

The relationship between ethylene biosynthesis and chilling tolerance in seedlings of rice (*Oryza sativa* L.)

Chun Chu and Tse-Min Lee

Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China

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Abstract. The level of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene production rate in rice seedlings was low under chilling stress (4°C). 1-(Malonylamino)cyclopropane-1-carboxylic acid (MACC) content in rice seedlings remained unchanged within 24 h at 4°C. After that, the MACC content of the chilling-sensitive cultivar (Taichung Native 1) decreased to 50% of the controls and that of the cold-tolerant cultivar (Tainung 67) decreased to 80% of the controls. Most of the decrease in MACC content occurred in root tissue. We also found that the MACC content in roots was higher than that in shoots, but the ACC content in roots was lower than that in shoots. The ACC content and ethylene production rate increased rapidly only after transfer to warmer temperature (25°C), and the ACC content reached the maximum at 9 h and ethylene production rate at 12 h. After warming, the chilled seedlings of the cold-tolerant cultivar showed a large increase in ACC and ethylene productions. Seedlings of chilling-sensitive cultivar also showed a large increase in ACC but little ethylene production after 2 days or longer chilling treatment. During the warming period, ACC content in shoots and roots increased in a similar trend. However, shoots showed a larger accumulation of ACC content than roots. The ACC-induced ethylene production of the chilling-sensitive cultivar decreased after 4 days of chilling treatment but that of the cold-tolerant cultivar remained unchanged even after 6 days of chilling treatment. These results indicated that the synthesis of ACC in two cultivars was enhanced by chilling stress, whereas the system of converting ACC to ethylene in the chilling-sensitive rice cultivar was easily damaged by exposing to longer chilling temperature.

Key words: 1-Aminocyclopropane-1-carboxylic acid; 1-(Malonylamino)cyclopropane-1-carboxylic acid; Chilling stress; Cold tolerance; Ethylene; Rice (*Oryza sativa* L.); Seedlings.

Introduction

Ethylene is an endogenous plant hormone which plays important roles in plant growth and development such as seed germination, fruit ripening, senescence, and extension growth (Abeles, 1985). It is also well

known that ethylene production is one of the stress responses. Stresses such as chilling stress, water-deficit, and mechanical wounding can stimulate ethylene production (Abeles, 1972). In higher plants, ethylene is known to be synthesized via the following biochemical sequences: methionine → SAM¹ → ACC → ethylene (Adams and Yang, 1979). The conversion of SAM to ACC is the limiting step of ethylene biosynthesis (Yang and Hoffman, 1984). ACC synthase and EFE¹ are two key enzymes in the regulation of ethylene biosynthesis. It has been reported that ACC synthase, which catalyzes the conversion of SAM to ACC (Boller *et al.*,

¹ **Abbreviations:** ACC, 1-aminocyclopropane-1-carboxylic acid; MACC, 1-(malonylamino)cyclopropane-1-carboxylic acid; EFE, ethylene-forming enzyme; SAM, S-adenosylmethionine.

1979; Yu *et al.*, 1979), and EFE, which mediates ACC to ethylene, are susceptible to environmental and chemical manipulations. Chilling can cause various physiological and biochemical modifications in chilling-sensitive plants (Lyons, 1973; Wang, 1982). One of them is the stimulation of ethylene production (Cooper *et al.*, 1969; Etani and Yoshida, 1987; Field, 1981, 1984; Vine *et al.*, 1968; Wang, 1987; Wang and Adams, 1980, 1982; Wang *et al.*, 1971; Wright, 1974). Wang and Adams (1982) suggested that the increase of ACC synthase activity and ACC content during warming periods after chilling are responsible for high ethylene evolution.

ACC content and ethylene production have been suggested to serve as an index of chilling sensitivity (Wang and Adams, 1982; Chen and Patterson, 1985). Guye *et al.* (1987) recently showed that genotypic differences in chilling tolerance/sensitivity also appear to be reflected by the level of chilling-induced ethylene production, and relatively high level of ethylene production generally indicates a high degree of chill-tolerance in *Phaseolus* spp.

MACC, the major metabolite and an end product of ACC (Amrhein *et al.*, 1981; Hoffman *et al.*, 1983), was found to be accumulated during high rate of ACC synthesis induced by water-deficit (Hoffman *et al.*, 1983) or by other factors (Amrhein *et al.*, 1982; Fuhrer and Fuhrer-Fries, 1985; Satoh and Esashi, 1984; VanLoon and Fontaine, 1984). Because MACC is a stable product of ACC metabolism, Hoffman *et al.* (1983) demonstrated that MACC level is related to the prehistory of water stress status. The effect of chilling stress on the change of MACC content has not been studied yet.

Chilling injury of rice seedlings in paddy field, especially the sensitive *indica* type, may frequently occur in the first crop season during the coldest months of January and February in the northern and central parts of Taiwan. The present study was carried out to investigate the effect of chilling stress on ethylene production and ACC level in the seedlings of chilling-sensitive and cold tolerant rice cultivars. Changes of MACC content under chilling treatment was also examined.

Materials and Methods

Plant Materials

The rice cultivars included in this study were cv.

Tainung 67 (*japonica* type) and cv. Taichung Native 1 (*indica* type). After sterilizing with 5% sodium hypochlorite for 10 min, seeds of rice were soaked in tap water for 2 days at 37°C and then cultivated in a 500-ml beaker containing half-strength Kimura (Yoshida *et al.*, 1976) B nutrient solution as described previously (Chu *et al.*, 1986). The solution was changed once in 4 days. Seedlings were grown in a glassroom of phytotron with natural light at 30°C day/25°C night and 90% relative humidity. Three days before chilling treatment, 11 day-old seedlings were transferred to a 25°C growth chamber under a photosynthetic photon flux density (PPF) of 500 $\mu\text{moles m}^{-2} \text{s}^{-1}$ provided by a combination of cool white fluorescent tubes and incandescent lamps. The photoperiod was 12 h. The 14 day-old seedlings were used for experiments.

Chilling Treatments

Intact seedlings were chilled at 4°C with a PPF of 500 $\mu\text{mole m}^{-2} \text{s}^{-1}$ for various periods. The photoperiod was 12 h. During chilling, relative humidity was kept about 75%–85%. After various days of chilling, seedlings were transferred to 25°C for post-chilling warming treatment. Ten seedlings were used for each sampling and the data presented were averages of 4 replicates.

Rating of Seedling Damage

Seedling damage was evaluated after seven days in 25°C mainly according to the index for cold tolerance of IRRI (1980): 1. seedlings dark green, 3. seedlings light green, 5. seedlings yellow with brown tip, 7. seedlings yellow but have new leaf growing, 9. seedlings brown and dead.

Measurement of Electrolyte Leakage

After chilling treatment, ten whole rice seedlings were immersed in 20 ml of 0.2 M mannitol solution in a container of 11 cm in diameter, and kept in darkness for 24 h at 25°C. The amounts of electrolyte leached into the solution were measured with a conductivity meter (TOA-Electronics, CM-24).

Determination of Ethylene

Ethylene production by seedlings was determined during 4°C treatment or after transfer from 4°C to 25°C. Ten seedlings were placed in a 78-ml glass cylinder (2.3 cm diameter \times 19 cm height) and sealed

with a silicone rubber cap. After incubation for 90 min under $168 \mu\text{mol m}^{-2} \text{s}^{-1}$. One ml of gas samples was withdrawn from the flask through the silicone rubber cap and then analyzed for ethylene at 80°C by a gas chromatograph (Carle Struments, CA, USA) equipped with a flame ionization detector, and fitted with an aluminum column, Porapak QS (6 ft* 1/BIN, 80/100 mesh). The ACC-induced ethylene production was determined after incubation for 60 min.

Determination of ACC and MACC

About 1.5 g shoots or roots were frozen in liquid nitrogen, then extracted with 10 ml of 80% ethanol at -20°C over night. The ethanol extracts were boiled in $80-85^\circ\text{C}$ water bath and reextracted with 10 ml 80% ethanol. The extracts were combined together, and then concentrated to dryness with a Savant speed vac concentrator (Savant Instruments, Fatmingdale, NY, USA). The residues was dissolved in 2 ml distilled water and chloroform (0.5 ml /1 g) was added to remove the pigments. The subsequent assay procedure was modified from the method of Lizada and Yang (1979). In a 12 ml test tube, 0.2 ml extract and 0.1 ml of $10 \mu\text{moles HgCl}_2$ were added. The solution was brought to 0.9 ml with dist. water and sealed with an injection cap. 0.2 ml of cold oxidizing agent (5.25% NaOCl : saturated NaOH = 2 : 1, v/v) was injected into the test tube. After shaking, 1 ml gas sample was withdrawn for ethylene measurement. The method was standardized with pure ACC (internal standard).

Quantification of MACC in 1 ml ACC extract was carried out by hydrolyzing in 2N HCl at 100°C for 6h as described by Hoffman *et al.* (1982). After hydrolyzing, the solution was neutralized with saturated NaOH in a total volume of 3 ml, and then centrifuged for 10 min at 10,000 g. The ACC content of hydrolyzed extract was assayed as described above. The difference in ACC content after and before HCl-hydrolysis was taken as the amount of MACC. ACC and MACC were expressed as nmoles per g fresh weight.

Results

Effects of Chilling on the Growth of Rice Seedlings

Intact rice seedlings cultivated with Kimura (Yoshida *et al.*, 1976) B nutrient solution were used for all experiments. The degree of chilling injury in rice seedlings was evaluated by the score of leaf damage (IRRI,

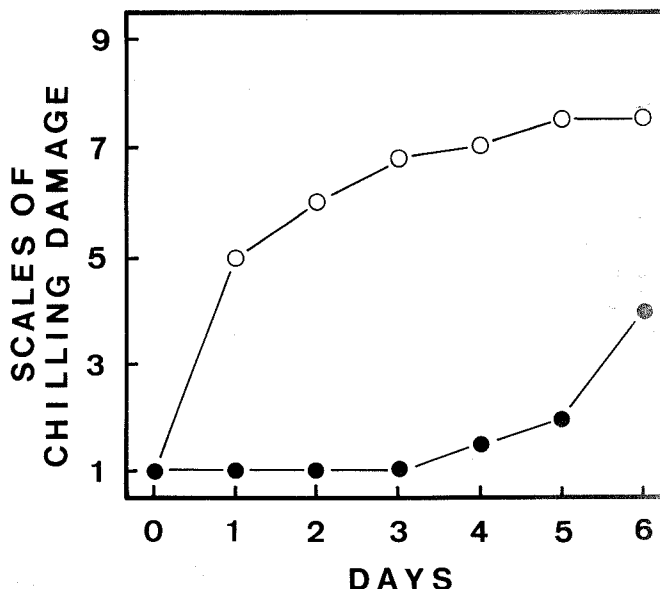


Fig. 1. The degree of chilling damage in the seedlings of two rice (*Oryza sativa* L.) cultivars. ○, Taichung Native 1. ●, Tainung 67. See materials and methods for the scale of chilling damage.

1980) as described in materials and methods. The results were presented in Fig. 1. The degree of chilling damage in Tainung 67 (*japonica* type) remained low even after 3 days at 4°C , and after that, elevated gradually to score of 5 of leaf damage. On the contrary, Taichung Native 1 (*indica* type) showed a rapid increase in the scale of leaf damage up to 5 after 1 day at 4°C . The results suggested that the chilling-sensitive *indica* rice seedlings suffered more serious damage than the cold-tolerant *japonica* cultivar under chilling stress. Sato and Park (1982) showed that electrolyte leakage of *japonica* rice plants was lower under chilling than that of *indica* rice plants. Our results also showed that the *japonica* rice seedlings (Tainung 67) had low electrolyte leakage than the *indica* rice (Taichung Native 1) (Fig. 2). The electrical conductivity of leakage in *indica* rice seedlings increased rapidly with the time of cold exposure, whereas an obvious increase in *japonica* rice seedlings occurred after 3 days at 4°C (Fig. 2). In the present study, the results showed that the changes in electrolyte leakage in the two types of rice cultivars were parallel to the degree of chilling damage.

Changes of Ethylene Production and ACC and MACC Contents in Rice Seedlings under Chilling Treatment

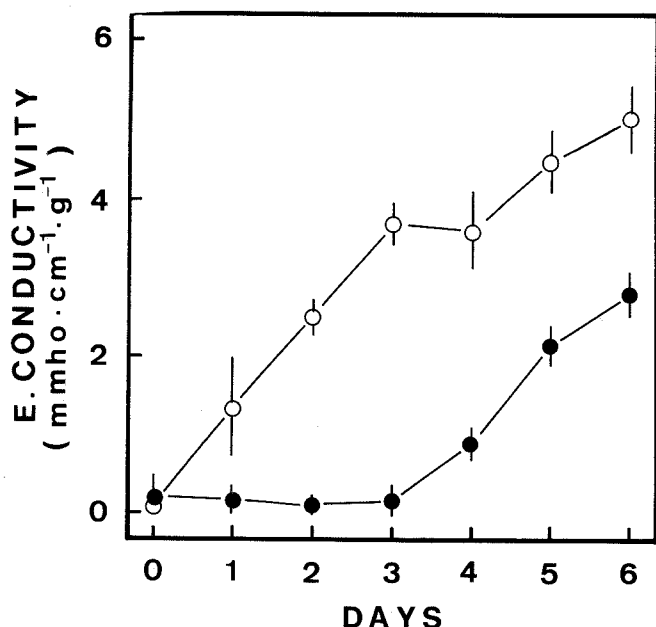


Fig. 2. Changes of electric conductivity of rice (*Oryza sativa* L.) seedlings during chilling (4°C). Bars indicated the standard deviation. ○, Taichung Native 1. ●, Tainung 67.

After transfer from 25°C to 4°C, both the cold-tolerant and chilling-sensitive cultivars showed a decrease in ACC level and ethylene production rate (Figs. 3 and 4). In comparison with the controls (25°C), the chilling-sensitive seedlings showed more rapid decrease in ACC and ethylene contents than the cold-tolerant seedlings (Fig. 3). The ethylene and ACC contents in chilled seedlings of the cold-tolerant cultivar decreased to about 30% after 6 h at 4°C. In the chilling-sensitive cultivar, the ethylene and ACC contents also showed a decrease to the level of 30% of the controls, whereas a significant decrease occurred only after 2 h. In present studies, we also determined the changes of ACC content in roots and shoots under 4°C condition. Fig. 4 showed that ACC content in both shoots and roots decreased to the minimum of 0.9 nmole/g f wt. Shoots of the cold-tolerant rice seedlings had a slower decreasing rate in ACC content than roots. The ACC content of shoots declined to the minimal level after 6 h at 4°C, whereas, that of roots occurred only 2 h at 4°C (Fig. 4). In the chilling-sensitive rice cultivar, both the root and shoot showed minimal

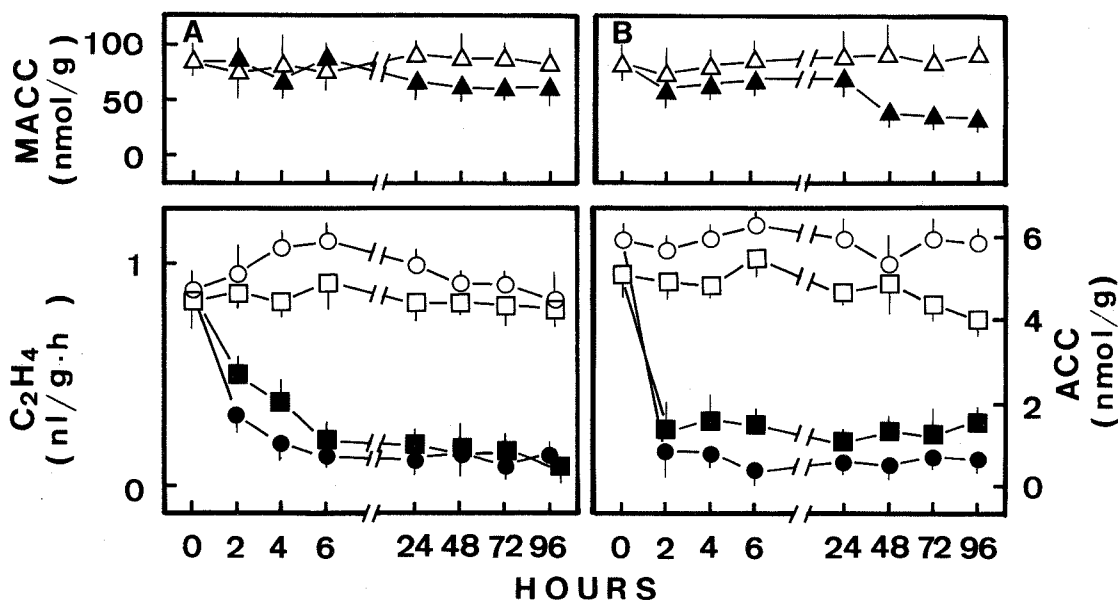


Fig. 3. Time course of changes in ACC and MACC contents and ethylene production rate of rice (*Oryza sativa* L.) seedlings grown at 4°C or at 25°C (controls). Intact plants (14 day-old seedlings) were used for ethylene production and then plants without seeds were used for ACC and MACC analysis. ○, ethylene production, 25°C; ●, ethylene production, 4°C; □, ACC content, 25°C; ■, ACC content, 4°C; △, MACC content, 25°C; ▲, MACC content, 4°C. A, Tainung 67; B, Taichung Native 1. Bars indicated the standard deviation.

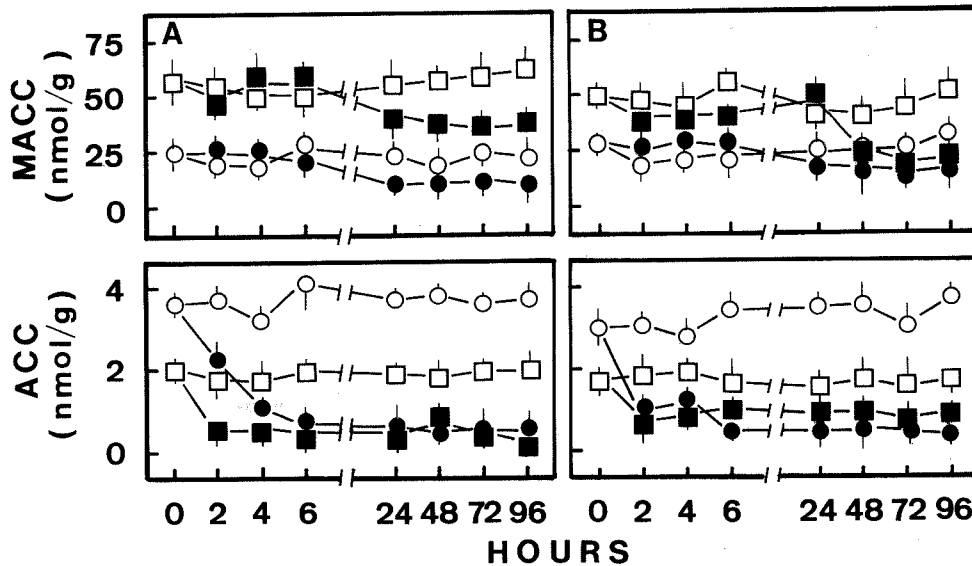


Fig. 4. Time course of changes in ACC and MACC contents of shoots and roots of rice (*Oryza sativa* L.) seedlings grown at 4°C or at 25°C (controls). □, shoot, 25°C; ■, shoot, 4°C; ○, root, 25°C; ●, root, 4°C. A, Tainung 67. B, Taichung Native 1. Bar indicated the standard deviation.

level of ACC after 2 h at 4°C (Fig. 4).

MACC is the ACC conjugate (Amrhein *et al.*, 1981, 1982; Hoffman *et al.*, 1983). It has been reported that MACC could accumulate under stress conditions (Amrhein *et al.*, 1982; Fuhrer and Fuhrer-Fries, 1985; Satoh and Esashi, 1984; VanLoon and Fontaine, 1984). Since changes of MACC content in plant tissues during chilling stress have not been reported, we examined the change of MACC content in rice seedlings under chilling temperature (4°C). The chilling treatment was found to have no effect on the MACC content in both cold-tolerant and chilling-sensitive rice seedlings in 24 h under 4°C condition (Fig. 3). After that, in the case of the chilling-sensitive seedlings, the MACC content decreased to about 50% of the controls after 24 h (Fig. 3). The MACC content in cold-tolerant seedlings only decreased to 80% of the controls (Fig. 3). The results of Fig. 4 showed that most of the decrease in MACC content took place in root tissue of both cultivars.

Our results also showed that ACC content in shoots was higher than that in roots; however, MACC content in roots was higher than that in shoots (Fig. 4).

Changes of Ethylene Production and ACC Content in Chilled Seedlings During Warming

After chilling for 2 days, the chilled rice seedlings were transferred to 25°C. A rapid increase in ethylene and ACC levels was detected after seedlings were transferred from 4°C to 25°C (Fig. 5 A and B). The greatest rate of ACC accumulation in both cultivars was occurred at 9 h after warming. After 9 h, the amount of ACC levelled off or decreased rapidly. The maximum amount of ethylene production in chilled seedlings occurred at 12 h during recovering at 25°C. In the present work, we also detected the influence of the longer chilling treatment on the changes of chilling-induced ACC and ethylene production in rice seedlings. For the cold-tolerant cultivar (Tainung 67), it was observed that ethylene production rate and ACC content increased to more higher levels during warming after 4 day-chilling treatment than those after 2 day-chilling treatment (Fig. 5 C). Though the ACC content in the chilling-sensitive rice seedlings also increased during warming after 4 day-chilling treatment the ethylene production rate was lower than that of the controls (Fig. 5 D). Fig. 5 A showed that the maximum ethylene production rate of 2 day-chilling treated *japonica* rice seedlings (Tainung 67) during recovery period was 2-fold of the control and the maximum ACC content was 3-fold higher than that of the controls. For the chilling-sensitive rice cultivar (Taichung Native 1), the chilling-induced ACC accumula-

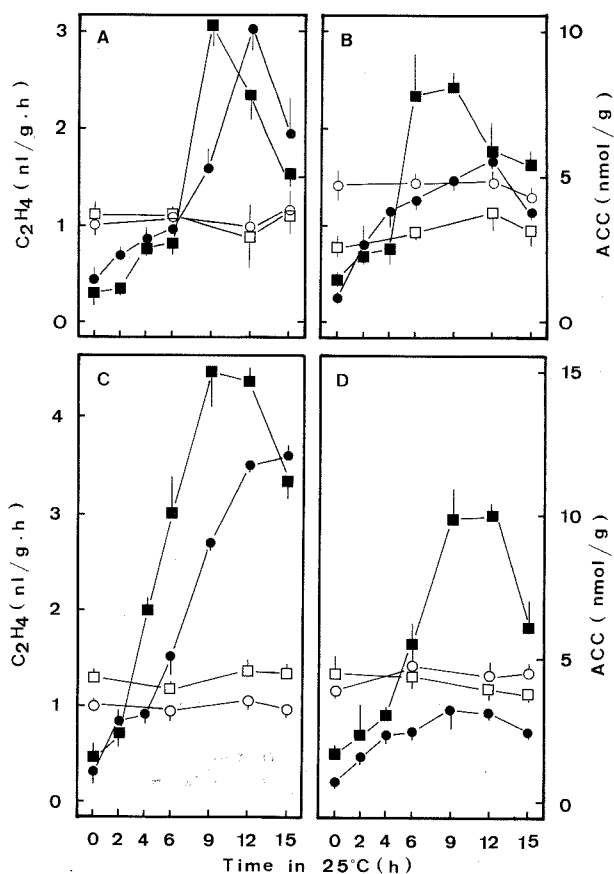


Fig. 5. Time course of changes in ACC content and ethylene production in chilled or control rice (*Oryza sativa* L.) seedlings. Plants were treated at 4°C for 2 days or 4 days and then transferred to 25°C for various hours. ○, ethylene production, 25°C; ●, ethylene production, 4°C; □, ACC content, 25°C; ■, ACC content, 4°C. A, Tainung 67, 2 days at 4°C; B, Taichung Native 1, 2 days at 4°C; C, Tainung 67, 4 days at 4°C; D, Taichung Native 1, 4 days at 4°C. Bars indicated the standard deviation.

tion was 2-fold of the controls, whereas the ethylene production was only slightly higher than those of the controls (Fig. 5 B). After 4 day-chilling treatment, the greatest rate of ethylene production and ACC accumulation in cold-tolerant rice seedlings were 3.7 and 3-fold of the controls, respectively. The maximum amount of 4 day-chilling-induced ACC production in chilling-sensitive rice seedlings was still 2-fold of the controls, but the ethylene evolution remained lower than controls during the warming period. As shown in

Fig. 6, no matter whether 2 or 4 day-chilling treatment, an obvious increase of ACC content was observed in shoots whereas a slight increase in roots.

To study the effect of chilling stress on the capacity of converting ACC to ethylene in rice seedlings, we applied exogenous ACC and analyzed the ACC-induced ethylene production. The rice seedlings were chilled at 4°C for various periods, and then ten intact rice seedlings were immersed in 5 mM ACC solution and vacuum-filtrated for 5 min. After ACC treatment, the rice seedlings were incubated at 25°C for 12 h in light and the ACC-induced ethylene production was determined. For the ethylene analysis, the ACC-treated and control seedlings were sealed in 78 ml glass cylinder in darkness and gas samples were taken for ethylene determination after 60 min incubation at 25°C. Exogenous ACC treatment stimulated large amount of ethylene release in control seedlings of both cultivars (Table 1). There was virtually no difference in the increase of ACC-induced ethylene in chilled and control seedlings of the cold-tolerant cultivar, Tainung 67. The chilling-sensitive cultivar, Taichung Native 1, however, showed a significant decrease in ACC-induced ethylene production after 4 day-chilling treatment (Table 1).

Discussion

It is well known that, in general, seedlings of *japonica* cultivar are much tolerant to chilling stress than those of *indica* cultivar (Chang, 1970; Chu *et al.*, 1986; Vergara *et al.*, 1976). Our previous work showed that electrolyte leakage could be a simple and rapid indicator of screening for cold tolerant identification in rice seedlings (Chu *et al.*, 1986). Sato and Park (1982) found that the degree of leakage by low temperature was *indica* type > *japonica* × *indica* > *japonica* type. They suggested that electrical conductivity of leakage induced by low temperature may be an index of cold-tolerance in rice varieties. In present studies, from the degree of electrolyte leakage and leaf damage (Figs. 1 and 2), seedlings of Tainung 67 (*japonica* type) were more tolerant to chilling stress than those of Taichung Native 1 (*indica* type). These results are in a good agreement with these previous reports.

The 14 day-old green rice seedlings produced about 1.5 nl ethylene per hour per gram fresh weight. Satler and Kende (1985) reported that etiolated rice

Table 1. ACC-dependent ethylene production of the chilled rice (*Oryza sativa* L.) seedlings

Tested cultivar	Chilling period (days)	Ethylene production rate (nl · g ⁻¹ f wt · h ⁻¹)			
		4°C		25°C	
		+ACC	–ACC	+ACC	–ACC
Tainung 67	2	12.24	3.36	9.03	1.15
	4	10.76	2.26	10.15	1.26
	6	9.05	2.15	9.49	1.07
Taichung Native 1	2	10.77	1.89	13.98	1.26
	4	4.02	1.00	14.17	1.75
	6	2.10	0.51	12.72	1.47

seedlings released ethylene at a rate of 0.9 pmole per hour per seedling. Their results also showed that shoots, seeds and roots of 80-h-old seedlings produced 0.057, 0.005 and 0.011 ethylene nmole per hour per gram fresh weight, respectively. In the present experiment, ethylene production rate in rice seedlings of the chilling-sensitive cultivar and cold-tolerant cultivar was similar to their results.

Our results showed that chilling stress inhibited the biosynthesis of ACC and ethylene both in chilling-sensitive and cold-tolerant rice seedlings (Fig. 2). The ethylene production was maintained at a constant rate of about 1 nl · g⁻¹ f wt · h⁻¹ in the controls. The chilled rice seedlings in both cultivars produced ethylene at a rate of about 0.28 nl · g⁻¹ f wt · h⁻¹ (Fig. 2). The ACC content in the control kept at the level of 4.5 nmol/g f wt, whereas decreased to 1.7 nmol/g f wt under chilling treatment. Our results agreed with previous studies in which the biosynthesis of ethylene and ACC in cucumber fruit (Wang and Adams, 1980, 1982) and seedlings (Wang, 1987) was inhibited under chilling temperature. Field (1984) also found that ethylene production in leaves of dwarf bean was inhibited at 5°C. Though Fig. 4 showed that no difference was found between the ACC content of shoots and roots under chilling treatment, the decreasing rate of ACC content in shoots was different in the two cultivars. The ACC level in the shoot of cold-tolerant rice cultivar reached the minimal level after 6 h, whereas, that of chilling-sensitive rice cultivar occurred within 2 h at 4°C. However, the ACC content of root tissues in the two tested cultivars decreased to the minimum

after 2 h of chilling treatment. It seems that the ACC metabolism in root tissues is more sensitive to chilling stress than that in shoot tissues. Our results also suggested that shoots of the cold-tolerant rice seedlings may be possessed partly ability of ACC synthesis or lowered ACC catabolism during 4 h after transfer to 4°C. A more detailed work is in great need, a research focused on ¹⁴C-SAM feeding and usage of ethylene synthesis inhibitors in chilled rice seedlings should be investigated further.

The chilling-induced ethylene production in rice seedlings only occurred after transfer from chilling to warmer temperature. After transfer from 4°C to 25°C, the ACC and ethylene production in intact rice seedlings increased rapidly (Fig. 5). Several results also indicated that ethylene production in a number of plants was enhanced by chilling stress when the plants were transferred from cold to warmer temperature (Chen and Patterson, 1985; Field, 1984; Ichii and Hamada, 1978; Wang and Adams, 1980, 1982). Wang and Adams (1982) suggested that the pathway for ethylene biosynthesis and related to chilling responses was similar in a number of higher plants and the step which was enhanced by chilling stress was the formation of ACC. Our results also showed that the ethylene and ACC levels were stimulated by chilling stress in seedlings of the rice varieties were similar to those of other plants. Fig. 5 showed that the chilling-induced ethylene production in rice seedlings increased to maximum following the maximum ACC content. Our results are consistent with previous reports which showed that the synthesis of ACC could be the rate limiting step in chil-

ling-induced ethylene production (Wang and Adams, 1982). The possible cause of the substantial increase in ethylene production following chilling stress might appear to be caused by an increase in ACC synthesis after transfer to warmer temperature and its subsequent enhanced conversion to ethylene (Field, 1984; Wang and Adams, 1982). By using RNA and protein synthesis inhibitors, cordycepin and cycloheximide, Wang and Adams (1982) have reported that the synthesis of the mRNA coding for ACC synthase remained normal under chilling stress, but its translation was inhibited by chilling. Therefore, the accumulated mRNA was translated rapidly during warmer temperature and evoked the increase in ACC synthase activity, ACC and ethylene production. The results of the present investigation showed that most of the chilling-induced increase in ACC occurred in shoots (Fig. 6). It is possible that shoots synthesized large amount of ACC itself or the ACC synthesized in roots was translocated to shoots during recovering at 25°C.

We found that cold-tolerant rice seedlings exhibited higher chilling-induced ACC content and ethylene production than chilling-sensitive rice seedlings. Wang and Adams (1982) have suggested that ACC content could be used to predict the sensitivity of chilling injury. Chen and Patterson (1985) recently also showed that the stimulation of ethylene evolution and ACC content in leaves was related to the sensitivity of plants to chilling. From present results with different rice varieties, ethylene release and ACC accumulation in seedlings after chilling could be an indicator for selection of cold-tolerant varieties.

After different periods of chilling treatment, a rapid increase in ethylene and ACC production in seedlings of the chilling-tolerant cultivar, Tainung 67, was detected. The ACC level in the chilling-sensitive variety (Taichung Native 1) was accumulated higher than that in controls, but the ethylene production was lower than that of controls. There was a remarkable difference between *indica* and *japonica* type rices. These results suggested that the step from ACC to ethylene was inhibited first by prolonged exposure to chilling. It has been reported that EFE in chilling-sensitive plants was liable and inactivated by prolonged chilling treatment (Etani and Yoshida, 1987; Guye *et al.*, 1987). Prolonged chilling caused a marked reduction of ethylene formation prior to a decline of the endogenously accumulated ACC level during warming (Wang and

Adams, 1980, 1982; Chen and Patterson, 1985). Wang and Adams (1982) also suggested that the system of converting ACC to ethylene was the first step to be readily damaged by chilling temperature. Chu *et al.* (1986) have reported that elevated electrical conductivity occurred in chilling-sensitive rice seedlings. In this experiment, we also found electric conductivity of chilling-sensitive increased to a large scale during chilling periods. Lyons (1973) proposed that chilling causes the alteration of membrane components resulting in loss of normal physical and chemical properties. It has been suggested that the system between ACC and ethylene might be associated with the membrane and was closely related to the integrity of membrane (Apelbaum *et al.*, 1981; Field, 1981), presumably, plasma membrane or tonoplasts is involved (Yang and Hoffman, 1984). Our results showed that visible responses of chilling injury such as increase of electrolyte leakage occurred after the same duration of chilling compared with ethylene release and ACC level. The relation between the change in electrolyte leakage in the two tested rice varieties and the accelerated ethylene and ACC productions provided indirect evidences to support the concept that the system between ACC and ethylene might be associated with the membrane. Chilling-sensitive rice cultivar, Taichung Native 1, the chilling-induced membrane phase transition might be irreversible after transfer to 25°C from chilling than in cold-tolerant rice cultivar, Tainung 67, with the results of poor conversion of ACC to ethylene (Fig. 5 D). Chilling more than 2 days might cause irreversible loss of the EFE activity in the chilling-sensitive rice seedlings. By adding exogenous ACC, the ACC-dependent ethylene release in the chilling-sensitive rice seedlings decreased 3-fold as compared with the controls growing at 25°C, whereas remained unchanged in the cold-tolerant rice seedlings (Table 1). The results suggest that prolonged chilling treatment cause the irreversible damage on the system between ACC and ethylene in chilling-sensitive rice cultivar. The cold-tolerant cultivar apparently maintained the system of converting ACC to ethylene normally. Etani and Yoshida (1987) also showed that the ACC-dependent EFE *in vivo* in hypocotyls of etiolated seedlings of mung bean (*Vigna radiata* L. Wilczek) was sensitive to chilling and progressively declined as a function of the chilling period.

In addition to converting ACC to ethylene, Amr-

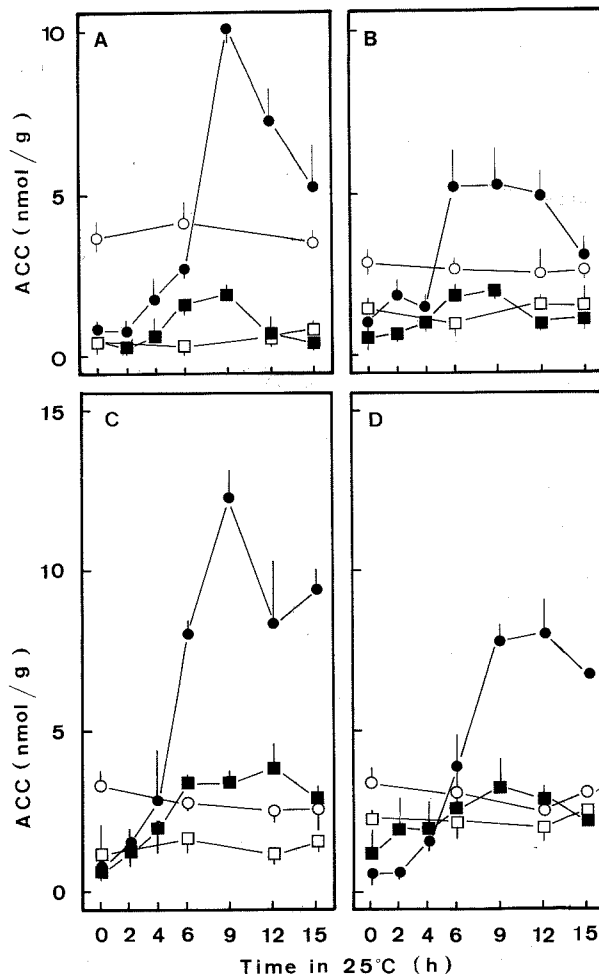


Fig. 6. Time course of changes in ACC level and ethylene production of shoots and roots in chilled or control rice (*Oryza sativa* L.) seedlings. ○, shoot 25°C; ●, shoot, 4°C; □, root, 25°C; ■, root, 4°C. A, Tainung 67, 2 days at 4°C; B, Taichung Native 1, 2 days at 4°C; C, Tainung 67, 4 days at 4°C; D, Taichung Native 1, 4 days at 4°C. Bars indicated the standard deviation.

hein *et al.* (1981) and Hoffman *et al.* (1982) have demonstrated that plant tissues are capable of metabolizing ACC to MACC which was regarded as a stable product. Hoffman *et al.* (1983) reported that MACC in excised wheat leaves accumulated under water deficit and MACC level could be used as a good indicator of the stress history. In the present work, it was showed that the MACC content in rice seedlings remained unchanged during 48 h of chilling treatment. After that, both of the chilling-sensitive and cold-tol-

erant cultivars exhibited a decrease in roots but not in shoots. It seemed that the MACC declined during the late period of chilling treatment could be caused by a leakage from roots into water which may be related to the alteration of membrane structure with increased permeability. The results which the chilling-sensitive rice seedlings (Taichung Native 1) showed a more decrease in MACC level than the cold-tolerant rice seedlings (Tainung 67) suggest that chilling stress may cause serious damage on the membrane structure in the root of the chilling-sensitive rice than the cold-tolerant cultivar. Changes of MACC content in chilling-treated seedlings after rewarming is presently under investigation.

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水稻幼苗乙烯生成與耐冷性之關係

朱 鈞 李澤民

國立台灣大學農藝學系

對於耐冷性(台農 67 號)及不耐冷性(台中在來 1 號)品種而言,三葉齡(14 天生育時間)水稻幼苗乙烯生成速率及 ACC 含量均會受低溫(4°C)處理之影響而有明顯的降低。雖然低溫處理 24 小時不會造成 MACC 含量顯著的變化,但較長之低溫處理時間卻會引起 MACC 含量的減少;其中不耐冷性水稻幼苗的減少量較明顯。試驗結果顯示全株幼苗 MACC 減少主要是發生於根部組織,我們推測這可能與冷害導致根部細胞膜通透性增加而致 MACC 流失有關。2 天低溫處理再回溫(25°C)之水稻幼苗,其乙烯生成速率及 ACC 含量會迅速增高。因此,冷害會刺激水稻幼苗合成大量之乙烯及 ACC,但是只有在回溫狀態始能表現。同時,本試驗指出耐冷性水稻幼苗的乙烯生成速率及 ACC 含量都高於不耐冷性水稻幼苗。雖然,4 天低溫處理也有相似之趨勢,但是不耐冷性水稻幼苗的乙烯生成速率卻低於未經低溫處理之對照組。另一方面,由外加 ACC 之試驗發現 4 天較長之低溫處理時間會造成不耐冷性水稻幼苗 ACC 轉變為乙烯反應明顯降低。顯然地,不耐冷性水稻幼苗 ACC 轉變為乙烯之反應較耐冷性水稻幼苗易受冷害之抑制。對於生長在 25°C 下之三葉齡水稻幼苗而言,雖然地上部 ACC 含量高於根部,但根部的 MACC 含量則高於地上部。