

Regulation of Chilling Tolerance in Rice Seedlings by Plant Hormones

Chun Chu and Tse-Min Lee

ABSTRACT : Since the major important factors limiting plant growth and crop productivity are environmental stresses, of which low temperature is the most serious. It has been well known that many physiological processes are alterant in response to the environmental stress. With regard to the relationship between plant hormones and the regulation of chilling tolerance in rice seedlings, the major physiological roles of plant hormones : abscisic acid, ethylene and polyamines are evaluated and discussed in this paper. Rice seedlings were grown in culture solution to examine the effect of such plant hormones on physiological characters related to chilling tolerance and also to compare the different responses among tested cultivars. Intact seedlings about 14 day-old were chilled at conditions of 5°C and 80% relative humidity for various period. Cis-(+)-ABA content was measured by the indirect ELISA technique. Polyamine content and ethylene production in leaves were determined by means of HPLC and GC respectively. Chilling damage of seedlings was evaluated by electrolyte leakage, TTC viability assay or survival test.

Our experiment results described here demonstrated the physiological functions of ABA, ethylene, and polyamines related to the regulation of chilling tolerance in rice seedlings.

Levels of cis-(+)-ABA in leaves or xylem sap of rice seedlings increased rapidly in response to 5°C treatment. The tolerant cultivars had significant higher level of endogenous ABA than the sensitive ones. The (±)-ABA pretreatment for 48 h increased the chilling tolerance of the sensitive indica cultivar. One possible function of abscisic acid is the adjustment of plants to avoid chilling-induced water stress. Accumulation of proline and other compatible solutes is assumed to be another factor in the prevention of chilling injuries by abscisic acid. In addition, the expression of ABA-responsive gene is reported in some plants and may be involving in the acclimation to low temperature.

Ethylene and its immediate precursor, 1-aminocyclopropane-1-carboxylic acid(ACC) increased significantly after 5°C treatment. The activity of ACC synthase which converts S-adenosylmethionine (SAM) to ACC enhanced earlier than the increase of ethylene and ACC. Low temperature increased ACC synthase activity, whereas prolonged chilling treatment damaged the conversion of ACC to ethylene. It was shown that application of Ethphon was beneficial to recovering from chilling injury in rice seedlings. However, the physiological functions of chilling-induced ethylene are still unclear.

Polyamines are thought to be a potential plant hormone and may be involving in the regulation of chilling response. Results indicated that chilling treatment induced a remarkable increase of polyamines, especially putrescine content in rice seedlings. The relative higher putrescine content

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was found in chilling-tolerant cultivar and the maximal level of enhanced putrescine in shoot of chilling cultivar(TNG. 67) was about 8 folds of controls at two days after chilling. The accumulation of polyamines may protect membrane structure or buffer ionic imbalance from chilling damage.

Stress physiology is a rapidly expanding field. Plant growth regulators that improve tolerance to low temperature may affect stress protein production. The molecular or gene approaches will help us to elucidate the functions of plant hormones related to the regulation of chilling tolerance in plants in the near future.

Abbreviation :

ABA : abscisic acid

ACC : 1-aminocyclopropane-1-carboxylic acid

ADC : arginine decarboxylase

ELISA : enzyme-linked immunosorbent assay

ODC : ornithine decarboxylase

Put : putrescine

SAMDC : S-adenosylmethionine decarboxylase

Spd : spermidine

Spm : spermine

TNG 67 : Tainung 67

TCN 1 : Taichung Native 1

TTC : triphenyl tetrazolium chloride

Key Words :

Chilling tolerance, Rice (*Oryza sativa* L.), Abscisic acid, Polyamine, Ethylene, ACC.

Because the population of the world increases and utilizes more land area for housing and commercial or industrial activities, agriculture and crop production are being forced into marginally productive areas. Temperature extremes, water stress, water flooding, nutrient deficiencies and toxicities, salinity, air pollution, and chemical interference are often encountered. Alteration of environments and climates may also result from human activities that increase the stressful conditions under which crop must grow and survive. Stress occurs as a result of aberrant metabolism and may be expressed as reduction in growth, yield, or value, or death of the plants.

Low temperature is a major limiting factor for crop growth and production. Various physiological and biochemical alterations occur in response to chilling stress. It has also been established that various physiological processes in response to environmental stress which are apparently regulated by plant hormones. Temperature or other stress are known to stimulate ethylene production in many plant species(Apelbaum & Yang, 1981 ; Chen & Patterson, 1985 ; Chu, et al., 1986 ; Chu & Lee, 1989 ; Yang, 1984, 1985 ; Yu & Yang, 1980). Ethylene is generally produced before visible chilling injury appear and it is easily and rapidly measured.

In general, high level of abscisic acid in plant tissues are correlated with increased resistance. The correlation has been demonstrated both by a rise in endogenous ABA during stress (Lee et al., 1991 ; Rikin et al., 1975, 1979 ; Rikin and Richmond, 1976)and by the application of exogenous ABA (Chen et al. 1983 ; Daie and Campbell, 1981 ; Emaus and Wilson, 1983 ; Wightman, 1979). ABA is also involved in the regulation of plant response to both freezing and chilling(Rikin & Richmond, 1976 ; Rikin et al., 1979, 1981 ; Titov, et al., 1985). Several lines of evidence suggest that

polyamines and their biosynthetic enzymes may play an important role in the regulation of plant growth and development (Galston & Kaur-Sawhney, 1987; Smith, 1985). Polyamine contents show a tremendous increase in response to chilling (Guye et al., 1986; Kramer & Wang, 1989, 1990; McDonald & Kushod, 1986; Nadeau et al., 1987; Wang). The putrescine and spermidine contents of primary leaves of bean has been observed to accumulate in chilling conditions and the relative changes of putrescine content is suggested to be correlated with chilling tolerance (Guye et al., 1986).

Chilling injury of rice seedlings in paddy field frequently occurs in the first crop season during the coldest months in Taiwan. Portions of our studies have been published (Chu et al., 1986; Chu & Lee, 1989) and a part of present work will be reported elsewhere (Lee & Chu, 1991; Lee et al., 1991). In this paper, the important roles of ABA, ethylene and polyamines in regulation of chilling tolerance of rice seedlings are presented. We also describe the possible correlation of the chilling tolerance with the function and metabolism of these plant hormones.

Materials and Methods

Plant materials: Rice cultivars including Taichung Native (TCN 1) (indica type) and Tainung 67 (TNG 67) (japonica type) were employed in this work. Seeds were soaked in distilled water for two days at 37°C and then cultivated in 500 ml beaker containing half-strength Kimura B solution described previously (Chu & Lee, 1989).

Chilling treatment: For most experiments, 14-day-old rice seedlings were chilled at condition of 5°C and 80% relative humidity for various periods.

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).

Measurement of electrolyte leakage: After 5°C treatment, ten seedlings were immersed in 20 ml of 0.2 M mannitol solution for 24 h at 25°C in darkness. The amount of electrolyte leaked into the solution was measured with a conductivity meter.

Triphenyl tetrazolium chloride (TTC) viability assay: The TTC assay which was employed to measure a tissue viability in response to stress was modified from the method of Steponkus and Lanphear (1967).

Determination of ethylene and ACC: Ethylene production in seedlings was determined during 4°C treatment or after transfer from 4°C to 25°C and analyzed by a gas chromatograph at 80°C with a flame ionization detector. ACC assay procedure was modified from the method of Lizada and Yang (1979).

Extraction and purification of ABA: Sample tissues was fixed with liquid nitrogen and lyophilized at -50°C. Liqueous MeOH (80%) were immediately used for extraction. ³H-ABA was added as an internal standard. A Waters HPLC equipped with a UV detector was employed in the purification of cis-(+)-ABA in sample extract.

Determination of cis-(+)-abscisic acid by indirect ELISA: Free ABA was analyzed by the indirect ELISA according to Walker-Simmons (1987) with some modification. ABA-4'-BSA conjugated was prepared by method of Weiler (1980). The detail procedure for ABA purification and assay were described elsewhere (Lee et al., 1991).

Extraction and determination of free polyamines: Free polyamines was extracted and benzoylated according to the method of Flore and Galston (1982) with some modifications. The separation and quantitation of benzoylated polyamines in plant tissue by

HPLC were modified from the method of Slocum et al. (1989).

Extraction and determination of enzyme activity : The assay method for enzyme activities of ADC (arginine decarboxylase), ODC (ornithine decarboxylase), and SAMDC (S-adenosylmethionine decarboxylase) were modified from the method of Cohen and Kende (1986). The extraction procedure and prep-

aration were described previously (Lee and Chu, 1991).

Result and Discussion :

Effect of chilling in the growth of rice seedlings : The degree of chilling injury in rice seedlings was evaluated by the score of leaf damage (IRRI, 1980), electrolyte leakage or survival ratio. The ability of TTC reduction

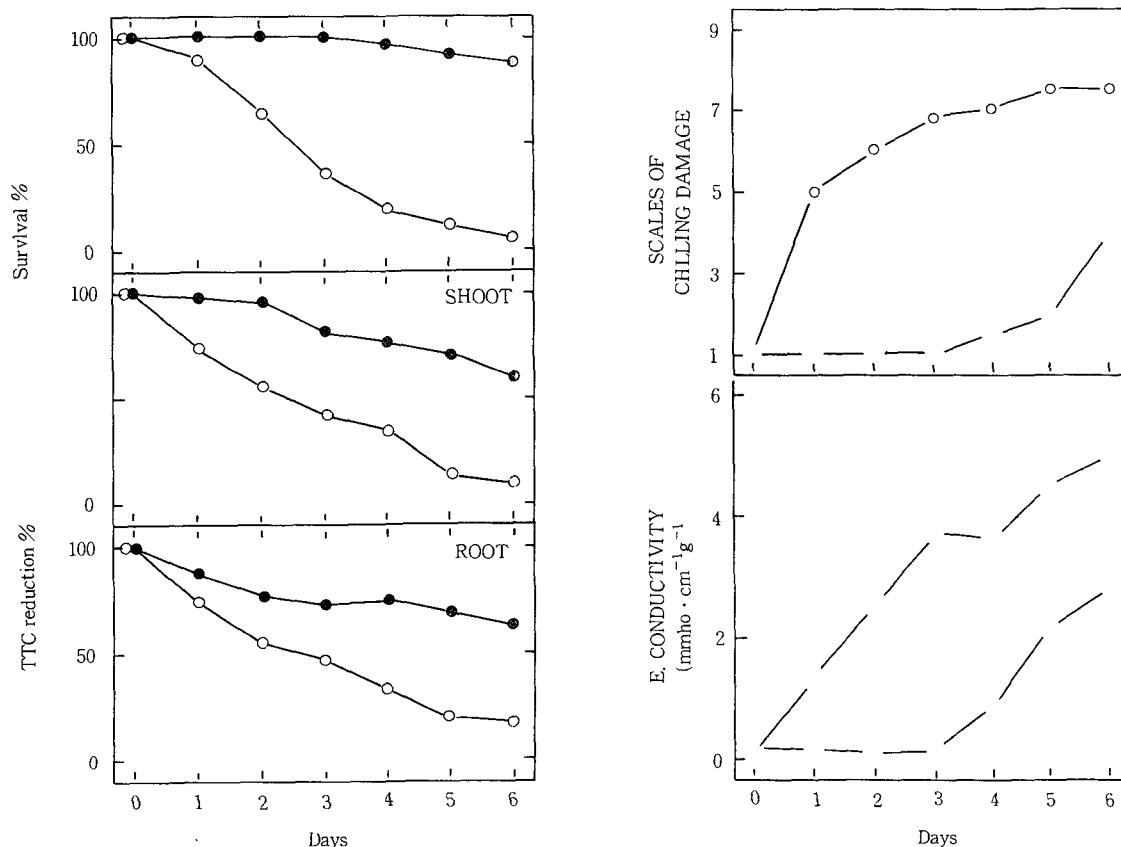


Fig.1 Evaluation of chilling tolerance in rice (*Oryza sativa* L.) seedlings by degree of chilling damage, electrolyte leakage, TTC reduction, and survival ratio. ●, TNG 67 ; ○, TCN 1.

Survival ratio of rice seedlings in response to chilling (5/5°C) was evaluated 7 days after recovery at 25/30°C.

The reduction of TTC in shoots and roots of chilled seedlings was presented as a percentage of 25/30°C controls.

The reduction of TTC in shoots and roots of chilled seedlings was presented as a percentage of 25/30°C controls. Scale of chilling damage was evaluated after 7 days in 25°C. For the determination of electrolyte leakage, leaves were immersed in 0.2M mannitol solution in darkness for 24 h at 25°C. The amount of electrolyte leached into the solution was measured with a conductivity meter.

was also used here to predict the viability of rice seedlings in response to chilling. Seedlings of TNG 67 preserved a high level of TTC reduction ability on exposure to chilling, whereas the TCN 1 seedlings showed a significant decline after chilling treatment. As showed in Fig.1, there is a remarkable difference in chilling tolerance between two cultivars, the chilling-tolerant cultivar, TNG

67, and chilling-sensitive one, TCN 1. Thus these two cultivars were chosen as plant materials in most work to investigate the relationship between plant hormone and chilling tolerance in rice seedlings.

Changes of ethylene and ACC content in chilled seedlings : The level of ACC and ethylene production rate in rice seedlings was low under chilling condition. However, the ACC content and ethylene production rate increased rapidly only after transferring to a warmer temperature (Fig. 2). The ACC content reached the maximum at 9 h and ethylene production rate at 12 h, respectively. After warming, the chilling seedlings of cold-tolerant cultivar showed a large increase in ACC and ethylene production. The chilling-sensitive seedlings showed a large increase in ACC but little ethylene production after chilling treatment. Similar results were obtained with rice seedlings of 4 cultivars which were chilled for 2 days (Chu et al., 1986). Several researches also indicated that ethylene production in a number of plants was stimulated by low temperature when the plants were transferred from chilling to warm condition (Chen & Patterson, 1985; Field, 1984, 1985; Ichii & Hanada, 1978; Wang & Adams, 1980, 1982). Chen & Patterson (1985) also showed that ethylene production rate and ACC level in leaves were dependent upon the sensitivity of plants to chilling. Ethylene evolution and ACC accumulation in seedlings after chilling could be an indicator for selection of cold-tolerant rice varieties.

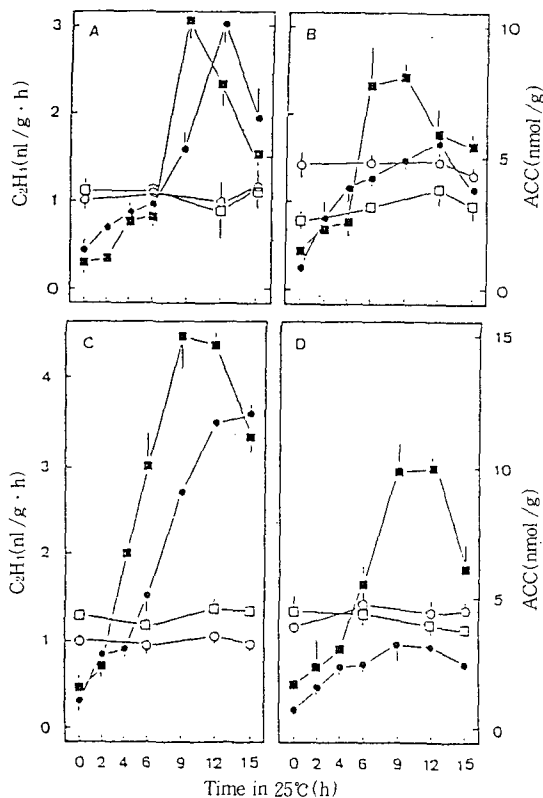


Fig. 2 Time course of changes in ACC content and ethylene production in chilled or control rice (*Oryza sativa* L.) seedlings. Plants were treated at 4°C for 2 days or 4 days and then transferred to 25°C for several hours. ○, ethylene production, 25°C; ●, ethylene production, 4°C; □, ACC content, 25°C; ■, ACC content, 4°C. A, TNG 67, 2 days at 4°C; B, TCN 1, 1.2 days at 4°C; C, TNG 67, 4 days at 4°C; D, TCN 1, 4 days at 4°C. Bars indicated the standard error.

Changes of cis-(+)-ABA content in relation to chilling tolerance : Chilling induced a fast increase of ABA content in rice seedlings (Fig.3). The maximal level of chilling-enhanced ABA increase was about 5 folds of controls at 72 h after chilling in shoot tissue of TNG 67. However, TCN 1 shoots only presented a slightly difference of ABA content in response to low temperature. In chilled roots, ABA content increased more rapid than

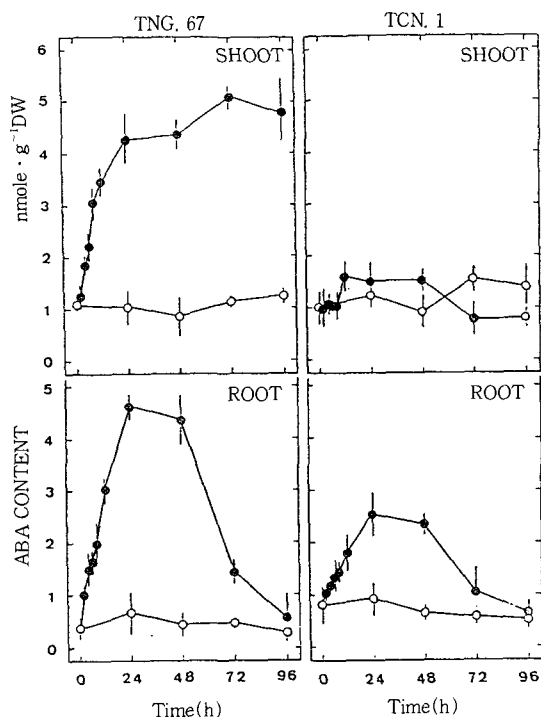


Fig. 3 Levels of endogenous ABA in shoots and roots of chilled rice (*Oryza sativa* L.) seedlings. The intact seedlings were chilled at 5°C. Bars indicated the standard error.

in shoots. There was a relatively small increase of ABA content in chilled roots of TCN.1 as compared with chilled roots of TNG. 67. It has been reported that levels of ABA in wheat accumulated in response to cold temperature in chilling-tolerant cultivar was specifically higher than those of chilling-sensitive cultivar (Wightman, 1979). Similar results were also observed in acclimated potato which cold-resistant cultivar showed a elevated ABA content and no significant changes occurred in cold-sensitive cultivar (Chen et al., 1983). Several mechanisms concerning enhancement of chilling tolerance of plants by ABA have been postulated (Reaney et al., 1989). One of them is the maintenance of water balance in chilling condition. There are abundant evidences which demonstrate that ABA can

induce stomatal closure (Zeevaart & Creelman, 1988; Zeiger, 1983). Alteration of cellular metabolism may mediate the enhancement of chilling tolerance by ABA. Proline content increases in response to chilling (Chu et al., 1974; Lalk & Dorffling, 1985) and has been suggested to protect plant to avoid damage (Lalk & Dorffling, 1985; Songstad et al., 1990; Withers & King, 1979). Since abscisic acid has been reported to induce proline accumulation, the increase of chilling-induced proline may be regulated by ABA (Eberhardt & Wegmann, 1989; Pesci, 1988; Steward & Voetberg, 1985). Polyamines are also found to accumulate under chilling conditions and show a close relation with chilling tolerance (Guye et al., 1986; Lee & Chu, 1991). Since ABA could change polyamine metabolism (Chen, 1990) and thus accumulated polyamines may be involved in the ABA-enhanced chilling tolerance.

Levels of polyamines in chilling rice seedlings : In present study, chilling induced a significant accumulation of polyamines, especially putrescine in rice seedlings. Seedlings of chilling-tolerant TNG 67 exhibited a higher putrescine response than that of chilling sensitive TCN 1 (Fig.4). The putrescine content in shoots of TNG 67 increased significantly only one day after chilling and reached the maximal level at 2 days after treatment. An increase of putrescine content was also found in TCN 1 shoots. However, the increased putrescine was relatively small at 3 days after chilling and then decreased subsequently even below controls (Fig. 4). Similar results as showed in shoots, a large accumulation of putrescine content occurred in roots of TNG 67. Levels of putrescine in roots of TCN.1 tremendously declined, on the contrary, during the chilling period of 3 days to 6 days. The chilling-induced polyamine accumulation has been investigated in several plant systems including fruits (Kramer & Wang, 1989, 1990; McDonald & Kushad, 1986), Citrus (Kushad & Yelenosky, 1985), wheat and

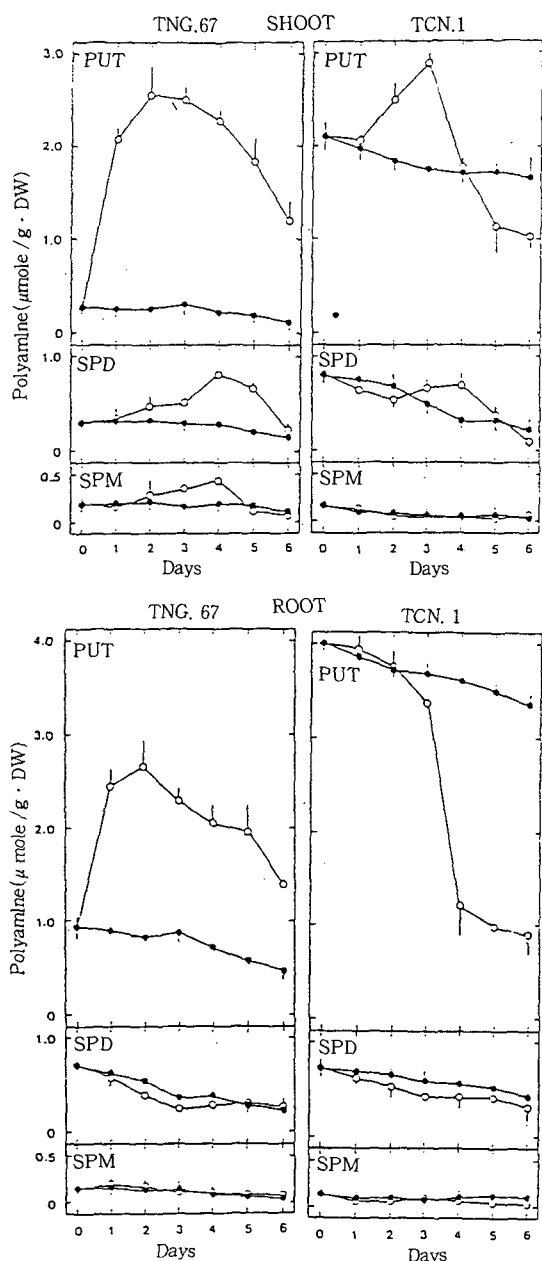


Fig. 4 Levels of endogenous polyamines in shoots and roots of chilled rice (*Oryza sativa* L.) seedlings. ●, 25/30°C; ○, 5/5°C. Bars indicated the standard error.

alfalfa seedlings (Nadeau et al., 1987) and beans (Guye et al., 1986). It seems that relative levels of putrescine, but not absolute

quantity, correlated closely with chilling tolerance of rice seedlings. Similar results from the comparison of chilling-induced polyamine accumulation of primary leaves between bean cultivars with different chilling tolerance indicated that the relative amount of accumulated putrescine has a close relation to chilling tolerance (Guye et al., 1986).

Changes of ADC, ODC, and SAMDC activities in chilled seedlings: To determine which biosynthetic pathway has been involved in chilling induced accumulation of putrescine, the activities of ADC and ODC were evaluated. In shoots, as shown in Fig. 5, the ADC activity of chilled TNG 67 raised rapidly up to a maximal level at one day after chilling (3 times of controls) and then decreased, while the chilled TCN.1 showed a small increase and decreased slightly at later chilling period. In roots, the ADC in chilled TNG 67 was also enhanced by low temperature treatment. However, the activity of ADC decreased slightly during prolonged chilling periods in roots of TCN 1. Chilling also resulted in a significant increase of SAMDC activity of TNG 67 shoots after exposure to chilling. On the contrary, the SAMDC activity of TCN 1 shoots showed little change in response to chilling. In root tissue, chilling, however, had no influence on SAMDC activity of TNG 67, but a tremendous decline was observed in chilled TCN 1 after 3 days of treatment.

Higher activity of ADC and lower ODC activity in chilled TNG 67 seedlings indicated that chilling-induced putrescine accumulation was synthesized through ADC pathway. These results were consistent with other data in which most of stress-induced accumulation of putrescine content was accompanied by a remarkable increase of ADC activity (Reggiani et al., 1989).

In conclusion, it has been showed that ethylene and ABA may act as stress hormones and mediate plant responses to low temperature. Polyamines are also found to accumulate in

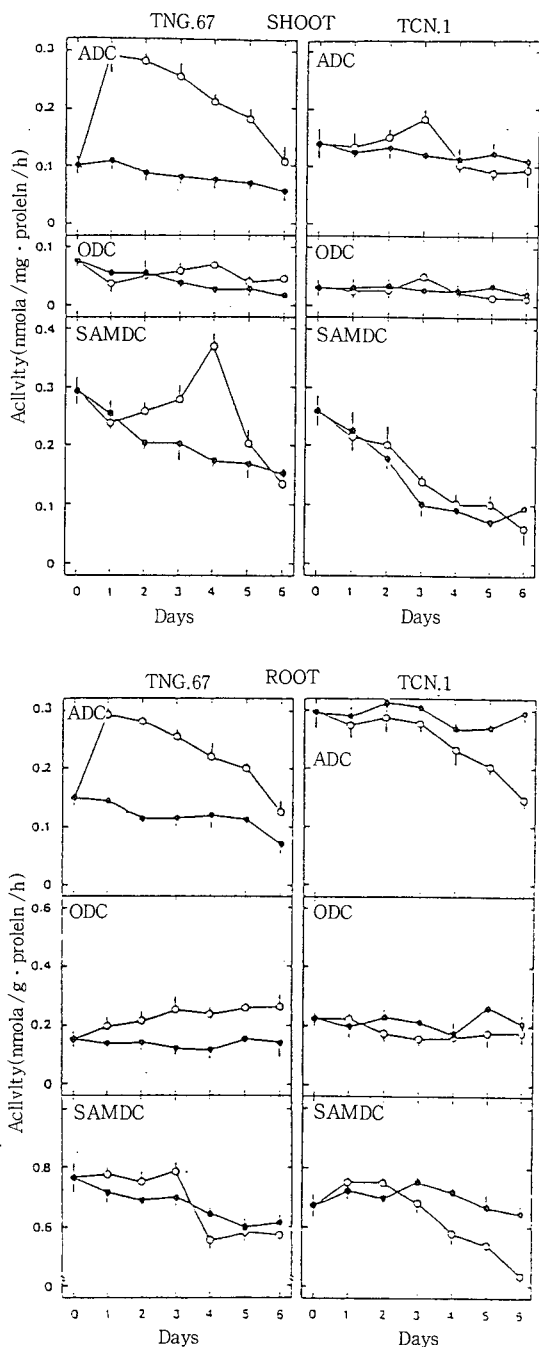


Fig. 5 Changes of specific activity of ADC, ODC, and SAMDC in shoots and roots of chilled rice (*Oryza sativa* L.) seedlings. ●, 25/30°C; ○, 5/5°C. Bars indicated the standard error.

chilling condition and show a close relationship with chilling tolerance. Plant hormones that improve tolerance to chilling may affect stress protein production, which may serve as a functional linkage between environmental stress and biochemical adaptation, molecular approaches would help us further to understand the mechanism of chilling tolerance in rice.

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