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# Sterility and Poor Pollination Due to Early Flower Opening Induced by Methyl Jasmonate

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**Abstract:** Flower opening in the early morning helps avoid sterility induced by heat stress at anthesis in rice (*Oryza sativa* L.). A pot experiment was conducted to reveal the effects of methyl jasmonate (MeJA) on flower opening time (FOT), sterility, pollination, and anther dehiscence. Four mmol L<sup>-1</sup> MeJA solution (3 mL per panicle) and water, as a control, was applied to the panicles of the japonica type rice cultivar ‘Hinohikari’ at 0900, 1000, or 1100. By photographing flowers at 4-min intervals, we determined the FOT. Flower sterility, pollination, and anther dehiscence were also examined. Application of MeJA solution at 0900 advanced FOT about 2 hr compared with that without application, and 1.5 hr compared with that after application of water. MeJA application at 1000 also advanced FOT, but that at 1100 did not. Application of MeJA solution significantly increased the numbers of flowers opening on the day of treatment and decreased that on the day after treatment. Application of MeJA solution at 0900 significantly increased flower sterility compared with that without treatment. More than 40% of the flowers that were treated with MeJA and opened on the day of treatment were those expected to open on the next day. These results suggest that sterility induced by application of MeJA is caused by the early opening of flowers expected to open the day after treatment.

**Key words:** Anther dehiscence, Flower opening in early morning, Flower opening time, Heat-induced sterility, Methyl jasmonate, *Oryza sativa*, Pollination, Sterility.

The global climate change is expected to cause a serious challenge to crop production around the world. Exposure of rice (*Oryza sativa* L.) to temperatures higher than 34°C at the time of flowering will lead to flower sterility and decrease yield, even in temperate regions such as southern Japan, unless the cropping season is changed to avoid such temperatures (Horie et al., 1996; Kim et al., 1996; Nakagawa et al., 2003). Using crop simulation models, Horie et al. (1996) suggested that yields of rice varieties currently grown in southern Japan would be reduced by up to 40% under future climate scenarios. In the summer of 2003, the Yangtze River rice-growing region in China experienced temperatures above 39°C, leading to a large reduction in rice production resulting from a low seed-set (30–70%; Wang et al., 2004).

Opening of rice flowers in the early morning is an adaptative measure to avoid sterility caused by heat stress at flower opening because the sensitivity of rice flowers to high temperatures decreases during the 1-hr period after flowering (Satake and Yoshida, 1978). Thus, sterility may be reduced if the flower opening time (FOT) is 1 hr earlier than the normal time, because it may lead to flower

opening before the air temperature reaches the critical level; air temperature can rise at a rate of >3°C hr<sup>-1</sup> starting at around 1000 (Nishiyama and Blanco, 1980). An experiment conducted under a controlled environment revealed that flowers of ‘Milyang 23’ that opened earlier in the morning had higher seed-set than those that opened later near at midday (Imaki et al., 1987).

FOT varies with the variety, weather condition, and the artificial treatment applied (Hoshikawa, 1989; Satake, 1995; Zeng et al., 1999). For example, the flowers of *Oryza glaberrima* Steud. open earlier than those of *Oryza sativa* L. (Nishiyama and Blanco, 1980; Jagadish et al., 2008), and the flowers of their interspecific hybrids open earlier than *O. sativa* (Nishiyama and Satake, 1981). Imaki et al. (1983, 1987) reported that the flowers of some cultivars of *O. sativa* opened 1–2 hr earlier than those of ‘Koshihikari’, a standard Japanese cultivar. In indica cultivars, FOT was about 45 min earlier at temperatures 7 C° higher than the normal temperature (Jagadish et al., 2007). Solar radiation from 0400 to 0800 influenced FOT of ‘Koshihikari’, but not that of EGO, a heading-time tester line (Nakagawa and Nagata, 2007). Strong winds (Tsuboi, 1961) as well as low

atmospheric pressure and rain (Hoshikawa, 1989) also affect FOT.

There are several artificial methods for advancing FOT in rice plants. Dark treatment for 1 hr before the natural FOT advances FOT by 2 hr or more (Nishiyama and Blanco, 1981). Light conditions can also influence FOT, since the duration of flower opening increases under continuous light or dark conditions (Nishiyama and Blanco, 1981; Hoshikawa, 1989). Subjecting rice panicles to some physical stimuli also advances FOT (Tsuboi, 1961). Applications of CO<sub>2</sub> and methyl jasmonate (MeJA) to panicles also advance FOT (Wang et al., 1989; Zeng et al., 1999; Song et al., 2001). Zeng et al. (1999) reported that MeJA induced flower opening in rice. They observed a response within 80 min after MeJA treatment and found clear flower-opening effects with 0.4 and 4 mmol L<sup>-1</sup> MeJA treatment.

Some artificial FOT advancements, however, are accompanied by undesirable effects such as anther indehiscence, poor pollination, and sterility (Tsuboi, 1961; Nishiyama and Blanco, 1981). Advancement of FOT caused by wind or physical stimulation of rice panicles reportedly resulted in anther indehiscence and poor pollination (Tsuboi, 1961). Advancement of FOT caused by 1-hr dark treatment has also been reported to result in anther indehiscence (Nishiyama and Blanco, 1981). Tsuboi (1961) speculated that such poor pollination may be due to flower opening before pollen maturation and preparation of anther dehiscence.

The effects of MeJA treatment on anther dehiscence and pollination in rice have not been examined in detail. Jasmonic acid (JA) is essential for anther dehiscence in *Arabidopsis*, and exogenous application of JA rescues some fertility-related mutations from sterility (Sanders et al., 2000; Stintzi and Browse, 2000). JA possibly plays a role in rice leaf senescence (Hung et al., 2006) and is important for tapetum, stamen, and pollen development in rice (Hirano et al., 2008). Spraying 2 mmol L<sup>-1</sup> MeJA solution increased seed-set in male sterile lines of rice (Song et al., 2001). On the other hand, 500 μmol L<sup>-1</sup> MeJA application to rice panicles had a slightly negative effect on flower fertility in the wild type and no rescue effects on flower fertility in the *aid1* mutant (Zhu et al., 2004). If MeJA application improves anther dehiscence, it might be useful for mitigating the negative effects of FOT advancement such as poor pollination and anther indehiscence.

The objective of this study was to clarify the effects of MeJA application on FOT as well as on anther dehiscence, pollination, and sterility in standard japonica rice 'Hinohikari' in order to develop artificial techniques for FOT control in rice. 'Hinohikari' was treated with 4 mmol L<sup>-1</sup> MeJA and water, as a control (referred to as "control water" hereafter), at 0900, 1000, or 1100. We specifically studied the effect of MeJA on flowering patterns and FOT;

the effect of MeJA on flower fertility, pollination, and anther dehiscence; and the correlations of FOT with sterility and anther indehiscence.

## Materials and Methods

### 1. Field sites, plant materials, and culture methods

In 2008, a pot experiment was conducted outdoors at Shimane University, Matsue, Japan (35°29'N, 133°04'E, altitude 4 m asl). The japonica type rice cultivar 'Hinohikari', which is one of the leading cultivars in Japan, was used. Surface-sterilized seeds were germinated at 32°C for 24 hr. Selected seeds were sown for uniformity on 15 June, 2008. Twenty germinated seeds were planted in each Wagner pot (1/5000 a) containing 3.6 kg equivalent to oven-dry soil (air-dried Andosol and a granitic sapolite Cambisol mixture, 1:1 volume) using the circular dense-culture method (Satake, 1972). The pots were watered to keep the field capacity level for ten days after sowing, and then kept flooded with 2–3 cm of water. Liquid fertilizer (0.15 g N, 0.15 g P<sub>2</sub>O<sub>5</sub>, and 0.15 g K<sub>2</sub>O) was applied every week until the start of the MeJA treatments. Tillers were removed as they appeared to obtain uniform plants. We measured air temperature every 5 min using a wireless weather station (Wireless Vantage Pro, Davis Instruments, Hayward, CA, USA) located at Shimane University (<http://www.ipc.shimane-u.ac.jp/weather/station/i/home.html>).

### 2. Methyl jasmonate application

MeJA was applied on 9 September. Panicles having several flowers that had opened before that day were selected for experiments. MeJA (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in a small amount of ethanol and then diluted with water to 4 mmol L<sup>-1</sup> (Zeng et al., 1999). The same amount of ethanol was also added to control water. Panicles were sprayed with MeJA solution and control water (approximately 3 mL per panicle) at 0900, 1000, or 1100. A no-spraying treatment was also added.

### 3. Measurements of flower opening time (FOT)

Physical stimuli, such as touch, may promote flower opening in rice (Tsuboi, 1961). To avoid this phenomenon, we photographed the panicles using a digital camera (Optio W30, Pentax, Tokyo, Japan) at 4 min intervals instead of physical inspection to determine the FOT and the daily flower opening pattern (Jagadish et al., 2007). The photographs were recorded automatically in the cameras. We put the camera on a tripod and used a built-in electronic timer to control the measurement intervals. We recorded FOT of 6–10 panicles per treatment. FOT was the time when 50% of the flowers opened on a given day.

After all flowers on the panicles closed, we marked the flowers that opened on the day of treatment with red paint

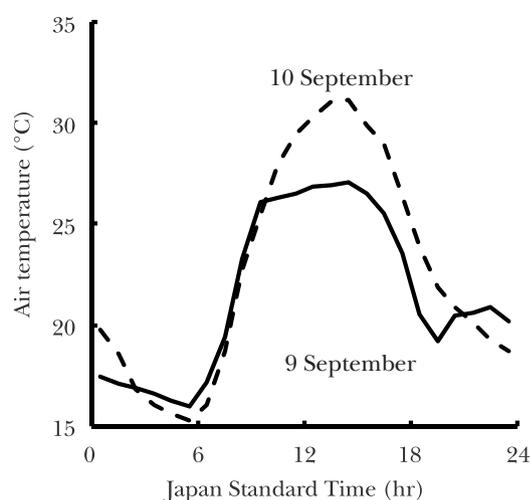


Fig. 1. Change in air temperature on the day of treatment (9 September) and the next day (10 September).

and those that opened the day after the treatment with white paint and counted the numbers of flowers (4 panicles per plot) that opened on the two d.

#### 4. Sterility, pollination, and anther dehiscence

We gathered and analyzed data on sterility of flowers that opened on the day of and the day after treatment as well as on pollination and anther dehiscence on the day of the treatment. To examine flower sterility, we sampled 4 panicles 17 d after treatment in each plot. Flowers of the collected panicles were marked red (flowering on treatment day) or white (flowering on the next day). We examined the sterility by manually inspecting each flower for ovary development.

To examine the stability of pollination, we samples 3 flowers per panicle (4 panicles in each plot) from the primary rachis-branches at 1600 on the day of treatment. The stigmata were collected from the flowers and stained with 0.1% aniline blue (0.1 g aniline blue, 2.12 g tripotassium phosphate, 75 mL of 1% ammonia solution, and 25 mL glycerol), and the total and germinated pollen grains on the stigmata were counted under a fluorescence microscope (OPTIPHOTO, Nikon, Tokyo, Japan).

To examine the percentage of dehisced thecae at the apical and basal parts, we samples 5 flowers from 4 panicles at 1600 on the day of treatment in each plot (Matsui et al., 2005). After air-drying, the number of indehiscent anthers at the apical and basal parts of the thecae were counted using a digital microscope (VH-8000, Keyence Corporation, Osaka, Japan).

#### 5. Data analysis

The percentage of flowers having more than 80 pollen grains on the stigmata (referred to as “>80-pollen-grain flowers” hereafter) was calculated for each treatment. The

Table 1. Effect of methyl jasmonate applied at three different time points on flower opening time (FOT) in rice (cv. ‘Hinohikari’).

	Flower opening time (Japan Standard Time)
MeJA9 <sup>2)</sup>	1016 a <sup>3)</sup>
MeJA10	1108 b
MeJA11	1244 d
CW9 <sup>3)</sup>	1214 c,d
CW10	1147 b,c
CW11	1148 b,c
No treatment <sup>3)</sup>	1212 c,d

<sup>2)</sup>MeJA9, MeJA10, and MeJA11 indicate the treatments with 4 mmol L<sup>-1</sup> methyl jasmonate solution at 0900, 1000, and 1100, respectively.

<sup>3)</sup>CW9, CW10, and CW11 indicate control-water treatments at 0900, 1000, and 1100, respectively.

<sup>3)</sup>No treatment indicates that neither methyl jasmonate solution nor control water were sprayed on rice panicles.

<sup>3)</sup>Values with the same letters were not significantly different at the level of P=0.05 with Tukey’s HSD test (n= 6–10).

percentage of >80-pollen-grain flowers strongly correlated with the number of pollen grains deposited on the stigmata and was thus used as a measure of the reliability of pollination under normal conditions (Matsui and Kagata, 2003; Matsui et al., 2005).

Differences between the mean values of treatments were analyzed by Tukey’s HSD test at a probability level of 0.05. FOT expressed as Japan Standard Time was converted to a value expressed as a decimal fraction to use Tukey’s HSD test (e.g. 1015 was converted to 10.25). Regression analysis of the mean data was performed to examine the relationship between FOT, flower sterility and characteristics of the anther. We conducted an analysis after arcsine conversion of the data expressed as percentages (fertility, <80 pollen-grain flowers, pollen grain germination, and anther dehiscence) using SPSS (Version 14J for Windows, SPSS Japan Inc., Tokyo, Japan).

## Results

### 1. Flower opening time (FOT) and flowering patterns

The temperature on the day of treatment (9 September) and the day after treatment (10 September) did not exceed 34°C (the threshold of heat-induced sterility; Fig. 1).

Flowers of ‘Hinohikari’ opened about 1 hr after the application of 4 mmol L<sup>-1</sup> MeJA solution when MeJA was applied at 0900 and 1000 (Table 1). Application of MeJA solution at 0900 advanced FOT about 2 hr and 1.5 hr compared with no application and application of control water, respectively. MeJA application at 1000 also advanced FOT, but that at 1100 did not. Applications of control water at 0900, 1000, and 1100 advanced FOT slightly, but not significantly, compared with no application.

Flowering-opening patterns after MeJA application at

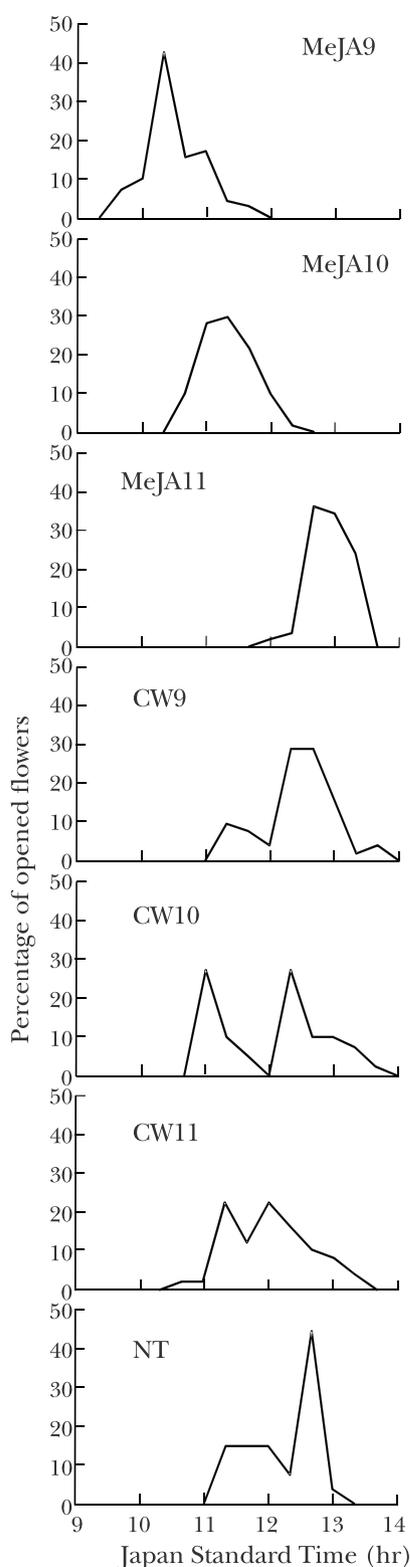


Fig. 2. Effect of methyl jasmonate applied at three different time points on the number of flowers opened at a different time in rice (cv. 'Hinohikari'). Values are percentage of flowers opened during the 20-min period on the treatment day.

MeJA9, MeJA10, and MeJA11 indicate treatments with methyl jasmonate solution at 0900, 1000, and 1100, respectively. CW9, CW10, and CW11 indicate control water treatments at 0900, 1000, and 1100, respectively. NT indicates that rice panicles were applied neither methyl jasmonate solution nor control water.

Table 2. Effect of methyl jasmonate applied at three different time points on the numbers of flowers opening on the day of treatment, the day after treatment, and on both days in rice (cv. 'Hinohikari').

	Numbers of opening flowers per panicle		
	Treatment day	Day after treatment	Total
MeJA9 <sup>2)</sup>	35.50 a <sup>w)</sup>	2.50 a	38.00 a
MeJA10	24.75 a,b	3.00 a,b	27.75 a
MeJA11	27.50 a,b	4.25 a,b	31.75 a
CW9 <sup>y)</sup>	17.50 b	16.00 c	33.50 a
CW10	21.25 a,b	11.25 a,b,c	32.50 a
CW11	23.50 a,b	13.00 b,c	36.50 a
No treatment <sup>x)</sup>	17.75 b	16.25 c	34.00 a

<sup>2)</sup>MeJA9, MeJA10, and MeJA11 indicate the treatments with 4 mmol L<sup>-1</sup> methyl jasmonate solution at 0900, 1000, and 1100, respectively.

<sup>y)</sup>CW9, CW10, and CW11 indicate control-water treatments at 0900, 1000, and 1100, respectively.

<sup>x)</sup>No treatment indicates that neither methyl jasmonate solution nor control water were sprayed on rice panicles.

<sup>w)</sup>Values with the same letters were not significantly different at the level of P=0.05 with Tukey's HSD test (n= 4).

0900, 1000, and 1100 were similar although the start of flower opening and FOT were different (Fig. 2). The flowering-opening patterns in the three MeJA treatments were very different from those subjected to control water application and no application. The number of opened flowers increased rapidly in the treatment with MeJA, but gradually in the controls (no application and control-water application; Fig. 2).

## 2. Effect of MeJA on the daily number of opened flowers

Applications of MeJA solution at 0900 significantly increased the number of flowers opening on the day of the treatment and decreased it on the next day, compared with application of water at 0900 (Table 2). Application of MeJA solution at 1000 and 1100 increased the number of flowers opening on the day of treatment and decreased it on the next day, compared with the application of control water at 0900 and 1000, respectively, though not significantly. Applications of control water at three different time points did not affect the number of flowers opening on either day. There was no significant difference among the seven treatments in the total number of flowers opening on the two examined days.

## 3. Effects of MeJA on fertility, pollination, and anther dehiscence

The flower sterility in the rice plants was increased by the application of MeJA solution at 0900 compared with the application of control water at 0900, though not significantly (Table 3). MeJA application at 1000 also increased flower sterility. Applications of control water at

Table 3. Effect of methyl jasmonate applied at three different time points on the percentages of sterile flowers, flowers with more than 80 pollen grains deposited on the stigmata (>80-pollen-grain flowers), germinated pollen grains, and dehisced anthers at the basal and apical parts of thecae in rice (cv. 'Hinohikari').

	Sterile flowers (%)	>80-pollen-grain flowers (%)	Pollen grain germination (%)	Basally dehisced thecae (%)	Apically dehisced thecae (%)
MeJA9 <sup>2)</sup>	49.6 a <sup>w)</sup>	41.7 a	22.8 a	19.8 a	15.3 a
MeJA10	20.4 a,b	41.7 a	13.8 a	63.1 b	57.3 b
MeJA11	8.7 b	45.8 a	22.3 a	21.8 a	21.1 a
CW9 <sup>3)</sup>	16.6 a,b	95.8 b	20.9 a	96.9 b	96.5 c
CW10	11.3 b	100.0 b	20.3 a	86.6 b	94.2 c
CW11	9.8 b	100.0 b	23.0 a	90.7 b	90.3 c
No treatment <sup>3)</sup>	5.3 b	95.8 b	30.2 a	100.0 b	99.2 c

<sup>2)</sup>MeJA9, MeJA10, and MeJA11 indicate the treatments with 4 mmol L<sup>-1</sup> methyl jasmonate solution at 0900, 1000, and 1100, respectively.

<sup>3)</sup>CW9, CW10, and CW11 indicate control-water treatments at 0900, 1000, and 1100, respectively.

<sup>3)</sup>No treatment indicates that neither methyl jasmonate solution nor control water were sprayed on rice panicles.

<sup>w)</sup>Values with the same letters were not significantly different at the level of P=0.05 with Tukey's HSD test (n= 4).

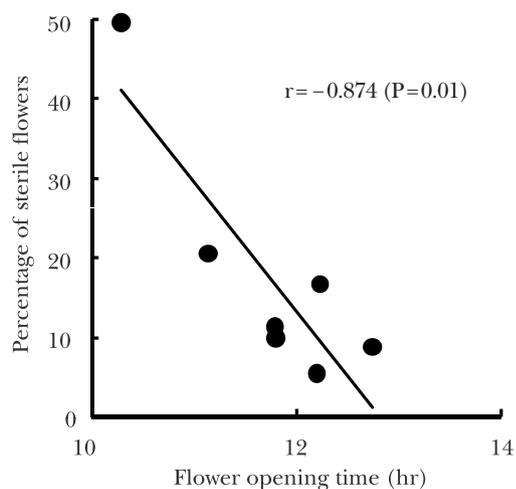


Fig. 3. Correlation of percentage of sterile flowers on the rice plants treated with methyl jasmonate or control water with flower opening time (Japan Standard Time).

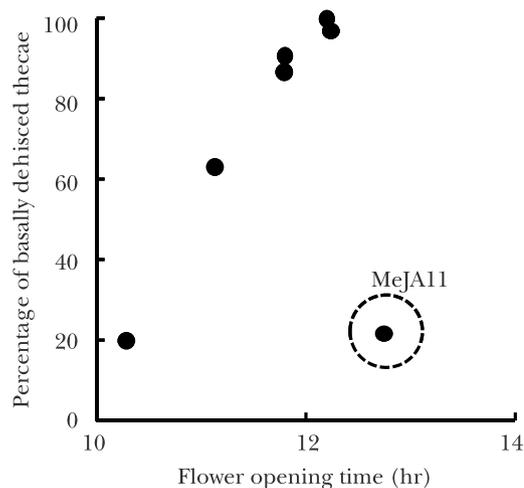


Fig. 4. Percentage of dehisced anthers at the basal part of thecae in rice plants treated with methyl jasmonate or control water at three different time points in relation to flower opening time (Japan Standard Time).

0900, 1000, and 1100 increased flower sterility slightly, though not significantly.

The percentage of >80-pollen-grain flowers was significantly decreased by applications of MeJA solution at three different time points. Applications of control water at three different time points did not affect the percentage of >80-pollen-grain flowers. Pollen germination was not affected by applications of either MeJA solution or control water.

Application of MeJA solution at 0900 and 1100 significantly decreased thecae with both basal and apical dehiscence. Application of MeJA solution at 1000 significantly decreased thecae with apical dehiscence. It also decreased thecae with basal dehiscence, but not significantly. Application of control water at either 0900, 1000, or 1100 decreased the number of apically and basally

dehisced thecae slightly, though not significantly.

#### 4. Correlation of FOT with sterility and anther dehiscence

There was a significant negative correlation ( $r=-0.874$ ,  $P=0.01$ ) between FOT and the percentage of sterile flowers (Fig. 3). The correlation between FOT and the percentage of thecae with basal dehiscence was not detected (Fig. 4). However, excluding the result of MeJA application at 1100, there was a significant positive correlation ( $r=0.988$ ,  $P<0.001$ ) between FOT and the percentage of thecae with basal dehiscence.

#### Discussion

Flowers of 'Hinohikari' treated with 4 mmol L<sup>-1</sup> MeJA

solution opened about 1 hr after the application at 0900 and 1000 (Table 1). Application of MeJA solution at 0900 advanced FOT about 2 hr compared with no application and control water application at 0900. In mid-August, the temperature at 1000, is  $>2^{\circ}\text{C}$  lower than that at 1200 in Matsue, when most of the flowers of japonica rice open under natural conditions. These results suggest that MeJA application before flower opening would allow earlier flower opening to mitigate heat-induced sterility. The flower-opening patterns after the MeJA application showed that MeJA triggered flower opening across a wide range of developmental stages and reduces the differences in FOT among flowers (Fig. 2). Zeng et al. (1999) reported that MeJA induced rice flower opening and is useful for synchronization of flower opening in cytoplasmic male sterile and restorer lines of hybrid rice.

Application of MeJA solution at 0900 increased the number of flowers opening on the day of the treatment and decreased that on the next day (Table 2). These results suggest that MeJA application induces the opening of flowers expected to open on the day after the application. Tsuboi (1961) speculated that flowers expected to open the next day but not prepared to develop mature pollen grains are induced to open by wind and other physical stimuli.

Although MeJA application at 0900 advanced FOT, it increased the percentage of sterile flowers (Table 3). MeJA application decreased the percentage of  $>80$ -pollen-grain flowers, which is used as a measure of reliability of pollination under normal conditions (Matsui et al., 2005). Such poor pollination was caused by anther indehiscence at the basal and apical parts of the thecae. However, rice plants treated with MeJA solution at 1100 had relatively high fertility, although the percentages of  $>80$ -pollen-grain flowers and of anther dehiscence at the basal and the apical thecae were low. Rice plants treated with MeJA at 1100 might be cross-pollinated because wind speed in the late morning of the treatment day was about  $5\text{ m s}^{-1}$ , and cross-pollination increases when wind speed is higher than  $2\text{--}3\text{ m s}^{-1}$  (Kato and Namai, 1987). In addition, only 3 to 4 pollen grains are sufficient for seed-set in rice (Namai and Kato, 1987).

The reason for sterility induced by MeJA application could be the opening of flowers expected to open the day after the treatment. Estimating from the data presented in Table 2, more than 40% of the flowers that were treated with MeJA and opened on the treatment day were those expected to open the next day. This value of "more than 40%" is similar to the percentage of sterile flowers on the plants treated with MeJA at 0900 (Table 3). However, flower sterility of plants treated with MeJA at 1000 and 1100 was lower possibly because their flower-opening period overlapped with those of the controls which allowed cross-pollination. On the day before flower

opening, development of the embryo sac is completed, and the egg cells become physiologically capable of being fertilized (Hoshikawa, 1989). Pollen grains, however, continue developing until flowers open and are filled with starch 1 day before flower opening. In pollen grains, starch is rapidly digested after the end of starch engorgement at the end of the grain opposite to the germ poles, 3–4 hr before anther dehiscence, and more than 70% of pollen grains become sugar-type grains by the time of anther dehiscence (Koike and Satake, 1987). This suggests that flowers expected to open the next day have egg cells physiologically capable of being fertilized and that flowers that opened after 1100 were cross-pollinated because the density of pollen grains in the air was high.

MeJA application, however, decreased the percentages of  $>80$ -pollen-grain flowers and of dehisced anthers (Table 3). In particular, the percentages of dehisced anthers at the basal and the apical parts of thecae in plants treated with MeJA at 0900 were as low as 20%. This suggests that these flowers that were expected to open on the treatment day also opened too early to develop pollen grains. Under normal conditions, during pollination, the flowers of rice, barley, and wheat normally open synchronously with anther dehiscence and filament extension, and pollen is subsequently scattered from extruded anthers (Honda et al., 2006). Pollen diameter in the flowers that were artificially opened by excising the top of the glumes before the expected natural flower-opening time increased rapidly, and the time of anther dehiscence coincided well with the time when pollen grains reached their maximum diameter (Matsui et al., 1999). The ability of pollen grains to swell rapidly in response to the artificial opening of flowers increases from 210 to 30 min before the natural flower-opening time (Matsui et al., 1999). The significant positive correlation ( $r=0.988$ ,  $P<0.001$ ; Fig. 4, excluding the MeJA application at 1100) between FOT and the percentage of thecae with basal dehiscence coincides with their result (Matsui et al., 1999) and suggests that flowers that were treated with MeJA 2–3 hr before the natural flower-opening time had a low ability to swell pollen grains. Although application of MeJA solution at 1100 delayed FOT in this experiment, it did not in preliminary experiments using 'IR72' and 'Hinohikari' (data not shown). The reason for this discrepancy is uncertain.

Recent studies using delayed dehiscence mutants (Sanders et al., 2000) clarified that JA is essential for pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. Male-sterile mutant of *Arabidopsis* can be rendered fertile by exogenous application of JA (Stintzi and Browse, 2000). From these results obtained in *Arabidopsis*, we speculate that MeJA application to rice panicles indirectly inhibits anther dehiscence because of the lack of mature pollen grains. MeJA has many functions such as induction of anther swelling and dehiscence (Zhu

et al., 2004) and flower opening through swelling lodicules (Zeng et al., 1999). The concentration of MeJA (4 mmol L<sup>-1</sup>) used in this experiment may have been so high that it induces opening of not only the flowers expected to open on the treatment day but also those expected to open on the next day. A lower concentration of MeJA such as 2 or 0.2 mmol L<sup>-1</sup> may induce flower opening only of the flowers expected to open on the treatment day but not of those expected to open on the next day. The jasmonic acid carboxyl methyltransferase enzyme converts JA to a volatile component, MeJA (Kim et al., 2009). In searching for substances that induce flower opening, only MeJA advanced FOT, and other JA substances did not (Y. Kamaro, personal communication, 2009).

In conclusion, application of MeJA solution advanced FOT but also increased flower sterility, poor pollination, and anther indehiscence. In this experiment, we could not separate the effects of MeJA on pollination and anther dehiscence of flowers expected to open on the treatment day from those expected to open the next day. Possible cross-pollination might have also affected the results of flower sterility. Although physical stimuli on flowers affect the characteristics of flowers, pollen, and anthers, plants need to be isolated to protect against cross-pollination in future experiments on FOT and MeJA. We also need to analyze data and classify the flowers expected to open on that day, taking into account the fact that the order of flower opening is roughly fixed within the panicle by the position of the flowers in rice (Hoshikawa, 1989).

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### References

- Hirano, K., Aya, K., Hobo, T., Sakakibara, H., Kojima, M., Shim, R.A., Hasegawa, Y., Ueguchi-Tanaka, M. and Matsuoka, M. 2008. Comprehensive transcriptome analysis of phytohormone biosynthesis and signaling genes in microspore/pollen and tapetum of rice. *Plant Cell Physiol.* 49: 1429-1450.
- Honda, I., Seto, H., Turuspekov, Y., Watanabe, Y. and Yoshida, S. 2006. Inhibitory effects of jasmonic acid and its analogues on barley (*Hordeum vulgare* L.) anther extrusion. *Plant Growth Regul.* 48: 201-206.
- Horie, T., Matsui, T., Nakagawa, H. and Omasa, K. 1996. Effects of elevated CO<sub>2</sub> and global climate change on rice yield in Japan. In K. Omasa, K. Kai, H. Taoda, Z. Uchijima and M. Yoshino eds., *Climate Change and Plants in East Asia*. Springer-Verlag, Tokyo. 39-56.
- Hoshikawa, K. 1989. *The Growing Rice Plant—An Anatomical Monograph*. Nosan Gyoson Bunka Kyokai, Tokyo. 1-310\*\*.
- Hung, K.T., Hsu, Y.T. and Kao, C.H. 2006. Hydrogen peroxide is involved in methyl jasmonate-induced senescence of rice leaves. *Physiol. Plant.* 127: 293-303.
- Imaki, T., Jyokei, K. and Yamada, I. 1983. Sterility caused by high temperature at flowering in rice plants. *Bull. Fac. Agric. Shimane Univ.* 17: 1-7\*.
- Imaki, T., Tokunaga, S. and Obara, N. 1987. High temperature sterility of rice spikelets at flowering in relation to flowering time. *Jpn. J. Crop Sci.* 56(Extra issue 2): 209-210\*\*.
- Jagadish, S.V.K., Craufurd, P.Q. and Wheeler, T.R. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *J. Exp. Bot.* 58: 1627-1635.
- Jagadish, S.V.K., Craufurd, P.Q. and Wheeler, T.R. 2008. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Sci.* 48: 1140-1146.
- Kato, H. and Namai, H. 1987. Floral characteristics and environmental factors for increasing natural outcrossing rate for F<sub>1</sub> hybrid seed production of rice *Oryza sativa* L. *Jpn. J. Breed.* 37: 318-330\*.
- Kim, E.H., Kim, Y.S., Park, S.H., Koo, Y.J., Choi, Y.D., Chung, Y.Y., Lee, I.J. and Kim, J.K. 2009. Methyl jasmonate reduces grain yield by mediating stress signals to alter spikelet development in rice. *Plant Physiol.* 149: 1751-1760.
- Kim, H.Y., Horie, T., Nakagawa, H. and Wada, K. 1996. Effect of elevated CO<sub>2</sub> concentration and high temperature on growth and yield of rice. II. The effect on yield and its components of Akihikari rice. *Jpn. J. Crop Sci.* 65: 644-651\*\*\*.
- Koike, S. and 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. *Jpn. J. Crop Sci.* 56: 666-672.
- Matsui, T., Kobayasi, K., Kagata, H. and Horie, T. 2005. Correlation between viability of pollination and length of basal dehiscence of the theca in rice under a hot-and-humid condition. *Plant Prod. Sci.* 8: 109-114.
- Matsui, T. and Kagata, H. 2003. Characteristics of floral organs related to reliable self-pollination in rice (*Oryza sativa* L.). *Ann. Bot.* 91: 473-477.
- Matsui, T., Omasa, K. and Horie, T. 1999. Rapid swelling of pollen grains in response to floret opening unfolds anther locules in rice (*Oryza sativa* L.). *Plant Prod. Sci.* 2: 196-199.
- Nakagawa, H., Horie, T. and Matsui, T. 2003. Effects of climate change on rice production and adaptive technologies. In T.W. Mew, D.S. Brar, S. Peng, D. Dawe and B. Hardy eds., *Rice Science: Innovations and Impact for Livelihood*. International Rice Research Institute, Laguna, the Philippines. 635-658.
- Nakagawa, H. and Nagata, A. 2007. Internal and environmental factors affecting the time of flower-opening in rice. *Jpn. J. Crop Sci.* 76(Extra issue 2): 280-281\*\*.
- Namai, H. and Kato, H. 1987. The number of pollen grains deposited upon a pistil assuring seed setting of male sterile seed parent in rice (*Oryza sativa* L.) *Jpn. J. Breed.* 37: 98-102.
- Nishiyama, I. and Blanco, L. 1980. Avoidance of high temperature sterility by flower opening in the early morning. *Jpn. Agric. Res. Quart.* 14: 116-117.
- Nishiyama, I. and Blanco, L. 1981. Artificial control of flower opening time during the day in rice plants. I. Preliminary experiments. *Jpn. J. Crop Sci.* 50: 59-66.
- Nishiyama, I. and Satake, T. 1981. High temperature damages in rice plants. *Jpn. J. Trop. Agric.* 25: 14-19\*\*.

- Sanders, P.M., Lee, P.Y., Biesgen, C., Boone, J.D., Beals, T.P., Weiler, E.W. and Goldberg, R.B. 2000. The *Arabidopsis* *DELAYED DEHISCENCE 1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *Plant Cell* 12: 1041-1061.
- Satake, T. 1972. Circular dense-culture of rice plants in pots, the purpose of obtaining many uniform panicles of main stems. *Proc. Crop Sci. Soc. Jpn.* 41: 361-362\*\*.
- Satake, T. and Yoshida, S. 1978. High temperature-induced sterility in indica rices at flowering. *Jpn. J. Crop Sci.* 47: 6-17.
- Satake, T. 1995. High temperature injury. In T. Matsuo eds., Science of the Rice Plant Vol. 2. Physiology. Food and Agriculture Policy Research Center, Tokyo. 805-812.
- Song, P., Xia, K., Wu, C.W., Bao, D.P., Chen, L.L., Zhou, X. and Cao, X.Z. 2001. Differential response of floret opening in male-sterile and male-fertile rices to methyl jasmonate. *Acta Bot. Sin.* 43: 480-485\*\*\*\*.
- Stintzi, A. and Browse, J. 2000. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 97: 10625-10630.
- Tsuboi, Y. 1961. Ecological studies on rice plants with regard to damages caused by wind. *Bull. Natl. Inst. Agric. Sci.* A 8: 1-156\*.
- Wang, C., Yang, J., Wa, J. and Cai, Q. 2004. Influence of high and low temperature stress on fertility and yield of rice (*Oryza sativa* L.): Case study with the Yangtze River rice cropping region in China. Abstract, World Rice Research Conference 2004, Tsukuba, Japan. 97.
- Wang, Z., Gu, Y.J. and Gao, Y.Z. 1989. Studies on the mechanism of rice glume-opening: I Effect of CO<sub>2</sub> on glume-opening. *Acta Agron. Sin.* 15: 59-66\*\*\*\*.
- Zeng, X.C., Zhou, X., Zhang, W., Murofushi, N., Kitahara, T. and Kamuro, Y. 1999. Opening of rice floret in rapid response to methyl jasmonate. *J. Plant Growth Regul.* 18: 153-158.
- Zhu, Q.H., Ramm, K., Shivakkumar, R., Dennis, E.S. and Upadhyaya, N.M. 2004. The *ANTHER INDEHISCENCE1* gene encoding a single MYB domain protein is involved in anther development in rice. *Plant Physiol.* 135: 1514-1525.

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\* In Japanese with English summary.

\*\* In Japanese.

\*\*\* In Japanese with English abstract.

\*\*\*\* In Chinese with English abstract.