

Comparison between Anthers of two Rice (*Oryza sativa* L.) Cultivars with Tolerance to High Temperatures at Flowering or Susceptibility

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Abstract : Anthers of rice cultivar, cv. Nipponbare, which showed floret fertility even when the temperature at flowering was high (tolerant) and cv. Hinohikari, which showed floret sterility at high temperatures (susceptible) were compared. The anther of Nipponbare had better developed cavities for dehiscence and thicker locule walls than Hinohikari. The well-developed cavities enable easy rupture of the septa in response to swelling of the pollen grains. The thick locule walls promote the swelling of pollen grains by retaining water in the locules. Since the swelling of pollen grains is the driving force to open the theca and the septum rupture is an indispensable process for the theca dehiscence, the well-developed cavities and the thick locule walls may be responsible for the tolerance to high temperatures in Nipponbare.

Key words : Anther dehiscence, Cavity for anther dehiscence, Floret fertility at high temperatures, High temperature-induced floret sterility, *Oryza sativa* L.

It has been predicted by simulation models that the future global warming may decrease regional rice production in Japan (Horie, 1993; Horie et al., 1996). The main cause of the yield decrease would be the floret sterility induced by high temperatures at flowering (Horie et al., 1996). One of the most effective countermeasures against such a climate change would be adoption of cultivars tolerant to high temperatures at flowering. Indeed, Horie et al. (1996) predicted using a simulation model that the adoption of such tolerant cultivars might mitigate the yield decrease. The tolerance correlated with the characteristics determining the anther dehiscence : the anthers of tolerant cultivars dehisce early and well at the anthesis under a high temperature condition (Satake and Yoshida, 1978; Mackill et al., 1982; Matsui et al., 1997).

On the other hand, Matsui et al. (1999a, b, 2000a, b, c) showed that in glamineous crops the driving force to open the theca is the rapid swelling of pollen grains in response to the floret opening. Matsui et al. (2000d) clarified that the main cause of the high temperature-induced sterility in rice is the disturbance of the swelling by high temperatures at flowering. They also clarified that the thecae of a high-temperature tolerant cultivar (Nipponbare) dehisce easily and homogeneously in response to the pollen swelling and that the pollen grains of the tolerant cultivar swell better than those of a susceptible cultivar (Hinohikari) under high temperature conditions (Matsui et al., 2000d).

Here, we compare the transverse sections of anthers between the two rice cultivars, one showing floret fertility and another showing floret sterility under high tempera-

tures at flowering and discuss the relationship between the tolerance to high temperatures and the structural characteristics of anthers.

Materials and Methods

Two japonica rice cultivars, Hinohikari (high-temperature susceptible) and Nipponbare (high-temperature tolerant) were used. Seedlings at the 5.2 leaf stage were transplanted in a circular pattern into four L pots, 20 seedlings per pot, on 15 June for Hinohikari and on 25 June for Nipponbare, and grown under submerged soil conditions outdoors (Experimental Farm of Kyoto University, Takatsuki, Osaka). The heading period of both cultivars was around 25 August. Each pot was provided with 0.5 g each of N, P₂O₅ and K₂O as a top-dressing at about 45 days before the heading. The tillers were removed during the vegetative stage when they appeared.

The third spikelets from the top of the first branch were fixed in FAA after removing the lemmas on the day before flowering. One anther from each floret was selected at random and the length of the anther was measured in situ with a micrometer. Fifteen florets were used for this measurement. Then the florets were dehydrated by a methanol-butanol series. One anther from each floret was selected at random and was infiltrated and embedded in paraffin. Eight-micron-thick sections were cut and stained with toluidine blue-O.

The thickness of the locule wall was measured at four points on the diagonal lines (dotted lines in Fig. 1) of the anther in each section (Fig. 1A). The number of cell layers separating the locule and the cavity for dehiscence

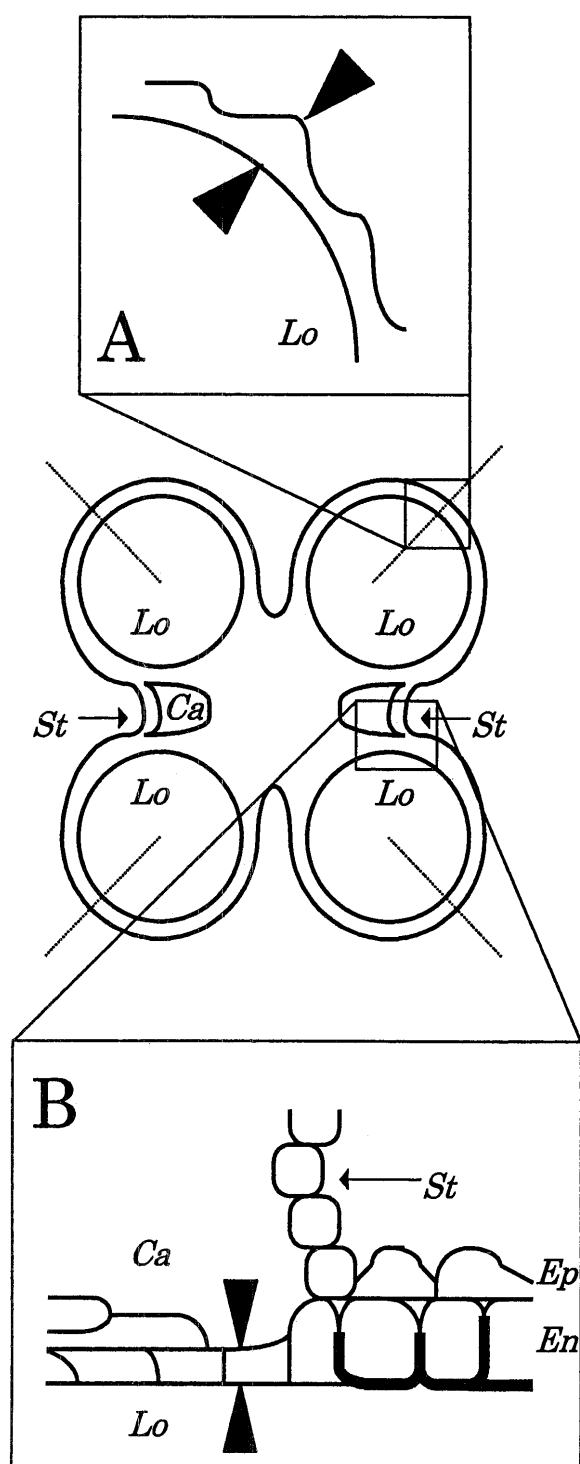


Fig. 1. Schematic transverse sections of anthers. **A**, Site for measurement of thickness of locule walls. The thickness was measured at four points on the diagonal lines (dotted lines) of the anther (between arrowheads). **B**, Number of cell layers that separates the cavity for anther dehiscence and the locule. The number was counted at the region where the number of cell layers was the smallest (probable breaking spot for septum rupture, between arrowheads). (*Ca*, cavity for anther dehiscence; *En*, endothecium; *Ep*, epidermis; *Lo*, locule; *St*, stoma)

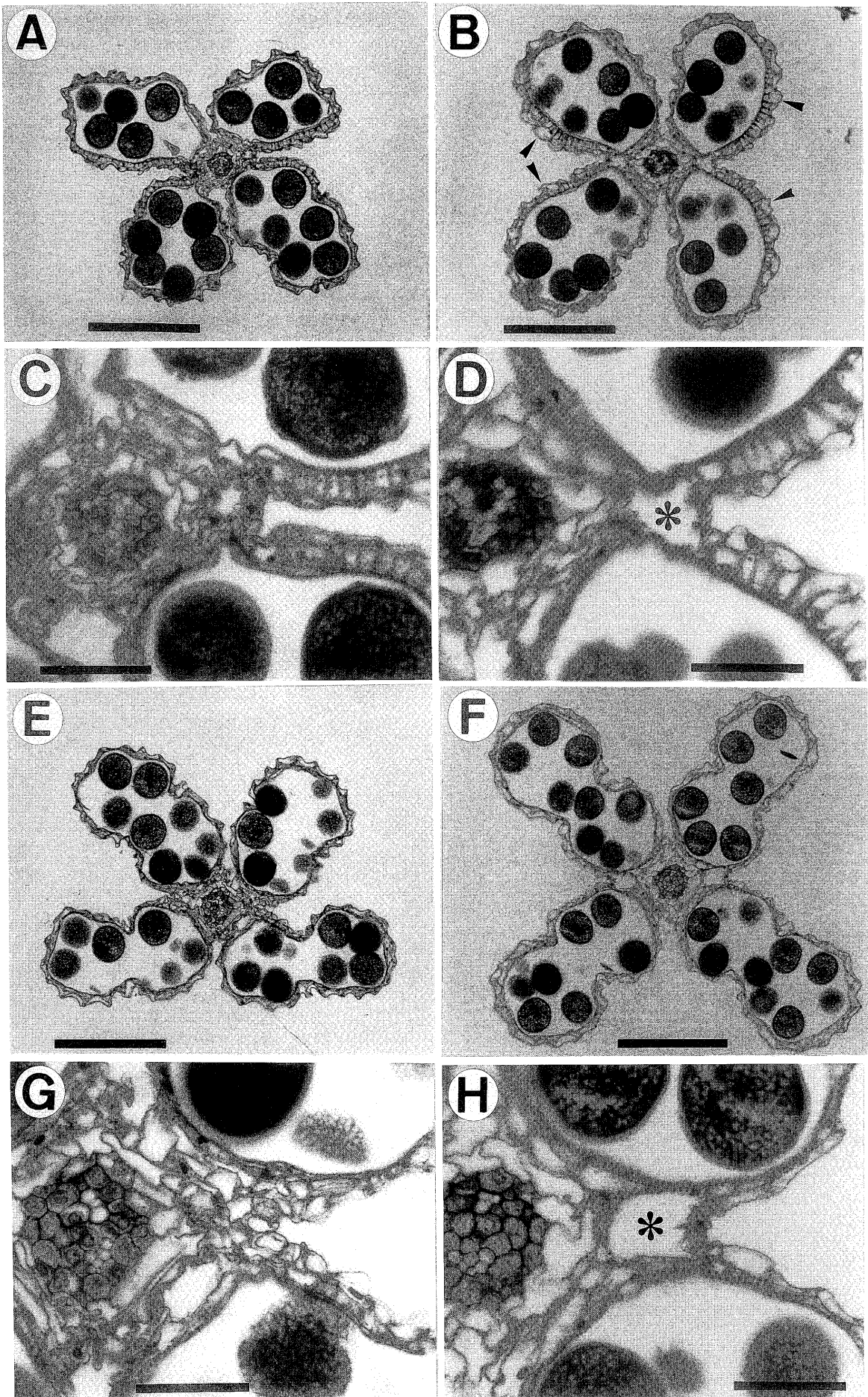
Fig. 2. Transverse section of anther of the Hinohikari (high-temperature susceptible cultivar) and Nipponbare (high-temperature tolerant cultivar). **A**, apical part of anther of Hinohikari. **B**, apical part of anther of Nipponbare. The locule walls have well-developed epidermal cells (arrowhead) and the walls are thicker than that of Hinohikari. **C**, magnification of poorly developed cavity for dehiscence in the apical part of anther of Hinohikari (detail of **A**). **D**, magnification of well-developed cavity (*) in the apical part of anther of Nipponbare (detail of **B**). **E**, middle part of anther of Hinohikari. **F**, middle part of anther of Nipponbare. **G**, magnification of poorly developed cavity for dehiscence in the middle part of anther of Hinohikari (detail of **E**). **H**, magnification of well-developed cavity for anther dehiscence (*) in the middle of anther of Nipponbare (detail of **F**). (Scale bars in **A**, **B**, **E**, and **F** indicate 100 μm and those in **C**, **D**, **G**, and **H** indicate 25 μm , respectively.)

at the part where the number of cell layers was the smallest (probable future breaking spot at the time of septum opening, Fig. 1B) was counted. The number was counted for all four locules in each section. These measurements were conducted on the three sections, at the apical (400 μm from the apex of anther), basal (400 μm from the base of short locule) and middle parts, of each anther. Fifteen florets per cultivar were used.

Results and Discussion

The length of anther was 1.98 ± 0.01 mm (mean \pm S.E.) in Hinohikari and was 1.94 ± 0.02 mm in Nipponbare. The anther of Nipponbare was shorter than that of Hinohikari on the average. In the studies on floral impotency type damage due to cool summers in rice, Hashimoto (1961), Suzuki (1982), and Tanno et al. (1999) showed that the tolerance to low temperature at the booting stage is positively correlated with the length of anther using various cultivars and in segregating generations of hybrids between tolerant and susceptible cultivars. However, the difference of 0.04 mm in the anther length observed in this experiment may be negligible (Suzuki, 1982; Tanno et al., 1999).

In rice anthers just before dehiscence, the septal cells and the tapetum remnants keep the adjacent two locules closed. An important difference which seems to directly relate to the characteristics of the theca dehiscence between the two cultivars was the form of the cavities for dehiscence which are formed in the septum (Fig. 2). The cavities in Hinohikari had various forms and were small (Fig. 2C, G), and were separated by about 1.5 cell layers from the locules (Fig. 3). In contrast, the cavities in Nipponbare were well developed (Fig. 2D, H) and separated by 0.5–0.9 cell layers (Fig. 3). Since the rupture of septum by the pollen swelling in response to the floret opening is an indispensable step for the theca dehiscence in rice (Matsui et al., 1999a, b), the strength of the septum would directly be related with the feasibility of the theca to dehiscence. Matsui et al. (2000d) reported that the thecae of Nipponbare dehiscence easily and homogeneously in response to the pollen swelling and that the easy and homogeneous dehiscing of the thecae is responsible for the tolerance to high temperatures in Nipponbare. The well-developed cavity in Nipponbare



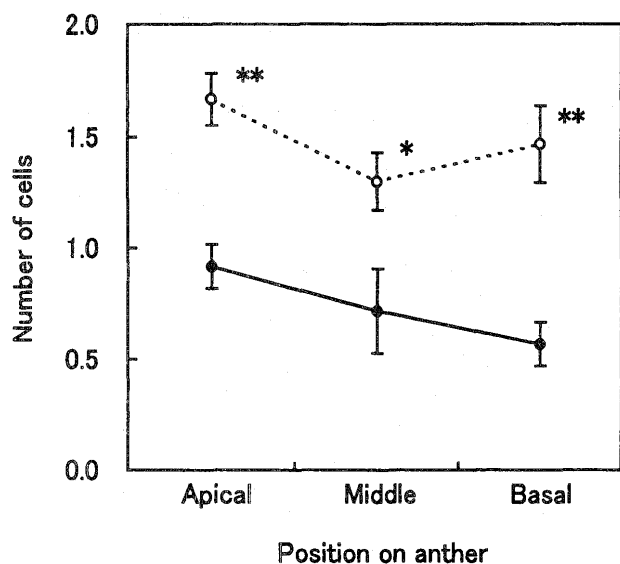


Fig. 3. The number of cell layers separating the locule and the cavity for dehiscence in Nipponbare (solid line) and Hinohikari (broken line). Vertical bars indicate S.E. ($n=15$). * and ** indicate statistically significant difference at 5 and 1 % levels, respectively at each region of the anther.

allows easy dehiscence of the theca. Under a high-temperature condition at flowering, delay of the theca dehiscence at the floret opening may inhibit the swelling of pollen grains by the loss of water in the locules, which is the driving force of the theca dehiscence, and may have a feed back effect on the delay of the dehiscence. Therefore, the strength of septa may be more important for the tolerance to high temperatures at flowering than the length of anther that has positive correlation with the number of pollen grains in the anther (Suzuki, 1981) and thus with the tolerance to low temperatures at the booting stage (Hashimoto, 1961; Suzuki, 1982; Tanno, 1999).

Suzuki (1978) showed that in a cultivar susceptible to low temperatures at the booting stage, the low temperature disturbs the development of the cavity and assumed that the poor development of cavity was one factor causing poor anther dehiscence even in the floral impotency-type damage due to cool summers. On the other hand, Mackill et al. (1982) found using indica cultivars that the cultivars tolerant to high temperature had more pollen grains deposited on the stigma even under a moderate-temperature condition. This is consistent with our theory that the physical characteristics of the anther that would affect anther dehiscence determine the high-temperature tolerance.

Another visible difference in the transverse sections between the two cultivars is the thickness of the locule walls. Nipponbare has a well-developed epidermis and thick endothecium (Fig. 1A, B), and thus thicker locule walls than Hinohikari especially in the apical parts (Fig. 4). Matsui et al. (2000d) reported that pollen grains of Nipponbare swell better than those of Hinohikari under high temperatures at flowering. The thicker wall may

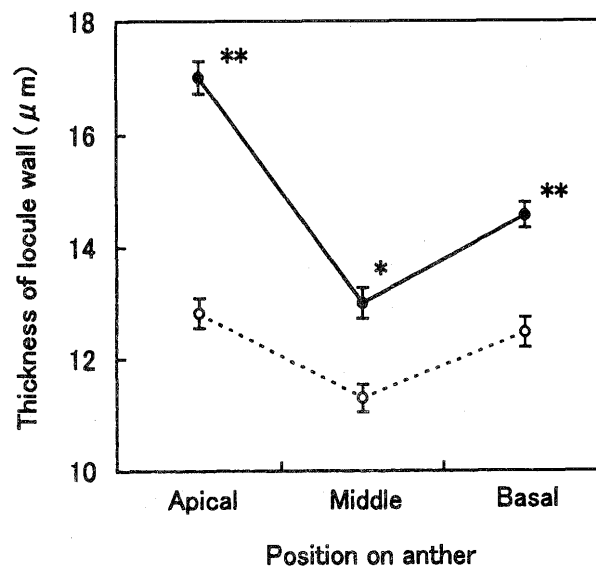


Fig. 4. Thickness of locule walls in Nipponbare (solid line) and Hinohikari (broken line). Vertical bars indicate S.E. ($n=15$). * and ** indicate statistically significant difference at 5 and 1 % levels, respectively at each region of the anther.

enable the pollen grains to swell under the high-temperature condition by retaining water in the anther locules.

It is not clear whether the well-developed cavity for dehiscence and the thick locule wall generally contribute to the dehiscence of theca because we used only two cultivars. Many cultivars need to be tested for generalization of the above theories.

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