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Opening of Rice Floret in Rapid Response to Methyl Jasmonate

X.-C. Zeng,[1,2] X. Zhou,[1,*] W. Zhang,[3] N. Murofushi,[4] T. Kitahara,[5] and Y. Kamuro[6]

[1]Agronomy Department, Nanjing Agricultural University, Nanjing 210095, China; [2]Agronomy Department, Jiangxi Agricultural University, Nanchang 330045, China; [3]Wuxian Agricultural Institute, Suzhou 215128, China; [4] Department of Applied Biological Chemistry, Akita Prefectural University, Japan; [5]Department of Applied Biological Chemistry, The University of Tokyo, Japan; and [6]BAL Planning Co. Ltd., Japan

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Abstract. Effects of methyl jasmonate (MeJA) on rice floret opening were investigated in seven cultivars or hybrid combinations covering various variety types. Intact or excised panicles, judged to have florets just before anthesis, were soaked in $4 \times 10^{-5} - 4 \times 10^{-3}$M MeJA solutions for 2 min at different temperatures. The results indicated that MeJA significantly induced opening of rice florets within about 30 min, with the most rapid induction occurring just 6 min after treatment. Numbers of induced opening florets are correlated with MeJA concentrations. Higher concentrations of MeJA induced more florets. pH values had no influence on MeJA effect, but MeJA required less time and induced more florets at 34°C than at 25°C. As far as we know, this is the first evidence that floret opening is induced by plant hormones. CO_2 evolution from panicles was also increased by MeJA treatment. Field experiments revealed that perfect flowering synchrony between the cytoplasmic male sterile (CMS) and restorer lines in hybrid seed production could be obtained by spraying MeJA solution on CMS line plants at the rate of 25 mg/m^2. As a result, many more hybrid seeds were harvested.

Key Words. *Oryza sativa* L—MeJA—Floret opening—Lodicule—Rice flowering—Hybrid seed production

Rice flowering refers to a series of events between the opening and closing of the floret and takes 6–10 days for the tens or hundreds of florets within the same panicle to complete flowering (Yoshida 1981). Rice flowering occurs at a certain time of the day. This time, termed flowering time, varies with varieties and weather conditions, and its regulation is vitally important in hybrid rice seed production. It has been suggested that the two lodicules become turgid at flowering time and force open the lemma and palea (Steward 1958). Lodicule swelling is promoted by high temperature (35–45°C) (Wang et al. 1988) and can be induced by carbon dioxide (Wang et al. 1989). However, little is known about the mechanism underlying this lodicule swelling, especially the function of plant hormones in this process. Many kinds of plant hormones, such as indole-3-acetic acid (IAA), gibberellin (GA), abscisic acid (ABA), and cytokinin (CTK), showed no effect on floret opening, but some novel plant hormones have not been tested yet. Jasmonate (JA) and its methyl ester, methyl jasmonate (MeJA) belong to a group of novel plant hormones called jasmonates (jasmonate and its derivatives) (Parthier 1991; Staswick 1995). JA and MeJA play remarkable roles in the regulation of plant morphogenesis, such as the induction of tuberization in potato (*Solanum tuberosum*) (Koda et al. 1991; Pelacho and Mingo-Castel 1991) and yam plant (*Dioscorea* spp) (Koda and Kikuta 1991), the stimulation of bulb formation in garlic (*Allium sativum*) (Ravnikar et al. 1993), and coil-induction of *Bryonia dioica* tendrils (Falkenstein et al. 1991; Weiler et al. 1993). The mechanism underlying these morphologic effects is thought to be that of JA and MeJA stimulation of the expansion of some specific cells (Abe et al. 1990; Matsuki et al. 1992; Takahashi et al. 1994, 1995). These results suggest that, in contrast to GA and IAA, which promote cell elongation, MeJA stimulates the expansion of plant cells. MeJA can also increase the respiration of plants (Cao et al. 1998). Based on this, we predicted that MeJA might cause swelling of lodicules and, consequently, the opening of florets. To test this idea, we investigated the effect of MeJA on the induction of floret opening in rice.

Abbreviations: ABA, abscisic acid; CMS, cytoplasmic male sterile; CTK, cytokinin; GA, gibberellin; IAA, indole-3-acetic acid; JA, jasmonate; MeJA, methyl jasmonate; PAR, photosynthesis active radiation.

*Author for correspondence: E-mail: zhish@nau.njau.edu.cn

Materials and Methods

Plant Materials and Reagents

Experimental cultivars or hybrid combinations included HanDao297 (hd297), Anxiang$_s$ × Zhu173 (az173, F_1 hybrid), XingLuA (xla, CMS line), HX-3 (hx-3) (*Oryza sativa* subsp. *Indica*), HanDao44 (hd44), AiA (aa, CMS line), and D15 (d15) (*Oryza sativa* subsp. *japonica*). MeJA (Wako Pure Chemical Industries. Ltd., Japan) was dissolved in a small amount of ethanol and then diluted to the desired concentrations. Control water received the same amount of ethanol alone. When needed, MeJA solutions were adjusted to desired pH values with 0.1 N HCl or NaOH. In this case, controls with corresponding pHs were included. Unless otherwise indicated, pH values of MeJA solutions were 6.0.

Experiments Using Panicles

Panicles judged to have florets nearing anthesis, in which several florets had flowered before the experiment date, were selected for tests. Experiments were conducted around 8 AM. Intact or excised panicles were carefully immersed in MeJA or control solution for 2 min. The attached culms of excised panicles were then mounted to 100-mL flasks containing tap water. For each panicle, the time lag between treatment and the occurrence of the first opening floret was carefully monitored, and the numbers of opening florets were recorded at fixed intervals. Photographs were taken to display the results. During the experiments, shaking or any form of contact stimuli was limited.

CO_2 Measurements

CO_2 evolution from panicles was measured with an LI-6200 portable photosynthesis system at 30°C, under light intensity of 280 μmolE/m^{2}s photosynthesis active radiation (PAR). For each measurement, two panicles were enclosed in a 1-L airtight leaf chamber, with their attached culm submerged in tap water in a 100-mL flask. CO_2 concentrations in the leaf chamber were monitored at 3-min intervals, and the accumulated CO_2 per gram panicle was calculated.

MeJA Application to Male Sterile Line in Hybrid Seed Production

In the field for hybrid seed production, two rows of restorer line (d15) and 10 rows of male sterile line (aa) plants were alternately arranged. Six 2-m^2 plots of male sterile line were designed, three for MeJA treatment, and the other three for control. Spraying of MeJA solution on male sterile line (aa) was done at 9:30 AM and 10 panicles from each plot were numbered; the numbers of opening florets were recorded at 30-min intervals. Numbers of opening florets from 10 panicles from the restorer line selected by the same criteria as the sterile line were recorded simultaneously. At harvest time, 10 hills of rice plants from each plot were randomly sampled, and their filled-spikelet percentage was surveyed.

Results and Discussion

Effect of MeJA on Rice Floret Opening

A cohort of florets in excised panicles from all 7 tested cultivars responded to the dip treatment of MeJA (1–4 mM). Each treated panicle had 12–54 opening florets within 80 min compared with the control where no opening florets could be found (Fig 1*a*). A cytoplasmic male sterile (CMS) line of Subsp. japonica, aa , was chosen for further analysis. The F_1 progeny of aa × d15 displayed a strong heterosis; however, the aa often showed a cleistogamous characteristic caused by the weak function of lodicules and could not be pollinated. To improve the lodicule expansion and induce the chasmogamy in aa, MeJA treatment was carried out. As shown in Fig. 1*b*, the critical role of MeJA in promoting floret opening in a male sterile line of rice is apparent. The cultivars used in these experiments represented various types of rice, including indica and japonica rice, upland and paddy

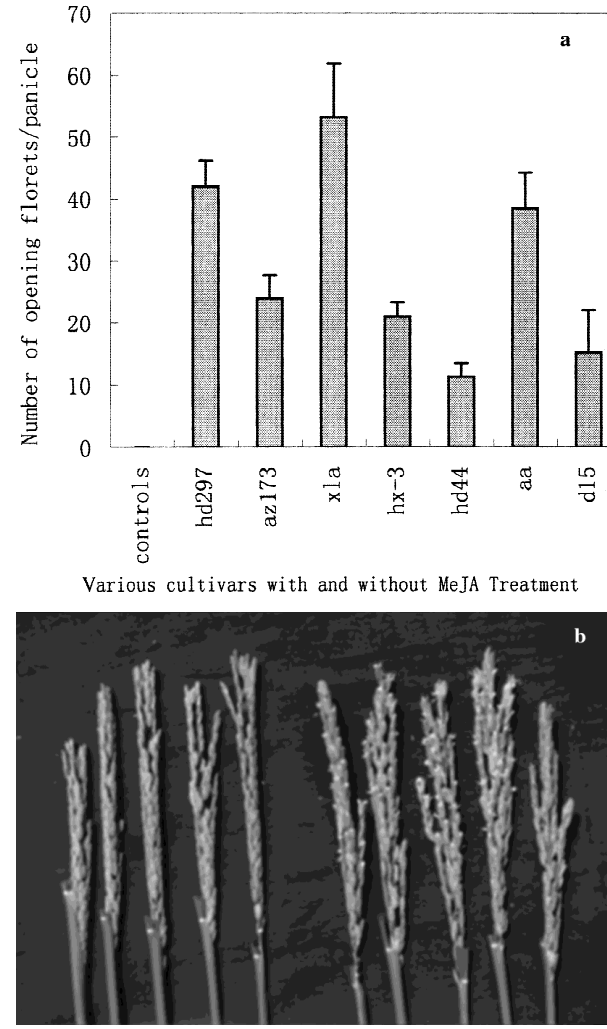

Fig. 1. Effect of MeJA on the induction of floret opening in rice. (*a)* Each data point represents the mean (with S.D.) of at least three experiments run in 10 replicates. Panicles taken from each cultivar (from left to right) were treated with 4, 4, 1, 4, 2, 4, and 4 mM MeJA, at 32, 32, 34, 26, 31, 34, and 26°C, respectively. (*b)* Left to right panicles from cv. aa dipped with MeJA solution at concentration of 0 and 4 mM respectively, taken at 80 min after the treatment.

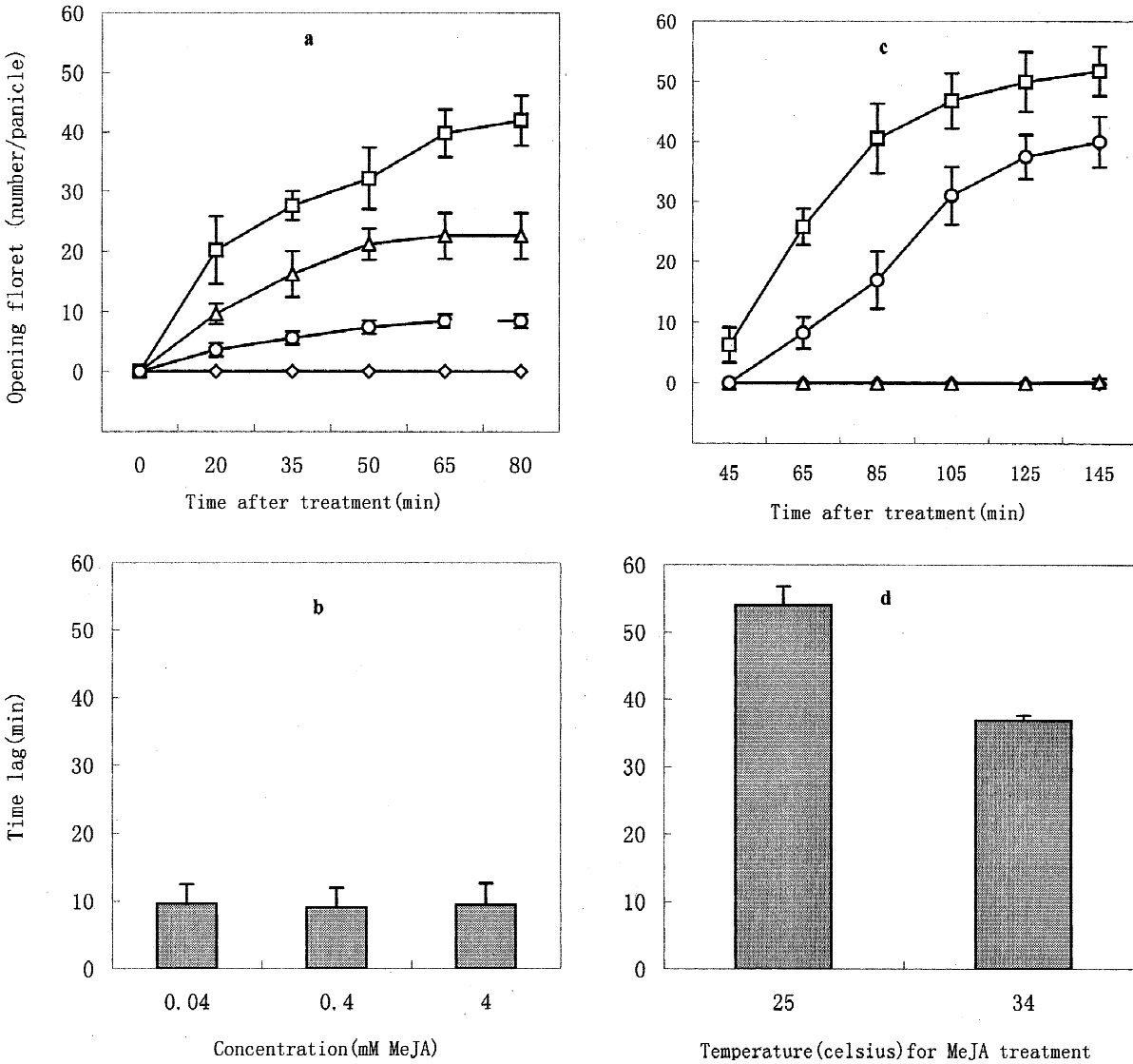

Fig. 2. Impact of concentration and temperature on MeJA action. Each data point represents the mean (with S.D.) of 10 replicates. (*a* and *c*) Time course of MeJA on floret opening. (*a)* Concentrations of MeJA were 0 (diamonds), 0.04 (circles), 0.4 (triangles), and 4 (squares) mM. (*c)* Diamonds denote control at 25°C; triangles control at 34°C, circles MeJA at 25°C, and squares MeJA at 34°C. (*b* and *d)* Time lag between MeJA treatment and occurrence of the first opening floret at various concentrations of MeJA (*b*) or at different temperatures (*d*). (*a* and *b*) Cultivar was hd297, temperature was 32°C. (*c* and *d)* Cultivar was aa, MeJA concentration was 4 mM.

rice, self-pollinated line and F_1 hybrid, and fertile and CMS lines. Therefore, we are confident in concluding that MeJA induces floret opening in rice.

Impact of Concentration and Temperature on MeJA Action

Furthermore, we tested the impact of concentration and temperature on the effect of MeJA on floret opening. The effect, in terms of number of opening florets per panicle, was concentration dependent in cv. Hd297 (Fig. 2*a*). This number increased slowly at the lower concentration (0.04 mM) of MeJA, and stopped increasing 65 min after treatment; at high concentrations (4 mM), the effect increased rapidly and was still increasing 80 min after treatment; the medium concentration (0.4 mM) effect was in between. However, in terms of the time lag between treatment and the occurrence of the first floret opening, there was almost no difference in effect among the three concentrations (Fig. 2*b*). These results imply that a panicle bears florets with different sensitivities to MeJA. Some of them are highly sensitive and respond to low concentrations of MeJA after only a short time. Others are less sensitive and require higher concentrations of

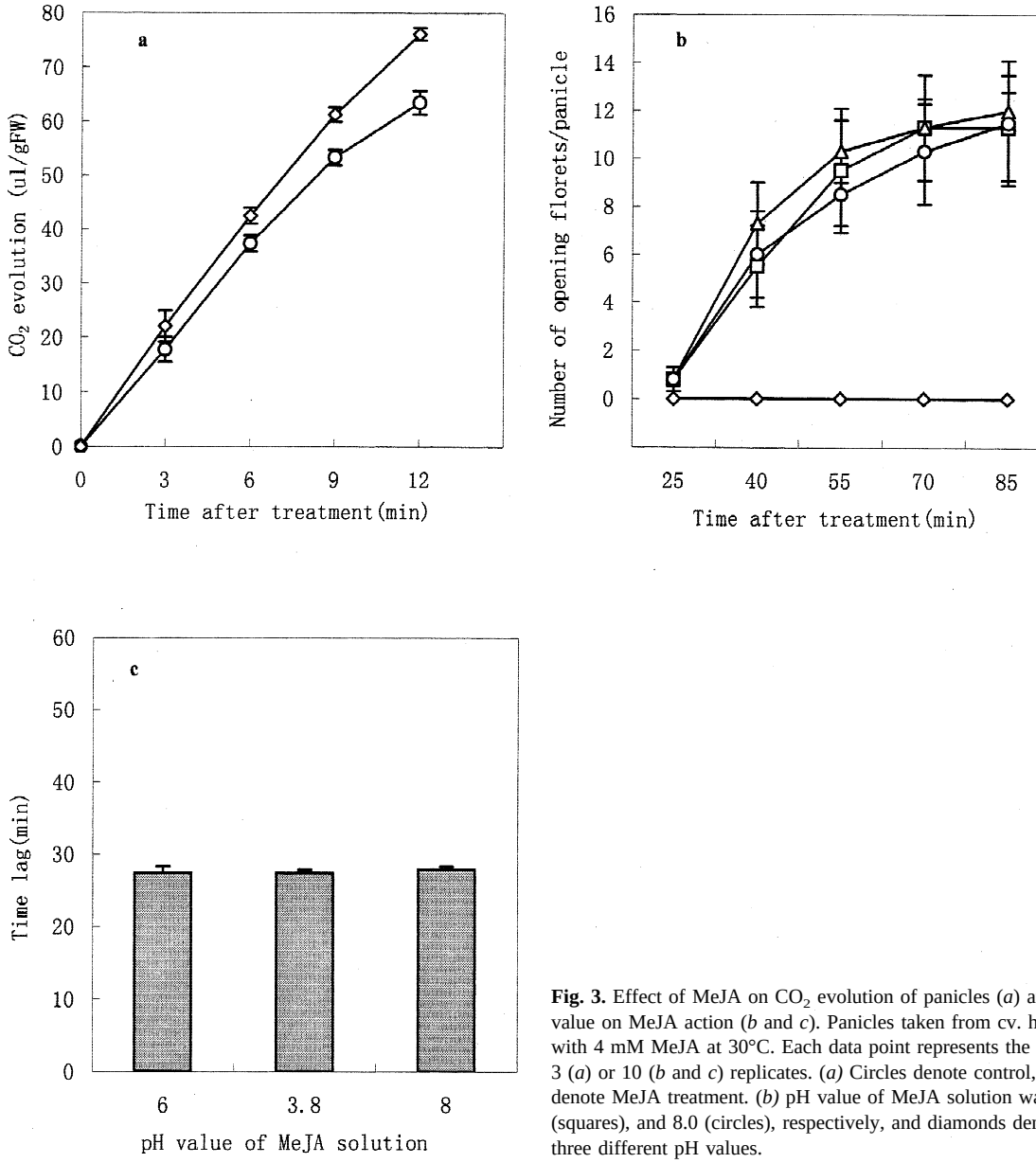

Fig. 3. Effect of MeJA on CO_2 evolution of panicles (*a*) and the impact of pH value on MeJA action (*b* and *c*). Panicles taken from cv. hd297 were treated with 4 mM MeJA at 30°C. Each data point represents the mean (with S.D.) of 3 (*a*) or 10 (*b* and *c*) replicates. (*a)* Circles denote control, and diamonds denote MeJA treatment. (*b)* pH value of MeJA solution was 3.8 (triangles), 6.0 (squares), and 8.0 (circles), respectively, and diamonds denote controls at the three different pH values.

MeJA and a longer response time. Temperature had a remarkable impact on the inductive action of MeJA on floret opening in cv. aa (Fig. 2*c*,*d*). When panicles were treated with 4 mM MeJA at 25°C, the average time lag and final number of opening florets per panicle was 54.0 min and 40.0 florets, respectively, whereas at 34°C, they were 36.8 min and 51.8 florets, indicating that MeJA is more effective at higher temperatures than at lower temperatures in the induction of floret opening.

Effect of MeJA on CO_2 Evolution, Impact of pH Value on MeJA Action

CO_2 promotes floret opening in rice, and it has been hypothesized that the mechanism underlying this promotion is that CO_2 causes "acid growth" and consequently the swelling of the lodicules (Wang et al. 1989). It has also been demonstrated that many kinds of acids promote opening of florets in rice (Xu et al. 1998). The promotion effects of MeJA on respiration and CO_2 evolution (Miszczak et al. 1995; Saniewski et al. 1998) have been well documented. To explain the mechanism underlying the inductive action of MeJA on floret opening, we tested whether MeJA might increase CO_2 evolution from panicles. Our results indicated that accumulated CO_2 from panicles in cv. hd297 was increased by 20% 12 min after treatment with 4 mM MeJA (Fig. 3*a*). However, this increase does not adequately account for the flowering induction because the CO_2 concentration required for flowering induction is at least 5% (Wang et al. 1988, 1989). Furthermore, the pH value of the MeJA solution

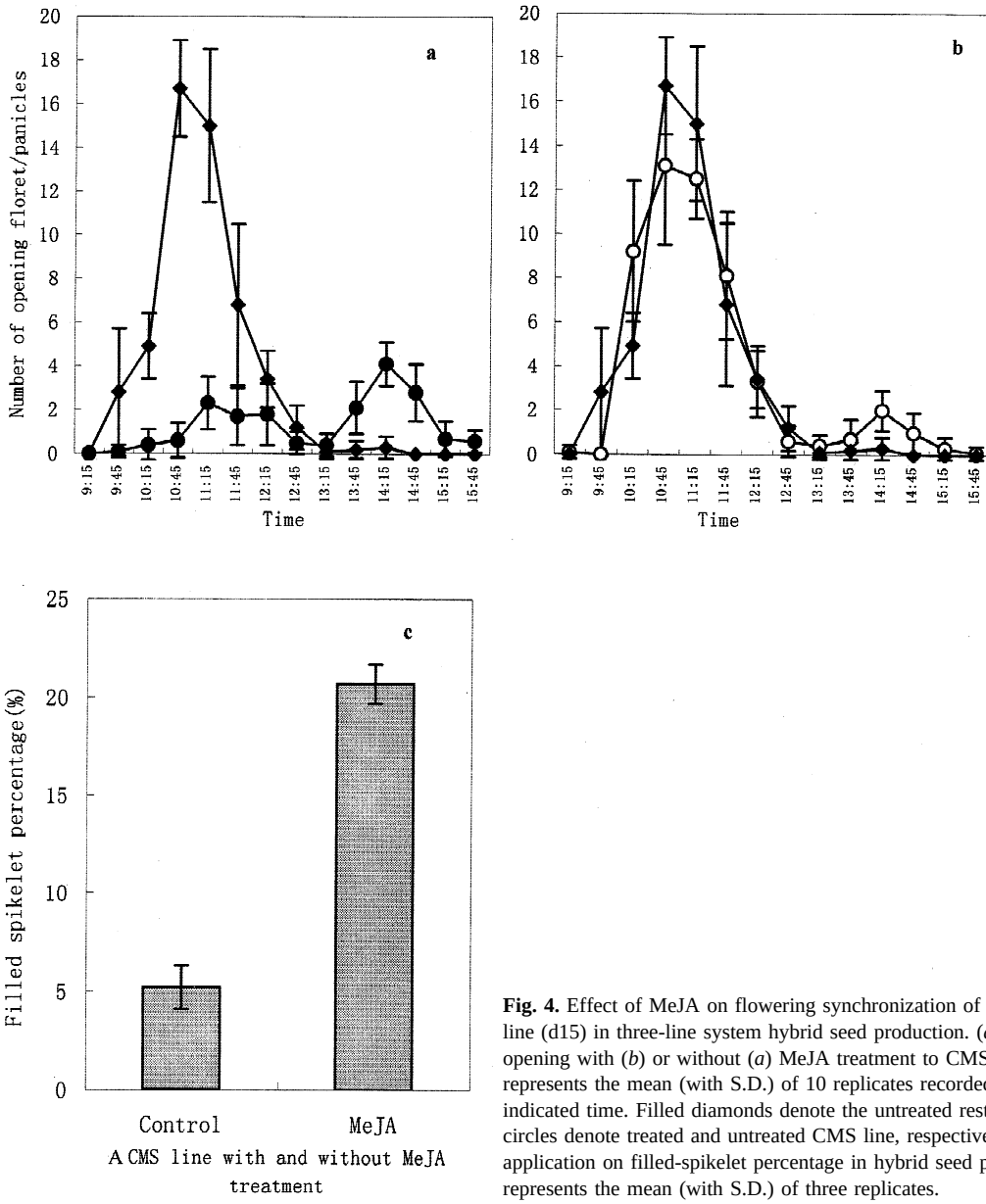

Fig. 4. Effect of MeJA on flowering synchronization of CMS line (aa) and restorer line (d15) in three-line system hybrid seed production. (*a* and *b*) Time course of floret opening with (*b*) or without (*a*) MeJA treatment to CMS line. Each data point represents the mean (with S.D.) of 10 replicates recorded 15 min before and after each indicated time. Filled diamonds denote the untreated restorer line; open and filled circles denote treated and untreated CMS line, respectively. (*c)* Effect of MeJA application on filled-spikelet percentage in hybrid seed production. Each data point represents the mean (with S.D.) of three replicates.

had no effect on MeJA action in flowering induction both in terms of the number of opening florets per panicle and the time lag (Fig. 3*b*,*c*). These results suggest that promotion of CO_2 evolution could not completely account for the mechanism underlying the flowering induction of MeJA. In other aspects, floret opening is caused by the lodicule expansion driven by the influx of water associated with the accumulation of K^+ in specialized distensible cells of the basal cushion of lodicules (Heslop-Harrison and Heslop-Harrison, 1996). K^+ is thought to be a sensitive osmotic mediator. It decreases water potential and drives cell expansion. Therefore, an alternative explanation for this MeJA action is that MeJA induced lodicule expansion by enhancing K^+ accumulation. It has been found that MeJA promotes cell expansion in tobacco and potato (Abe et al. 1990; Takahashi et al. 1995), but the response was not as rapid as in lodicule expansion. This implies mechanisms underlying MeJA action in various plant systems might be different. It is likely that MeJA-induced cell expansion in lodicules is caused by osmotic regulation, whereas in other plant systems such as tobacco and potato, some other processes, such as microtubule reorientation or disruption, might be involved.

Effect of MeJA on Synchrony of Flowering Time in Hybrid Rice Seed Production

Finally, we investigated the field application of MeJA for the seed production of hybrid rice. The hybrid, aa × d15, possesses very strong heterosis in yield (11,100 kg/ha).

But its hybrid seed production has been limited because of lack of coordinated flowering times between the two parents caused by the weak and scattered flowering characteristic of cv. aa, the CMS line (Fig. 4*a*). As shown in Fig. 4*a*, flowering in the restorer line (d15) was concentrated in the period from 10:15 to 12:15, but only a few florets opened in the CMS line (aa) during that same period. This problem was eliminated by spraying a MeJA solution (at a dosage of 25 mg/m^2) on the CMS line (aa)(Fig. 4*b*). As shown in Fig. 4*b*, the number of opening florets in the MeJA-treated CMS line almost doubled and the flowering course of both parents matched perfectly. As a result, the percentage of filled spikelets of the female parent increased about 400% (Fig. 4*c*).

Here, we have demonstrated that exogenous MeJA induced floret opening in rice. This is, to our knowledge, the first evidence that floret opening is induced by plant hormones. However, we still do not know whether endogenous MeJA functions as an inducer of floret opening, and further investigations are needed to test whether MeJA also elicits floret opening in other gramineous plants. Our results also indicated that MeJA could regulate the flowering time of the male sterile line, synchronizing flowering of the two parents of a hybrid combination. This provides a promising method for flowering synchrony in seed production of hybrid rice. Further study on the effects of JA and its other analogs are needed to make practical use of this MeJA function. Furthermore, this rapid and remarkable reaction would also offer a good experimental system for the signal transduction of MeJA.

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