Rapid Swelling of Pollen Grains in the Dehiscing Anther of Two-rowed Barley (*Hordeum distichum* L. emend. LAM.)

T. MATSUI*†, K. OMASA‡ and T. HORIE§

†*Experimental Farm, Kyoto University, 12-1 Hatchonawate, Takatsuki, Osaka 569-0096, Japan, ‡Graduate School of Agriculture and Life Science, The University of Tokyo, Tokyo 113-8657, Japan and §Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

The role of rapid swelling of pollen grains in anther dehiscence in *Hordeum distichum* L. emend. LAM. and the mechanism of this swelling were examined. Artificial opening of the floret induced rapid swelling of pollen grains and thecae dehiscence. The theca dehisced as pollen grains became swollen and dehisced anthers had larger pollen grains than indehisced anthers. Septa in the anther segments dehisced as a result of water-induced pollen pressure. These results strongly support the theory that the rapid swelling of pollen grains is a driving force for anther dehiscence. On the other hand, potassium was detected in pollen grains from dehisced anthers, but not in pollen grains in indehisced anthers. This suggests that potassium ions function as a turgor regulator in the rapid swelling of pollen grains. The mechanism of anther dehiscence is discussed in relation to the swelling of pollen grains, as is the possible mechanism of this swelling.   © 2000 Annals of Botany Company

Key words: Anther dehiscence, *Hordeum distichum* L. emend. LAM., pollen swelling, potassium ion, two-rowed barley.

INTRODUCTION

Historically, anther dehiscence has generally been regarded as a desiccatory process (Schmid, 1976). However, some workers have challenged this assumption (Cheng *et al.*, 1979; Keijzer, 1987; Bonner and Dickinson, 1990). Using Liliaceae Keijzer (1987) reported that the dehiscing process of the anther consists of the following four stages: (1) enzymatic rupture of the septum between two locules; (2) mechanical rupture of the tapetum that kept the locules close together by swelling of pollen grains; (3) mechanical opening of the stomium by the centripetal force of the highly turgescent epidermis and endothecium using the epidermal cells of locule walls adjacent to the stomium as a fulcrum; and (4) outward bending of the locule walls by the centrifugal force of the dehydrating epidermis and endothecium.

Previously, Matsui *et al.* (1999*a*,*b*) showed that floret opening in rice (*Oryza sativa* L.) induces rapid swelling of pollen grains, which causes the theca to bulge, disrupting the septum and resulting in theca dehiscence. The next question is whether this mechanism evolved to effect anther dehiscence in rice, a species that pollinates even in a wet or rainy monsoon season. This paper reports the mechanism of anther dehiscence, based on pollen grain swelling, in two-rowed barley (*Hordeum distichum* L. emend. LAM.), a cleistogamous poaceous plant, and shows that such a mechanism is not peculiar to rice. In addition, we discuss the possible involvement of potassium ions in the rapid swelling of pollen grains.

* For correspondence. Fax +81 726 83 1532.

MATERIALS AND METHODS

Plant materials

Hordeum distichum L. emend. LAM. ('Saikaikawa 24', cleistogamous two-rowed barley) grown under field conditions (Experimental Farm of Kyoto University, Osaka, Japan) from November to May was used for all experiments. Heading was from the end of March to mid-April.

Transverse sections of anthers

Florets with lemmas removed were fixed in FAA immediately after emergence. The fixed materials were dehydrated with a methanol–butanol series, and embedded in paraffin. Ten-micron-thick transverse sections were cut and stained with toluidine blue-O.

Process of anther dehiscence

Florets were detached from panicles at the heading stage before pollination, and the lemmas were removed immediately (artificial floret opening) at 20°C, and 60% relative humidity (R.H.). Within a few minutes, anthers in some florets dehisced. The process of anther dehiscence was observed under a stereo-microscope.

Diameter of pollen grains and anther dehiscence

Several days after emergence of the panicles, florets on one side of the panicles (Fig. 1A–L) were detached and their lemmas were immediately removed at 20°C, and 60% R.H.

FIG. 1. The notation of floret position on a panicle.

After 4 min, the percentage of dehisced thecae was examined. The anthers were then broken open using tweezers and the diameters of scattered pollen grains were immediately measured under a stereo-microscope. Eighteen pollen grains from three anthers per floret were examined.

Florets on the other side of the panicles (a–k in Fig. 1) were also detached and their lemmas were immediately removed. In this group, anthers in one floret were broken every 2 min after checking whether they had dehisced, and the diameters of six pollen grains per anther were measured.

Role of pollen grain swelling

Anthers that did not dehisce by artificial floret opening were detached from a panicle several days after emergence. Both ends of each anther were excised leaving the middle part (about 0·3 mm) in a 10% sucrose solution. The cut piece was again transversely cut in half, each with four locule tubes (about 0·15 mm in length). Pollen grains in one half were carefully brushed out of the locule tubes and left in the sucrose solution. Pollen grains were left intact in the other half of the anther. After this treatment, both anther segments were immersed in water. Thirty minutes later, septum rupture was observed. Five to ten minutes were required for anther cutting and pollen removal. This experiment was repeated ten times.

Detection of potassium ions in pollen grains

Florets were detached from panicles several days after their emergence, and the lemmas of the florets were removed at 20°C and 60% R.H. After 2 min, pollen grains were sampled from dehisced and indehisced anthers. Both pollen grains were stained with Macallum's modified solution (Toriyama, 1955) and embedded in glycerin.

RESULTS

Anther structure

The anther of two-rowed barley consists of two thecae, bound together by connective tissue (Fig. 2A, B). Each theca is separated by small epidermal cells (stomium) and a septum. In the centre of the septum the cavity for dehiscence is formed, while some of the septum cells still persist; the septum cells and the deteriorated tapetum appear to keep the locules close together (Fig. 2C). The U-shaped cell-wall thickening in the endothecium became thicker in the upper part of the locule, especially adjacent to the stomium, than in other parts.

Process of anther dehiscence

After artificial floret opening, anthers began to dehisce within a few minutes. The first detectable change relating to anther dehiscence was the rapid expansion of the whole theca, which implies rupture of the septum (Fig. 3A, B). As a result of this expansion, the stomium bulged outwards and became flat except at the apical part of the theca; here, the locule walls adjacent to the stomium remained bent inward (Fig. 3B). Subsequently, the stomium split at the apical end of the thecae (Fig. 3C). After a few minutes, the locule walls adjacent to the split straightened (Fig. 3D), causing the split on the stomium to widen and the pollen grains to overflow from the widened split.

Diameter of pollen grains and anther dehiscence

Figure 1 shows the notation of floret position on a panicle, and Fig. 4 the relationship between the position of florets on the panicle and the diameter of the pollen grains in the floret 4 min after artificial floret opening, together with the dehiscence of thecae at that time. Anthers in flowers on the apical and basal parts of the panicle did not dehisce. Pollen grain diameters in the dehisced anthers were over 45 μm, whereas those in the indehisced anthers were smaller. The stomium of indehisced thecae had not risen, which means that the septum of such thecae remained unruptured.

Figure 5 shows the time course of changes in the diameters of pollen grains and the thecae dehiscence after artificial floret opening. The diameter of pollen grains in the florets on the middle part of the panicle increased within 2 min, whereas those in the florets near the apical and basal ends of the panicle did not. Only anthers with large pollen grains dehisced.

Rupture of septum in water and potassium in pollen grains

In anther segments with locules holding pollen grains, 80% of the septa between locule tubes ruptured in water, but no septa ruptured in the anther segments holding no pollen grains.

Figure 6 shows the potassium salt crystals in pollen grains formed in Macallum's modified solution. The pollen grains from dehisced thecae had crystals (Fig. 6A), whereas those from indehisced thecae had few crystals (Fig. 6B).

DISCUSSION

Pollen grains in florets in the middle part of the panicle showed rapid swelling in response to artificial floret opening, whereas grains in florets at the apical and basal ends did not. Florets on a panicle of two-rowed barley complete pollination from the middle part of the panicle to either ends. Thus, the swelling ability of pollen grains coincides with flowering order, which indicates that there is a natural maturation gradient of pollen grains in a panicle
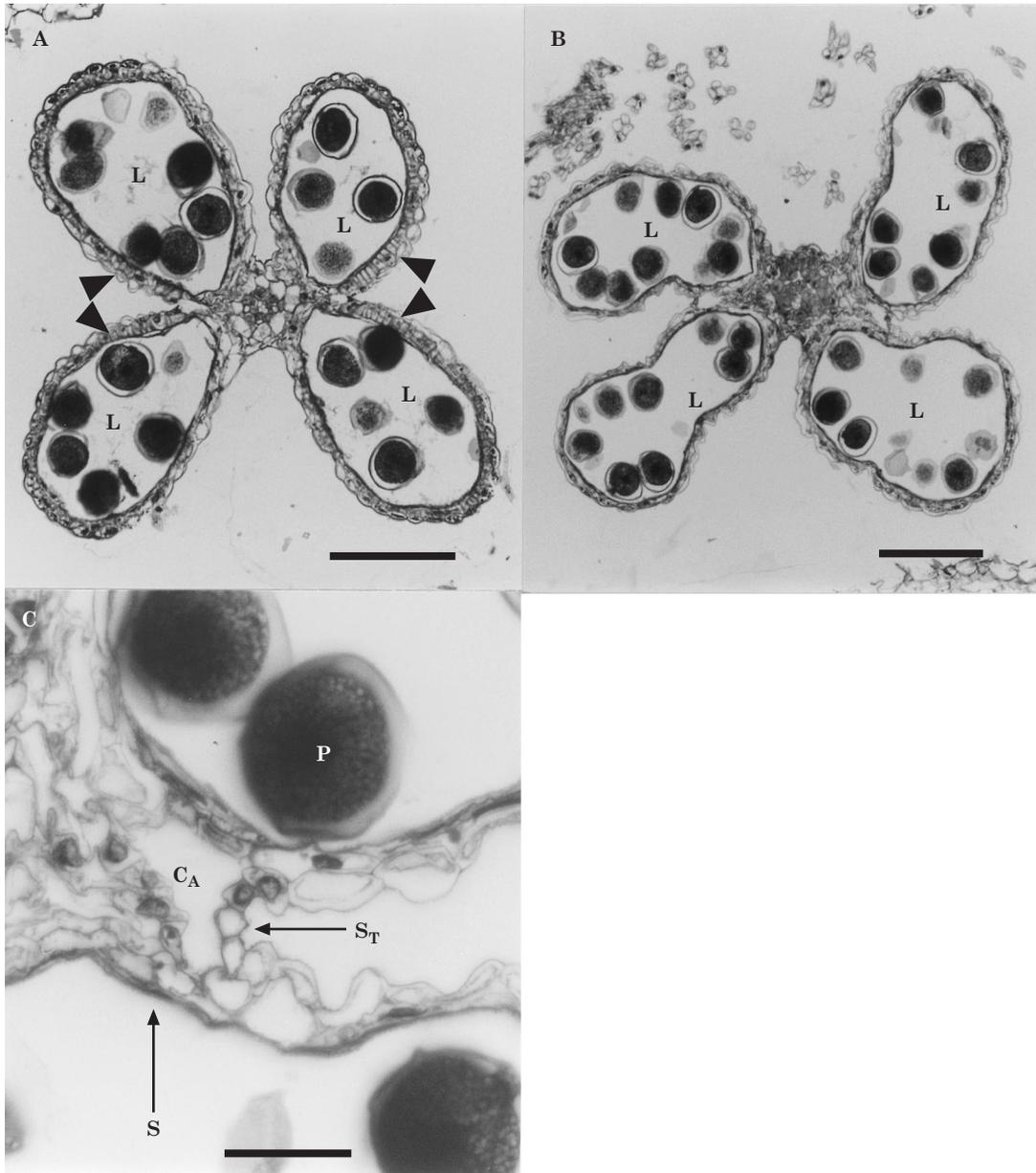
FIG. 2. Cross-sections of an anther stained by toluidine blue O. A, Cross-section of apical end. Thickened U-shaped walls are observed in the endothecial cells (arrowheads). B, Cross-section of middle region. C, Details around a septum. S, Septum; S_T, stomium; L, locule; C_A, cavity for anther dehiscence. Bars = 100 µm (A and B) or 25 µm (C).

and that the swelling ability of the pollen grains depends on the maturation stage of the pollen grains.

Dehisced anthers had larger pollen grains than indehisced anthers, and the anthers dehisced as the pollen grains became swollen. Transverse sections of the anther before dehiscence show that some of the cells in the septum still persist and keep the two adjacent locules closed together with the deteriorated tapetum. Dehiscence, therefore, includes rupture of the septum and splitting of the stomium, and the close relationship between the swelling of pollen grains and anther dehiscence suggests that swelling of pollen grains is important in these two events.

Since the last century the mechanism of septum rupture has been the subject of debate (Keijzer, 1987). The

mechanisms presented by earlier investigators can be categorized into mechanical rupture and enzymatic breakdown of the tissue. According to the mechanical rupture theory, outward bending of locule walls by desiccation and pollen pressure were the driving forces.

Keijzer (1987) showed that desiccation of the endothecium did not cause opening of the septum in Liliaceae. Matsui *et al.* (1999*b*) also showed disturbance of septum rupture by desiccation in the anther of rice. Moreover, Cheng *et al.* (1979) showed that maize septum rupture does not involve endothecial fibrose wall thickening. In the present observation, floret opening caused rupture of the septum only in the florets in the middle of the panicle. This observation suggests that septum rupture is not a simple
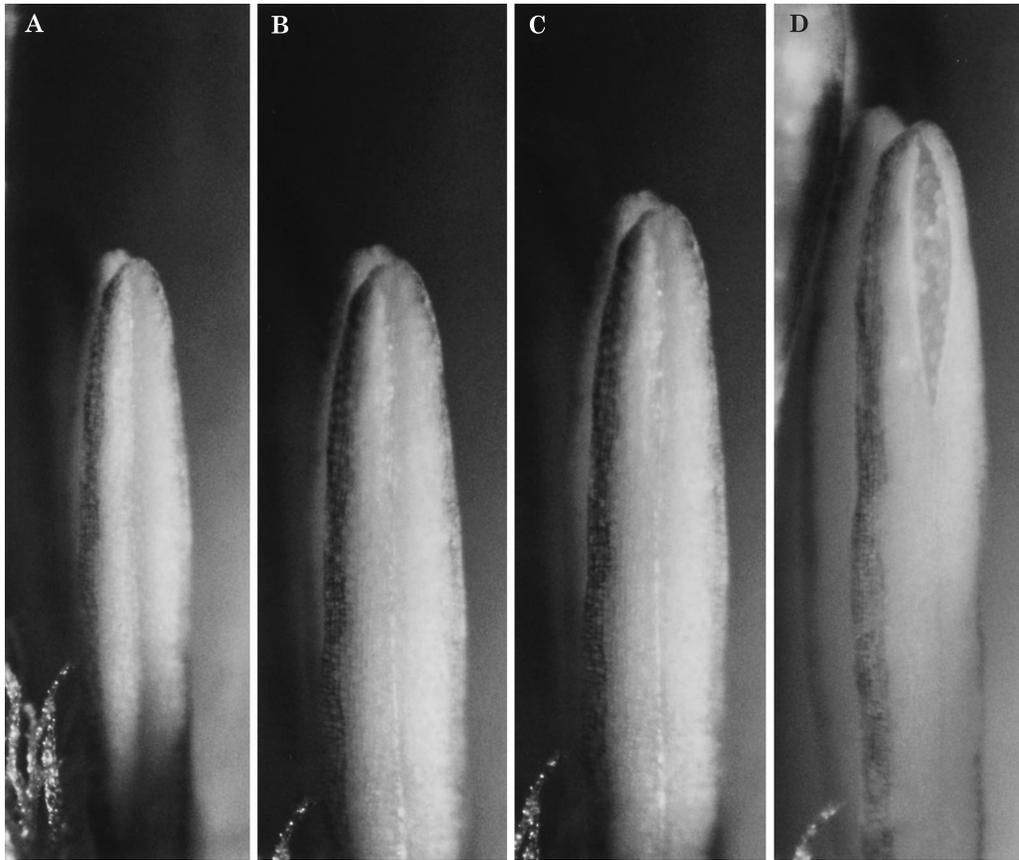
FIG. 3. The process of anther dehiscence. A, The beginning of the anther dehiscence process; the stomium is rising. B, Just after the rising of stomium. C, The beginning of stomium opening. Locule wall keeps the slit at the opening stomium. D, Locule wall adjacent to the stomium begins to bend outward. The slit on the stomium begins to widen.
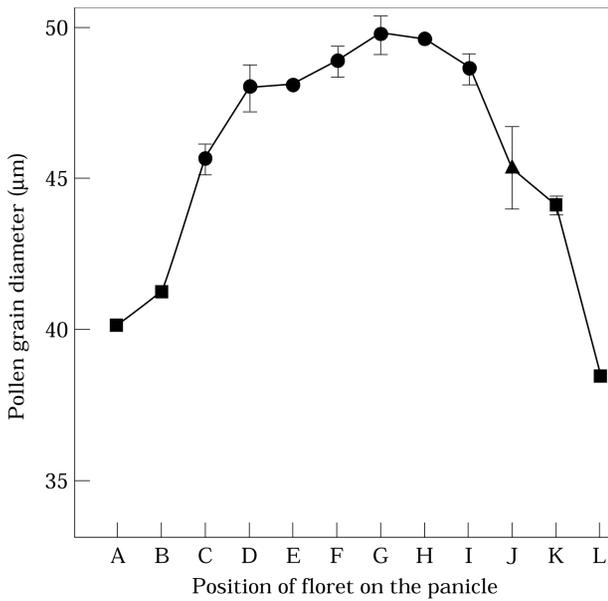
FIG. 4. Relationships between the position of the floret on the panicle and the diameter of pollen grains, and the percentage of dehisced thecae in the anthers 2 min after artificial floret opening. ●, All thecae dehisced; ▲, half of the thecae dehisced; ■, no thecae dehisced. Vertical bars indicate ±s.e. of three anthers.
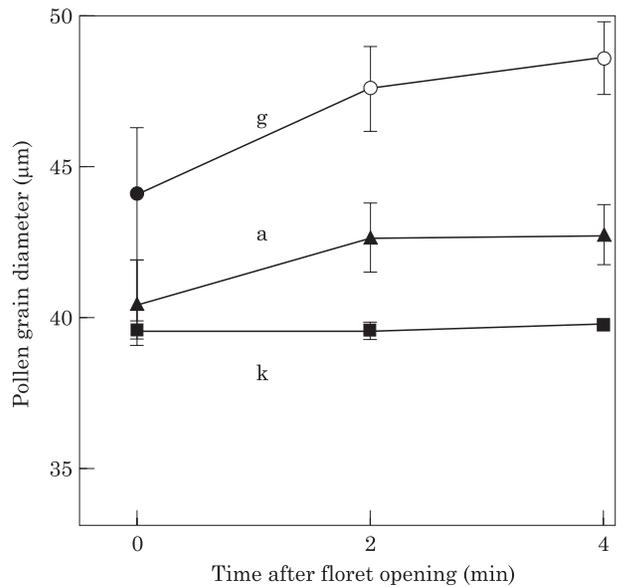
FIG. 5. Typical change in the diameter of pollen grains after artificial floret opening. Letters indicate the position of the floret on the panicle (see Fig. 1). Open and solid symbols indicate dehisced and indehisced anthers, respectively. Vertical bars indicate ±s.e. of six pollen grains.
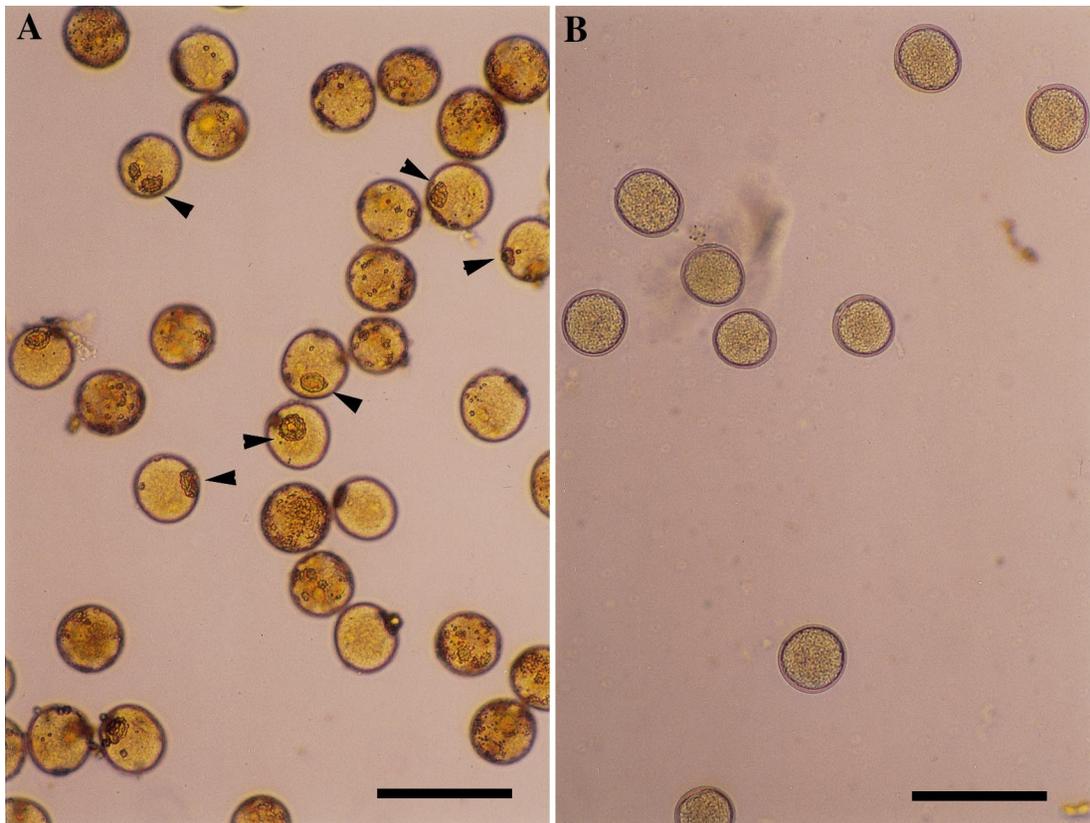
FIG. 6. Pollen grains from dehisced (A) and indehisced anthers (B). Both materials were treated with Macallum's modified solution and embedded in glycerin. Arrowheads indicate the crystals of potassium salts (yellow crystals). Bars = 100 μm.

desiccatory process because the U-shaped thickening of the cell wall in the endothecium, which generates an outward- and inward-bending force of locule walls with the osmotic properties of the endothecium, is already completed at the panicle emergence. This is in agreement with the above reports.

Keijzer (1987) showed that the septum in *Gasteria verrucosa* opened normally after removal of the pollen from the locules. The septum in male sterile anthers lacking expanding pollen in *Aloe vera* was also found to open (Keijzer and Cresti, 1987). From these results and cytological changes in the anther of *G. verrucosa*, Keijzer (1987) concluded that enzymatic breakdown of cell wall material leads to the opening of the septum. In maize, Keijzer *et al.* (1996) found enzymatic lysis of the septum preceded theca dehiscence. Cheng *et al.* (1979, 1986) assumed that the shortening of the outer epidermal wall from the microspore stage to the time of anther dehiscence generates the tensile force responsible for the rupture of the septum. The enzymatic lysis and the tensile force, however, cannot explain the synchronism between floret opening and septum rupture. Our artificial water-induced rupture of the septum proves that pollen pressure can rupture the septum and cause the theca to bulge in two-rowed barley. In this experiment, septa that had not dehisced as a result of artificial floret opening, probably because of immaturity of the pollen, dehisced in water. This indicates that rupture does not depend on a change in physical character of the

tissue but on the ability of pollen grains to swell. Pollen pressure would complete the rupture of the septum weakened by enzymes.

In our observations on the anther dehiscence process, the stomium bulged outward in the whole theca simultaneously after artificial opening of the floret. The stomium, however, ruptured only at the apical part of theca. This indicates that the swelling of pollen grains alone cannot split the stomium, although there is a close relationship between the diameter of pollen grains and thecae dehiscence. Since the locule walls adjacent to the splitting stomium remained bent inward, it is supposed that bulging of the theca as a result of pollen pressure, combined with the centripetal force of the locule walls adjacent to the stomium on the apical part of thecae, stretches the stomium more severely than in other parts and splits it. In transverse sections of the anther, the locule walls adjacent to the splitting stomium had well-developed cell wall thickening. Since such locule walls bend outward by desiccation and inward due to water uptake by the endo-thecium and epidermis (Keijzer, 1987), the locule walls would remain bent by the turgor in the latter two tissues. Previously, Matsui *et al.* (1999b) reported a similar role of locule walls in the splitting of the stomium on the apical part of the rice anther. They compared anther dehiscence under different relative humidities, and found that desiccation was not necessary for stomium splitting. Keijzer (1987) reported that epidermal cells of the locule walls adjacent to the stomium act as a fulcrum in *G. verrucosa*. Such a role of the

epidermis was not confirmed in our observation; the locule walls adjacent to the splitting septum did not touch each other. On the other hand, Keijzer *et al.* (1996) found cell wall lysis in the stomium of the apical part of the anther preceded dehiscence in maize.

Swelling is caused by imbibition by the pollen grains. It is generally accepted that active water movement in plants depends on the gradient of water potential that is mainly regulated by saccharide or cation (especially potassium ion) content. In Poaceous pollen grains, accumulated starch in the grains breaks down just before pollination (Watanabe, 1961; Koike and Satake, 1987). The dissolution of starch into saccharides will decrease the osmotic potential in pollen grains. The observed pollen grain swelling, however, occurred within 2 min. Since starch breakdown is a relatively slow process (a few hours is needed for rice pollen to complete breakdown; Koike and Satake, 1987), it is difficult to explain the swelling by a change in saccharide concentration alone. In contrast, movement of potassium ions as osmotic regulators is rapid: various rapid actions have been shown to be caused by the movement of potassium ions in plants (e.g. pulvinus in *Mimosa pudica*; Toriyama, 1955).

Potassium was detected in pollen grains from dehisced anthers, but not in pollen grains in indehisced anthers, which suggests the participation of potassium ions in the swelling of pollen grains. There is good evidence that the plasma membrane of pollen grains has K^+ 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 K^+ influx is regarded as a process which initiates pollen grain germination (Weisenseel, Nuccitelli and Jaffe, 1975; Obermeyer and Blatt, 1995), it seems to be required for active water uptake by pollen grains. We hypothesize that the inward current of potassium ions from the inter-pollen space (locular fluid) into pollen grains decreases the water potential in pollen grains and leads to their swelling.

The mechanism of anther dehiscence after artificial floret opening may be summarized as follows: (1) floret opening triggers rapid swelling of pollen grains, which may be caused by K^+ accumulation; (2) the swelling causes the theca to bulge and the whole septum to rupture; (3) at the apical end of the theca, the pressure of the swollen pollen combined with the centripetal force of the locule walls adjacent to the stomium splits the stomium.

A similar mechanism, from the rapid swelling of pollen grains to stomium split, has been observed in the apical part of rice thecae (Matsui *et al.*, 1999*a,b*). Such mechanisms may be common in Poaceous plants. However, the two-rowed barley used in this study is cleistogamous, and

normally the anthers dehisce without floret opening. It is somewhat surprising, therefore, that artificial floret opening triggered anther dehiscence as seen in chasmogamous rice. How anther dehiscence is triggered will be the subject of future research.

LITERATURE CITED

Bonner LJ, Dickinson HG. 1990. Anther dehiscence in *Lycopersicon esculentum*. II. Water relations. *New Phytologist* **115**: 367–375.

Cheng PC, Greyson RI, Walden DB. 1979. Comparison of anther development in genic male-sterile (*ms*10) and in male-fertile corn (*Zea mays*) from light microscopy and scanning electron microscopy. *Canadian Journal of Botany* **57**: 578–596.

Cheng PC, Greyson RI, Walden DB. 1986. The anther cuticle of *Zea mays*. *Canadian Journal of Botany* **64**: 2088–2097.

Keijzer CJ. 1987. The process of anther dehiscence and pollen dispersal. I. The opening mechanism of longitudinally dehiscing anthers. *New Phytologist* **105**: 487–498.

Keijzer CJ, Cresti M. 1987. A comparison of anther tissue development in male sterile *Aloe vera* and male fertile *Aloe ciliaris*. *Annals of Botany* **59**: 533–542.

Keijzer CJ, Leferink-ten Klooster HB, Reinders MC. 1996. The mechanics of the grass flower: anther dehiscence and pollen shedding in maize. *Annals of Botany* **78**: 15–21.

Koike S, Satake T. 1987. Sterility caused by cooling treatment at the flowering stage in rice plants. II. The abnormal digestion of starch in pollen grain and metabolic changes in anthers following cooling treatment. *Japanese Journal of Crop Science* **56**: 666–672.

Matsui T, Omasa K, Horie T. 1999*a*. Rapid swelling of pollen grains in response to floret opening unfolds locule in rice (*Oryza sativa* L.). *Plant Production Science* **2**: 196–199.

Matsui T, Omasa K, Horie T. 1999*b*. Mechanism of anther dehiscence in rice (*Oryza sativa* L.). *Annals of Botany* **84**: 501–506.

Obermeyer G, Blatt MR. 1995. Electrical properties of intact pollen grains of *Lilium longiflorum*: characteristics of the non-germinating pollen grain. *Journal of Experimental Botany* **46**: 803–813.

Obermeyer G, Kolb HA. 1993. K^+ channel in the plasma membrane of lily pollen protoplasts. *Botanica Acta* **106**: 26–31.

Obermeyer G, Lutzelschwab M, Heumann H-G, Weisenseel MH. 1992. Immunolocalization of H^+-ATPases in the plasma membrane of pollen grains and pollen tubes of *Lilium longiflorum*. *Protoplasma* **117**: 55–63.

Schmid R. 1976. Filament histology and anther dehiscence. *Botanical Journal of the Linnean Society* **73**: 303–315.

Toriyama H. 1955. Observational and experimental studies of sensitive plants. VI. The migration of potassium in the primary pulvinus. *Cytologia* **20**: 367–377.

Watanabe K. 1961. Studies on the germination of glass pollen. II. Germination capacity of pollen in relation to the maturity of pollen and stigma. *Botanical Magazine Tokyo* **74**: 131–137.

Weisenseel MH, Jaffe LF. 1976. The major growth current through lily pollen tubes enters as K^+ and leaves as H^+. *Planta* **133**: 1–7.

Weisenseel MH, Nuccitelli R, Jaffe LH. 1975. Large electrical currents traverse growing pollen tubes. *Journal of Cell Biology* **66**: 556–567.