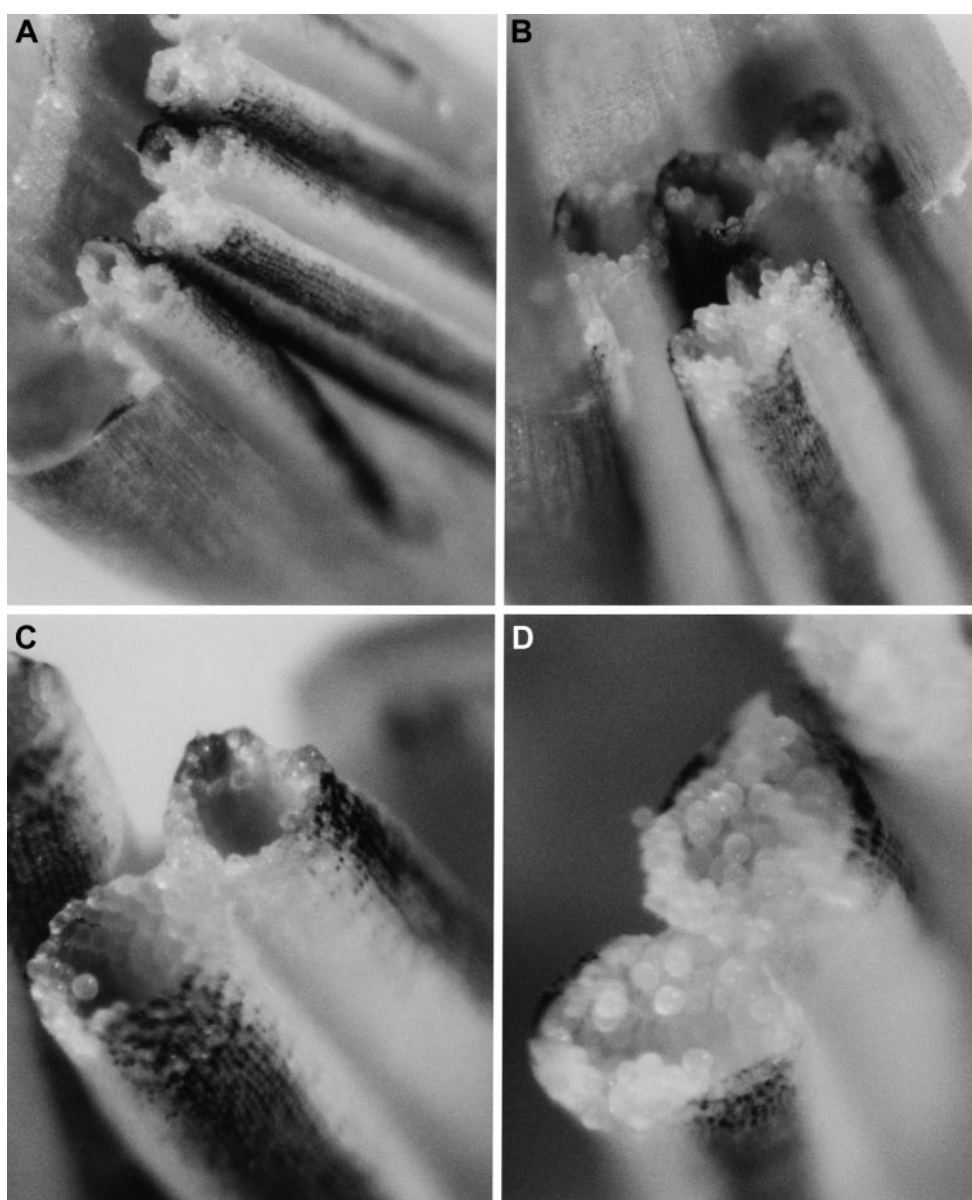

FIG. 1. Unfolding process of the decapitated anther. A, Anthers prior to rupture of the septum. B, Anthers at the beginning of septum rupture. The septum of the lower theca has been ruptured. C, Anther just after septum rupture. The pollen grains adhere to the locule wall in an orderly manner. D, Anther a few minutes after rupture of the septum. The pollen grains are released from the locule wall.

Mature anthers were also sampled to record the moisture condition of the thecae. In one group, thecae were artificially opened immediately after sampling, while in the other group, thecae were opened after the spontaneous rupture of the septum. In both groups, moisture condition was recorded immediately after artificial opening of the thecae.

Detection of potassium ions in the anther

Florets were detached from the panicle several days after emergence. Subsequently, the lemmas of the florets were removed and the anthers were decapitated at 20°C and 70 % RH. After 5 min, both dehisced (mature) and indehisced (pre-mature) anthers were stained with Macallum's modified solution (Toriyama, 1955) at 0°C for 48 h. The materials were thoroughly washed with cold water and fixed in 70 % ethanol before being dehydrated in a methanol–butanol series, and embedded in paraffin. Ten-micron-thick transverse sections were prepared and observed, without staining, under a microscope.

RESULTS

Behaviour of pollen grains and moisture condition in anther locules during the unfolding process

After decapitation of the anthers, the septa ruptured normally. The pollen grains remained stuck to the tapetum remnants in an orderly manner during the rupture (Fig. 1A–C). After the emergence of the stomium, the pollen grains were released from the tapetum and dispersed into the thecae (joined locules) (Fig. 1C, D). Figure 2 shows the interior of a theca opened artificially. Before the septum ruptured, the insides of the locules of the mature anther were wet and the pollen grains adhered closely to the locule wall (Fig. 2A). In contrast, in anthers whose septa had ruptured spontaneously after removal of the lemma, the insides of the locules were dry and the pollen grains were released from the locule walls (Fig. 2B).

Distribution of potassium in transverse sections of the anther

In transverse sections of the locule in indehisced anthers, potassium salt crystals were formed on the surface of the pollen grains and the remnants of the tapetum (Fig. 3A). In contrast, the crystals were formed inside the pollen grains in the fused locule in dehisced anthers (Fig. 3B).

DISCUSSION

Previously, Matsui *et al.* (1999*a*, *b*, 2000) reported that, in rice and two-rowed barley, pollen grains swell rapidly causing the locules to unfold at the start of anther dehiscence, and that this unfolding causes the two adjacent cylindrical locules to join into a single pollen cavity. At the time the septum widens, the volume of the theca approximately doubles (Matsui *et al.*, 1999*b*, 2000). In contrast, the difference in pollen grain diameter between indehisced and dehisced anthers was less than 20 % (less than 75 % in volume) (Matsui *et al.*, 1999*a*, 2000). Given this difference between the rate of volume increase of the theca and the pollen grains, the reports that pollen swelling is the driving force behind unfolding of the locules remained inconclusive.

The present study has shown how the pollen grains unfold the locules. At the time when the two locules fuse, the pollen grains still adhere to the tapetum in an orderly manner. This suggests that the pollen grains paving the
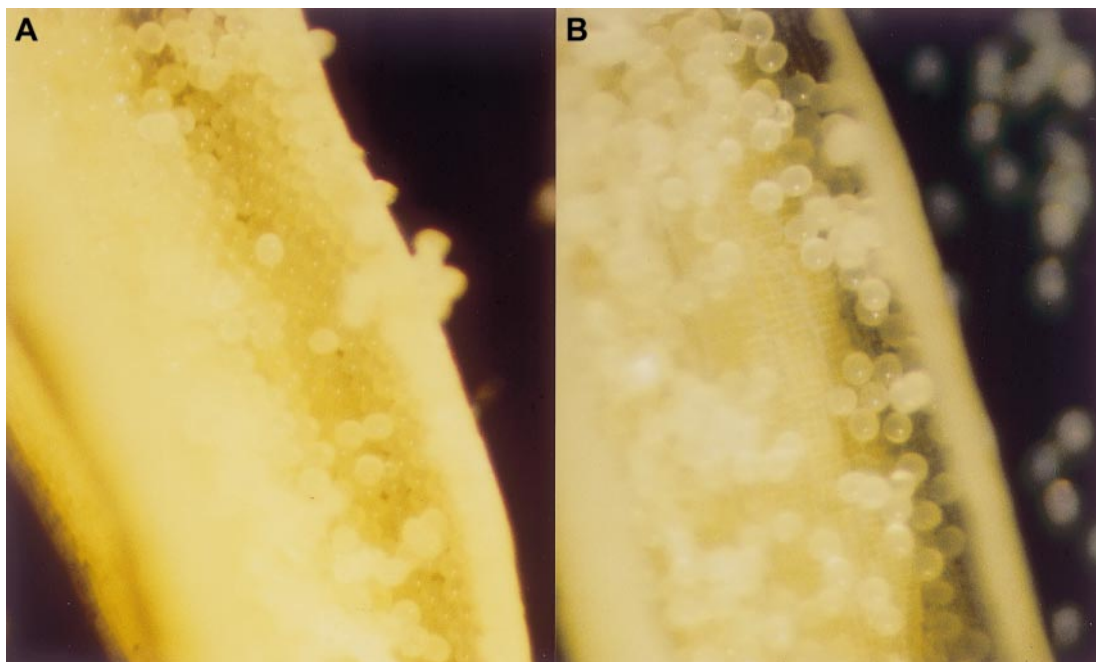

FIG. 2. Interior of the thecae before (A) and after (B) rupture of the septum.
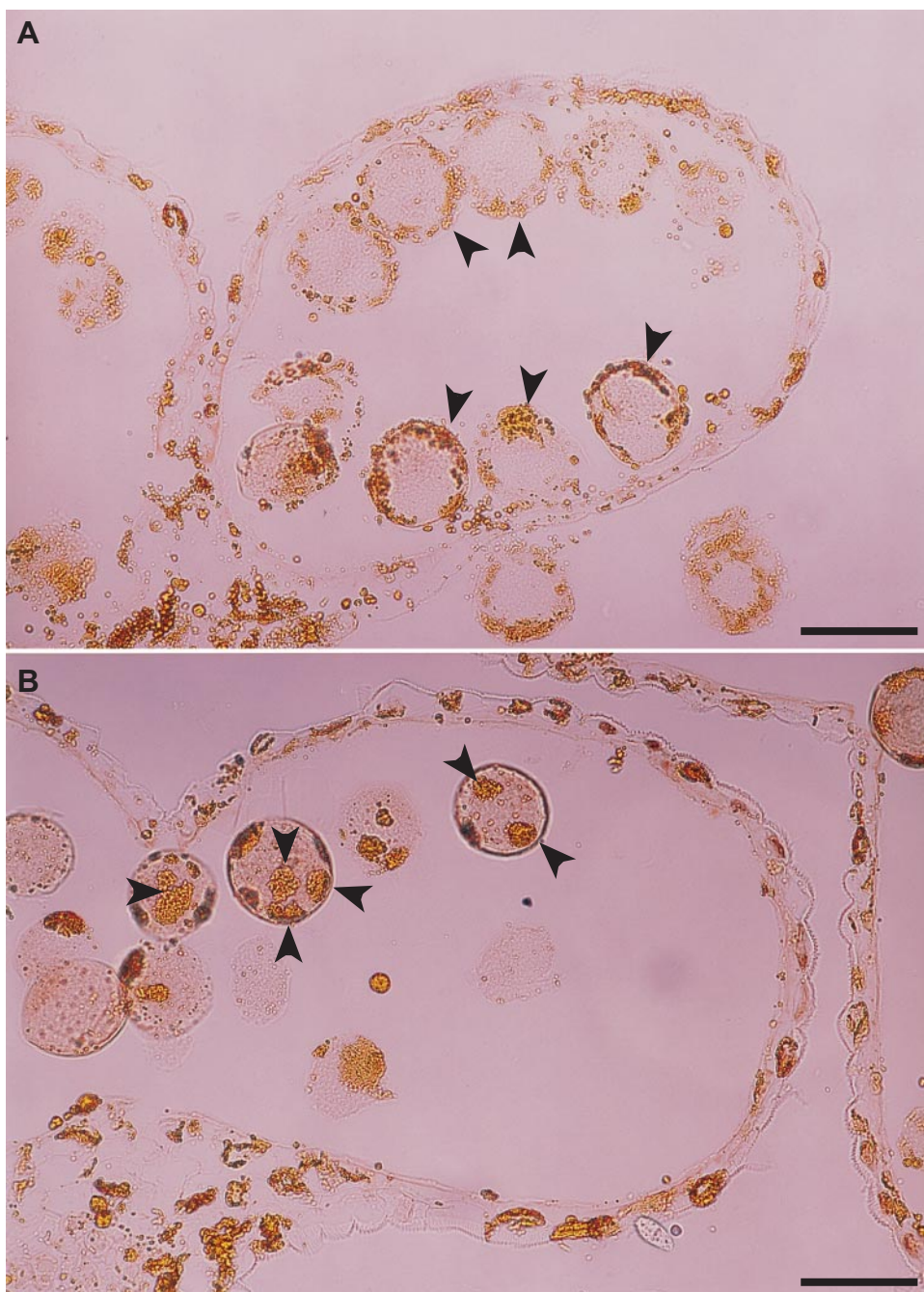
FIG. 3. Distribution of potassium in transverse sections of indehisced (A) and dehisced (B) thecae. Arrowheads indicate the potassium salt crystals formed outside (A) and inside (B) the pollen grains. Bars = 50 μm.

inner wall of the locule bend the locule wall outward by the combination of the fixed circumference of the locule and tangential swelling of pollen grains attached to the locule wall. In this way, a 14 % increase in the diameter of pollen grains can unite the two locules into a cylindrical cavity when the pollen grain diameter is 25 % of the locule diameter. Moreover, Keijzer *et al.* (1996) reported that the pollen grains of maize are tightly packed against the remnants of the tapetum and that the mutual pressure forces the pollen grains to lose their spherical shape 2 d

before anthesis. A similar change in the shape of pollen grains in the locule as a result of mutual pressure has also been reported in rice (Takeoka *et al.*, 1993). The change in shape would also help unfold the locule with less increase in diameter.

Matsui *et al.* (2000) detected potassium in pollen grains from dehisced anthers, but not in those from indehisced anthers, suggesting the participation of potassium ions in the swelling of pollen grains. The present observation of the distribution of potassium salt crystals stained with

Macallum's modified solution revealed that almost all the potassium in indehisced anthers was found outside the pollen grains and that much of the potassium in dehisced anthers was present inside the pollen grains. This strongly supports the theory that potassium ions move from the inter-pollen space into the pollen grains, resulting in pollen swelling.

Moreover, before the rupture of the septum, the inside of the locule is wet and the pollen grains adhere to the locule wall in an orderly manner. However, after rupture of the septum, the inside of the locule is dry and the pollen grains are detached from the walls. This suggests that water moves from the inter-pollen space into the pollen grains at the time of locule unfolding; this is also in agreement with the theory that the movement of potassium ions from the inter-pollen space into the pollen grains causes pollen swelling. Since the surface of the pollen grains and inner surface of the locule wall are hydrophobic (Heslop-Harrison, 1968; Keijzer, 1987*b*) and poaceous pollen grains are stuck to the tapetum with tiny amounts of pollenkitt (Keijzer *et al.*, 1996), adhesion between them would be lost rapidly upon dehydration of the locule and a decrease in contact area between the tapetum and the grain as a result of a change in pollen grain shape. At the same time, expansion of the locule after the rupture of the septum would release the tightly-packed pollen grains, which would then detach from the tapetum.

There is solid evidence that the plasma membrane of pollen grains has potassium ion channels (Obermeyer and Kolb, 1993) and a proton pump (Weisenseel and Jaffe, 1976; Obermeyer *et al.*, 1992). Moreover, Obermeyer and Blatt (1995) showed that K^+ influx and H^+ efflux are present in quiescent, ungerminated pollen grains in *Lilium longiflorum*. Although such a K^+ influx is regarded as one of the processes of pollen grain germination (Weisenseel *et al.*, 1975; Obermeyer and Blatt, 1995), it may also regulate the osmotic potential for the swelling of pollen grains in poaceous plants.

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