

## Rice (*Oryza sativa* L.) Cultivars Tolerant to High Temperature at Flowering: Anther Characteristics

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We examined the relationship between morphological characteristics of anthers and fertility in japonica rice cultivars subjected to high temperature (37.5/26 °C day/night) at flowering. Percentage fertility was negatively correlated with the number of cell layers that separated the anther locule from the lacuna that formed between the septum and the stomium. The cell layers consisted of the remaining septum and degraded tapetum, and serve to keep the adjacent two locules closed. Anther dehiscence therefore requires the rupture of the cell layers. We conclude that the tight closure of the locules by the cell layers delayed locule opening, and decreased fertility at high temperatures. © 2002 Annals of Botany Company

**Key words:** Anther dehiscence, anther size, high temperature tolerance, lacuna between septum and stomium, *Oryza sativa* L., rice, septum, tapetum.

### INTRODUCTION

Rice is grown mainly in tropical and subtropical zones, and a high temperature at flowering can induce floret sterility and can limit grain yield (Osada *et al.*, 1973; Satake and Yoshida, 1978; Matsushima *et al.*, 1982). Since the 1980s, an increase in the concentration of greenhouse gases, such as carbon dioxide, in the atmosphere is thought to have been responsible for increasing the air temperature (Hansen *et al.*, 1984). Amongst other things, global warming is expected to result in the occurrence of high temperature-induced floret sterility in rice.

Crop scientists have attempted to assess the effects of increasing temperature and high carbon dioxide in the atmosphere on the growth and yield of rice using simulation models (Boote *et al.*, 1994; Horie *et al.*, 1996, 1997; Matthews *et al.*, 1997). Horie *et al.* (1996) suggested that the anticipated high temperature would induce floret sterility and increase the instability of the rice yield even in temperate regions. These authors also showed that adoption of high temperature-tolerant cultivars is one of the most effective countermeasures to maintain high productivity and stability of rice under the anticipated climate in temperate regions (Horie *et al.*, 1996).

The main cause of floret sterility induced by high temperatures at flowering is anther indehiscence (Satake and Yoshida, 1978; Mackill *et al.*, 1982; Matsui *et al.*, 1997a, b, 2001). High temperatures at flowering inhibit swelling of the pollen grains (Matsui *et al.*, 2000b), which is the driving force behind anther dehiscence in rice (Matsui *et al.*, 1999a, b). Anthers of high temperature-tolerant cultivars dehisce more easily than those of susceptible cultivars and contribute to pollination under high-tempera-

ture conditions (Satake and Yoshida, 1978; Mackill *et al.*, 1982; Matsui *et al.*, 2000b, 2001). However, the factors determining the degree of anther dehiscence are unknown.

Here, we examine the relationship between morphological characteristics of the anther and floret tolerance to high temperature at flowering in japonica rice cultivars in relation to sterility. We also investigate the morphological characteristics of anthers in tolerant cultivars and discuss why their anthers dehisce so readily.

### MATERIALS AND METHODS

#### *Floret sterility due to high temperatures during the flowering period*

Data concerning floret tolerance to high temperatures at flowering have been reported previously (Matsui *et al.*, 2001). Nine japonica rice cultivars, including two tolerant to, and two susceptible to, high temperature were used (Table 1). Seeds were sown from March to June 1995 so that panicles would emerge late in August. Seedlings at the 5.0–5.5 leaf stage were transplanted in a circular pattern into 4.1 pots, with 20 seedlings per pot, and were grown outdoors under submerged soil conditions (Experimental Farm of Kyoto University, Osaka, Japan). Each pot was provided with 0.4 g N, 0.4 g P<sub>2</sub>O<sub>5</sub> and 0.4 g K<sub>2</sub>O as a top dressing about 45 d before heading (about 15 d before panicle initiation). Tillers were removed during the vegetative stage as they appeared.

Sun-lit phytotrons were used for high-temperature treatments. Plants at the middle heading stage were exposed to 37.5 °C for 6 h (1000–1600 h) for six consecutive days. The night-time temperature (1800–0800 h) was 26.0 °C. Relative humidity was 60 % in the daytime (1000–1600 h) and 80 % at night. Air temperature and humidity were

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TABLE 1. Tolerance of rice cultivars to high temperature at flowering based on the studies of Matsui et al. (2001)

	Cultivar	Control 35.0/26.0 °C*	Percentage fertility under high temperature		Tolerance to high temperature
			37.5/26.0 °C	40.0/26.0 °C	
1	Nipponbare	94.6	85.3	35.0	Tolerant
2	Akitakomachi	93.4	80.0	50.3	Tolerant
3	Aichinokaori	96.1	78.2	19.8	Moderate
4	Yumehikari	91.5	74.3	22.9	Moderate
5	Kinmaze	95.4	65.2	22.2	Moderate
6	Akihikari	92.6	65.9	13.2	Moderate
7	Aoinokaze	91.4	58.4	19.9	Moderate
8	Minamihikari	92.7	45.7	19.2	Susceptible
9	Hinohikari	93.4	44.1	13.7	Susceptible

\* Day/night temperature

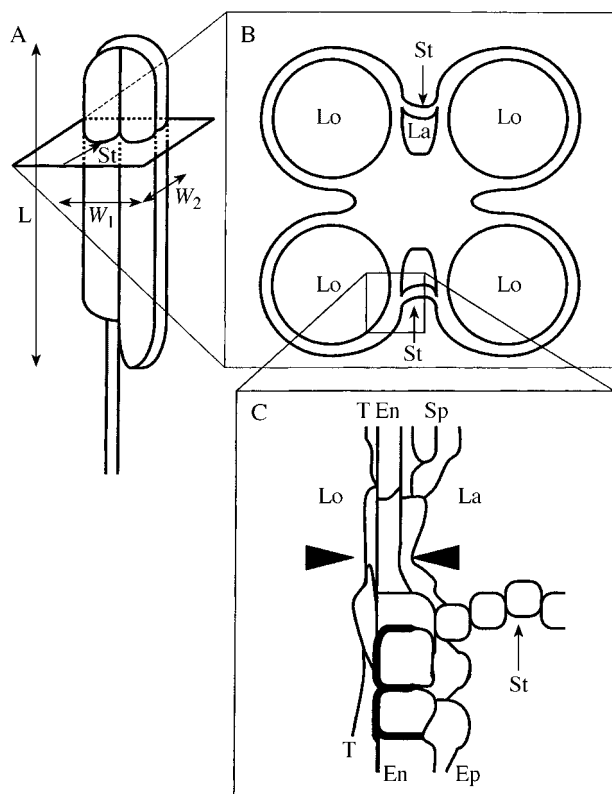


FIG. 1. Measurement of anther characteristics. A, Exterior of rice anther. Anther length (L) was taken as the length of the long locule. Anther width was calculated as the mean width taken in two directions  $[(W_1+W_2)/2]$ : one parallel to the stoma ( $W_1$ ) and the other ( $W_2$ ) at right angles. B, Schematic transverse section of anther. C, Magnification of B around the cell layers separating the locule from the lacuna formed between the septum and the stoma. The number of cell layers separating the lacuna from the locule between the septum and the stoma was counted at the point where the number of cell layers was smallest (the probable breaking point when the septum opens; between arrowheads). La, Lacuna formed between the septum and the stoma; En, endothecium; Ep, epidermis; T, tapetum; Lo, locule; St, stoma.

at 1800 h, 6 d later. Three pots of each cultivar were used for each treatment.

The fertility of all florets on panicles on which florets started to open on the first day of treatment and finished opening before the last day of treatment was examined at maturity. More than four panicles (over 150 florets) were examined per pot. Means of the three pots were used for regression analysis.

#### Morphological characteristics of anthers

The same nine cultivars were grown in 2000 under the conditions described above. On the day before florets opened, the third florets from the top of the first branches were fixed in FAA after removing the lemmas. Six florets per cultivar were fixed.

Three florets (18 anthers) per cultivar were used for measurement of anther sizes. The length and width of anthers were measured with a micrometer under a stereomicroscope. Width was taken as the mean of measurements made parallel and at right angles to the stoma (Fig. 1A).

The other three florets were dehydrated in a methanol-butanol series before being infiltrated and embedded in paraffin. Transverse sections, 8  $\mu$ m thick, were cut from the florets of 18 anthers and stained with toluidine blue-O. The number of cell layers separating the anther locule from the lacuna that formed between the septum and the stoma was counted at the point where the number of layers was smallest (probably the point that will break when the septum opens; Fig. 1C, cf. Fig. 4). The number of cell layers was recorded every 80  $\mu$ m for all four locules in five sections, from 480  $\mu$ m to 800  $\mu$ m from the apex of the anther. Mean anther length, width, length  $\times$  width and the number of cell layers were calculated for each floret. Means of the three florets of each cultivar were then used for regression analysis. Predictor variables in the multiple regression analysis were determined by a stepwise regression procedure.

## RESULTS

altered gradually between 0800 and 1000 h, and between 1600 and 1800 h. The treatments started at 1800 h and ended

Percentage fertility under a day/night temperature regime of 37.5/26 °C was significantly correlated with the number of

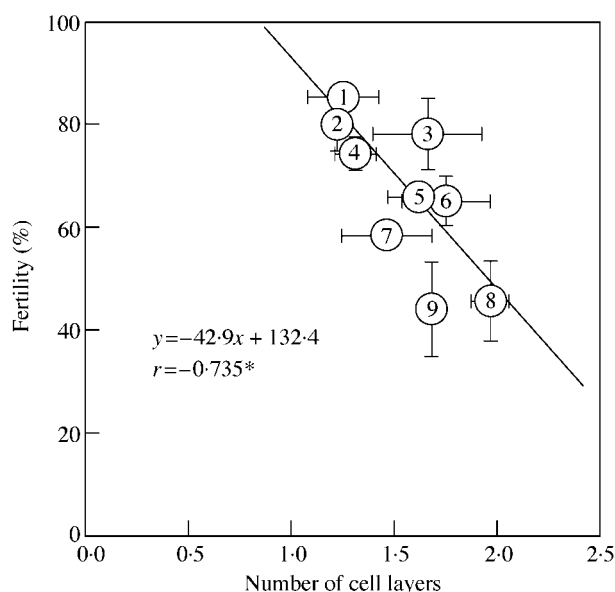


FIG. 2. Relationship between the number of cell layers separating the locule from the lacuna formed between the septum and the stomium and percentage fertility. Numbers represent cultivars (see Table 1). Vertical bars indicate  $\pm$  s.e. of three pots. Horizontal bars indicate  $\pm$  s.e. of three florets.

cell layers separating the locule from the lacuna that formed between the septum and the stomium (Fig. 2). There was no correlation between anther length, width or length  $\times$  width and percentage fertility, but anther length  $\times$  width was significantly correlated with the difference between measured percentage fertility and percentage fertility estimated from the number of cell layers separating the locule from the lacuna ( $r = 0.750$ ,  $P < 0.05$ ). Under high temperature, 85 % of the variance in the percentage fertility among the nine cultivars was explained by multiple regression with the number of cell layers and the product of anther length and width (Fig. 3).

When the locule is separated from the lacuna between the septum and the stomium by three or more layers, then these layers consist of parenchyma cells and endothecium cells in the septum, and tapetal cells (Fig. 4). If it is separated by one or two layers only, then these consist of endothecium cells and/or degrading tapetal cells.

## DISCUSSION

The difference in the sterility-inducing daytime temperature between the most tolerant cultivar, Akitakomachi, and the most susceptible cultivar, Hinohikari, used in the present study has been reported to be about 3 °C (Matsui *et al.*, 2001). Using a crop simulation model, Horie *et al.* (1996) showed that even a difference of 1.6 °C in the sterility-inducing temperature could have a serious effect on rice yield in a temperate region under anticipated global warming. The present results suggest that the 3 °C difference in tolerance was mainly related to the number of cell layers separating the locule from the lacuna that formed

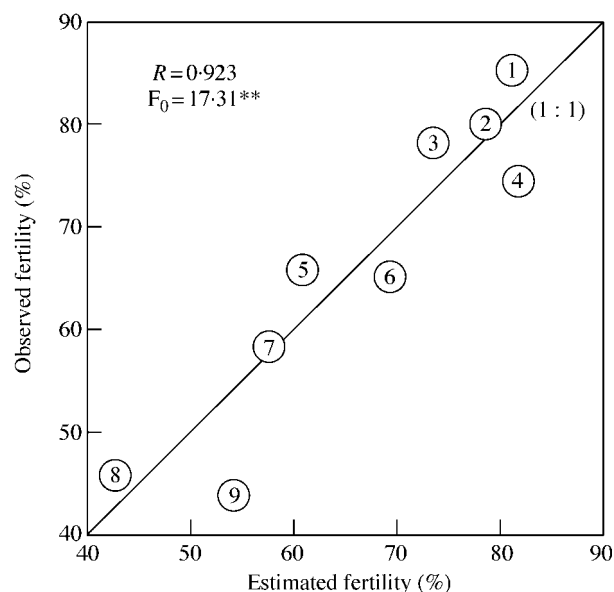


FIG. 3. Comparison between observed and estimated fertility in rice under a daytime temperature of 37.5 °C. Numbers represent cultivars (see Table 1). The estimation was made by:  $y = -57.6x_1 + 160.5x_2 + 12.03$ , where  $x_1$  and  $x_2$  represent the number of cell layers separating the locule from the lacuna between the septum and the stomium, and the product of length and width of anther, respectively. \* and \*\* indicate significance at 5 and 1 % levels, respectively.

between the septum and the stomium, and secondarily related to anther size.

It has been reported that anther length is positively correlated with tolerance to floret sterility induced by a low temperature at the booting stage (e.g. Hashimoto, 1961; Suzuki, 1981, 1982; Tanno *et al.*, 1999), which is the most serious cool-weather damage in rice. Using cultivars with a large variation in anther width, Tanno *et al.* (1999) showed that the product of anther length and width was correlated more closely with tolerance to low temperature at the booting stage than was length alone. The number of pollen grains in the anther is positively correlated with anther length in rice cultivars (Suzuki, 1981), and the inner wall of the anther locule in Poaceae is lined with pollen grains before the septum opens (Keijzer *et al.*, 1996; Matsui *et al.*, 2000a). Thus, anther width would also be positively correlated with the number of pollen grains in the anther. Since the direct cause of floret sterility induced by low temperature at the booting stage is the decreased number of germinated pollen grains caused by anther indehiscence (Shimazaki *et al.*, 1964), it has been assumed that anther size would be correlated with tolerance to low temperature because the reduction in the number of dehiscent anthers would be compensated by a large number of pollen grains per anther (Suzuki, 1981; Tanno *et al.*, 1999). Anther size might be correlated with tolerance to high temperature-induced sterility in a similar way, because the direct cause of spikelet sterility induced by high temperature at flowering is also a decrease in the number of germinated pollen grains caused by poor anther dehiscence (Satake and Yoshida, 1978; Mackill *et al.*, 1982; Matsui *et al.*, 1997a, b, 2001).

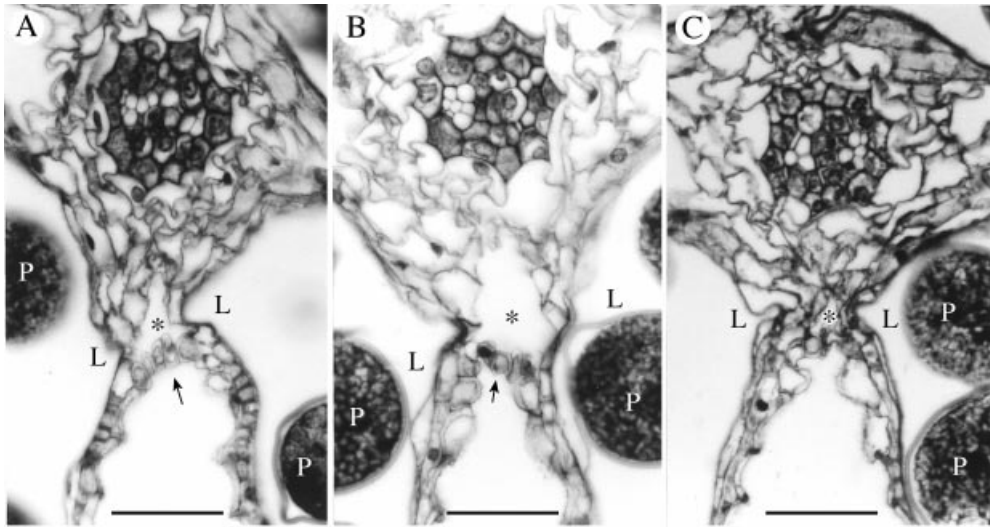


FIG. 4. Transverse section of the septum in which one or two (A, B), or three (C) cell layers separate the locule from the lacuna between the septum and the stomium. A, Degrading tapetal cells separate the lacuna from the locule ('Nipponbare', high-temperature tolerant); B, degrading endothecium and degraded tapetal cells separate the lacuna from the locule ('Akitakomachi', high-temperature tolerant); C, degrading tapetal cells, endoecium cells and parenchyma cells separate the lacuna from the locule ('Minamihikari', high-temperature susceptible). L, Anther locule; P, pollen grain; arrowhead, stomium; \*, lacuna formed between the septum and the stomium. Bars = 25  $\mu$ m.

The number of cell layers separating the locule from the lacuna between the septum and the stomium was more closely related to fertility under the high-temperature treatment than was anther size. Keijzer *et al.* (1996) reported that the stomium dissociates from the septum 2 d before anthesis in maize, and this dissociation ruptures the tapetum membranes. Thus, adjacent maize locules become one cavity before anthesis (Keijzer *et al.*, 1996). In rice, the stomium dissociates from the septum before anthesis, but the locules are kept closed until anthesis by parenchyma and endothecium cells in the septum, often supported by the degrading tapetum (Matsui *et al.*, 1999b). The lacuna that is surrounded by the septum and stomium cells is formed in this way. Since the locule remains closed until anthesis, opening of the septum (i.e. locule opening) at flowering is an indispensable process for anther dehiscence (Matsui *et al.*, 1999b). The number of cell layers separating the locule from the lacuna between the septum and the stomium is the number of cell layers responsible for keeping the locules closed. These cell layers might be responsible for the high-temperature susceptibility of rice through the strength of the septum and thus the degree of anther dehiscence.

The fundamental cause of poor anther dehiscence induced by high temperatures is disturbance of pollen swelling (Matsui *et al.*, 2000b), which is the driving force for anther dehiscence (Matsui *et al.*, 1999a, b). Although it is not known whether the disturbance is caused by water loss, water loss in the locule would impede pollen swelling because swelling occurs as a result of water migration into pollen grains. Indeed, low atmospheric humidity disturbs anther dehiscence in rice (Matsui *et al.*, 1999b). Thus, delayed anther dehiscence at floret opening might inhibit the swelling of pollen grains through loss of water in the locule under high temperatures, and might have a feedback effect delaying dehiscence further. Therefore, the strength of the

septum might be more important for high-temperature tolerance than the size of the anther which is the dominant factor in tolerance to sterility induced by low temperature at the booting stage. However, it must be noted that the range of anther sizes used in the present experiment (1.82–2.24 mm in length and 0.405–0.455 mm in width) was not as wide as that used in studying tolerance to low temperature at the booting stage, when tolerance was attributed to anther size (e.g. anther lengths from 1.59 to 2.54 mm, and widths from 0.395 to 0.515 mm; Tanno *et al.*, 1999). Thus, the narrow range of anther sizes may have overemphasized the importance of the number of cell layers in high-temperature tolerance.

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