ELSEVIER

Plant Science 126 (1997) 1–10

PLANT SCIENCE

Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings. II. Modulation of free polyamine levels

Tse-Min Lee [a,*], Huu-Sheng Lur [b], Chun Chu [b]

[a] *Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung, 80424, Taiwan, ROC*
[b] *Department of Agronomy, National Taiwan University, Taipei, Taiwan, ROC*

Received 9 December 1996; received in revised form 17 March 1997; accepted 18 March 1997

Abstract

We have investigated the role of abscisic acid (ABA) in the regulation of polyamine biosynthesis in rice (*Oryza sativa* L.) seedlings exposed to chilling (5°C). In a chilling-tolerant cultivar (cv. Tainung 67, TNG.67), levels of free putrescine and activity of arginine decarboxylase (ADC, EC 4.1.1.19) in both shoots and roots, and levels of free spermidine/spermine and activity of *S*-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) in shoots increased after exposure to chilling. In a chilling-sensitive cultivar (cv. Taichung Native 1, TN.1), level of free putrescine and activity of ADC in shoots increased slightly after exposure to chilling while those in roots decreased. Activity of ornithine decarboxylase (ODC, EC 4.1.1.17) in both cultivars remained unchanged after exposure to chilling. α-Difluoromethylarginine (DFMA), an irreversible inhibitor of ADC, but not α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, at 0.5 mM inhibited free putrescine accumulation in chilled TNG.67 and resulted in a decrease in chilling tolerance; a decrease in survival and an increase in electrolyte leakage. The effects of DFMA were partially reversed by the addition of 0.5 mM putrescine. In TNG.67, chilling induced an increase of free ABA levels first, then ADC activity and finally free putrescine levels. Fluridone, an inhibitor of ABA synthesis, at 0.5 mM inhibited the increase of free ABA levels, ADC activity and free putrescine levels in chilled TNG.67, and resulted in a less tolerance of TNG.67 to chilling; these effects could be reversed by the pre-chilling treatment of 10 μM ABA. Application of 10 μM ABA for 48 h before chilling not only rendered TN.1 tolerant to chilling but also induced a change of polyamine pattern to be similar to chilled TNG.67. It seems that, for the materials used in this study, one of strategies of rice seedlings to resist chilling stress is to raise ABA levels and, in turn, one of ABA's functions is to enhance the ADC-mediated putrescine synthesis. © 1997 Elsevier Science Ireland Ltd.

Keywords: ABA; Chilling tolerance; *Oryza sativa*; Polyamine

* Corresponding author. Tel.: +886 7 5252000, ext. 5110; fax: +886 7 5255100; e-mail: tmlee@mail.nsysu.edu.tw

PII S0168-9452(97)00076-9

1. Introduction

Polyamines (putrescine, spermidine and spermine) have been implicated in the regulation of plant growth and development [1,2]. There is also an association of polyamines with plant responses to many stresses [1,2]. In higher plants, putrescine could be directly synthesized from ornithine via ornithine decarboxylase (ODC; EC 4.1.1.17) [1–4]. Besides, putrescine could be indirectly synthesized from arginine; arginine is decarboxylated to agmatine by arginine decarboxylase (ADC; EC 4.1.1.17), then hydrolyzed to *N*-carbamoylputrescine by agmatine iminohydrolase (EC 3.5.3.12) and finally *N*-carbamoylputrescine is converted to putrescine by *N*-carbamoylputrescine aminohydrolase (EC 3.5.1.-) [3,4]. Spermidine/spermine are synthesized from putrescine by the addition of propylamine group from decarboxylated *S*-adenosylmethionine that is derived from *S*-adenosylmethionine (SAM) by the action of *S*-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) [3,4].

The role of polyamines in chilling tolerance has been studied in several plants [5–8]. By using 11 cultivars, the chilling-induced changes in free putrescine levels are positively associated with the cultivar resistance to chilling ($r = 0.95$) [6]. In the system of roots cultured in vitro, free putrescine was also found to be an important factor in the resistance of rice roots to low temperature [10]. Tajima and Kabaki [9] showed that application of spermidine could ameliorate chilling injury of rice seedlings.

Abscisic acid (ABA) is essential for rice seedlings to resist chilling stress [11] and controls intricate functions with chilling tolerance, such as the maintenance of water balance through the closure of stomata and the enhancement of root conductivity, as well as the prevention of membrane damage [11,12]. Recently, we have found that the chilling-induced increase of ABA rose before that of putrescine in rice seedlings of chilling-tolerant cultivars [6]. It raises the possibility that ABA may influence polyamine metabolism in respect to chilling tolerance. The relationships between ABA and polyamines have been studied in several plant systems [13–18]. In cucumber cotyledons [13] and rice embryos [14], ABA decreases polyamine levels and ADC activity. In contrast, ABA above 3.78 μM increases putrescine levels in peeled oat leaf segments [15]. Putrescine levels were also observed to increase in the ABA-induced senescent rice leaves [17]. Aurisano et al. [18] showed that, in wheat seedlings, ABA could induce a stress-like pattern of polyamine metabolism.

In this study, the changes of polyamine levels and their biosynthetic enzyme activities and the putative role of ABA in chilling-induced changes in polyamine metabolism were determined in two cultivars with different chilling tolerance. Since fluridone, an ABA biosynthesis inhibitor [19], has been reported to inhibit the chilling-induced ABA accumulation in rice seedlings [11], its effects on polyamine biosynthesis were determined. Besides, since ABA increases chilling tolerance of chilling-sensitive rice seedlings [10], the effects of ABA acclimation on polyamine biosynthesis were also determined in chilling-sensitive rice seedlings.

2. Materials and methods

2.1. Plant materials and treatments

A chilling-tolerant (cv. Tainung 67, TNG.67, Japonica type) and a chilling-sensitive (cv. Taichung Native 1, TN.1, Indica type) cultivar were used. Seeds were sterilized with 5% sodium hypochloride for 10 min and then rinsed with distilled water. After germination for 2 days at 37°C, seedlings were cultured in 250 ml beakers containing half-strength Kimura B solution and incubated in phytotron with natural light at 30°C day/25°C night and 95% relative humidity as described previously [11]. Seedlings with 3 leaves were chilled at 5°C day/5°C night (85% relative humidity) for 96 h. The photoperiod was 12 h and the light strength was 325 μmol m^{-2} s^{-1}, achieved by a mixture of cool-fluorescent (FL40D, 40 W, China Electric Apparatus, Taoyuan, Taiwan, ROC) and incandescent (I60, 60 W, China Electric Apparatus) light. For ABA pretreatment, (±)-ABA at 10 μM (pH 5.0) was applied hydroponically to roots of rice seedlings for 48 h before chilling.

2.2. Determination of survival ratio, electrolyte leakage and triphenyl tetrazolium chloride (TTC) viability

Survival and relative degree of electrolyte leakage of chilled rice seedlings were determined according to Lee et al. [11]. The TTC assays were determined according to Steponkus and Lanphear [20].

2.3. Extraction and determination of free polyamines

Levels of free polyamines were determined by HPLC according to Lee and Chu [21]. The recovery of polyamines by evaluating diaminopropane in these procedures ranged from 74 to 89%. The polyamine levels were the average of four replicates and expressed as micromoles per gramme of dry weight.

2.4. Determination of polyamine biosynthetic enzyme activity

Activities of the polyamine biosynthetic enzymes were determined according to Lee and Chu [21]. Tissue (about 0.4 g fresh weight) was frozen in liquid nitrogen, ground to a fine powder and homogenized with 1.5 ml extraction buffer containing 25 mM potassium-phosphate, 50 μM ethylenediaminetetraacetic acid, 100 μM phenylmethylsulfonylfluoride and 25 mM ascorbic acid (pH 8.0). The homogenate was then centrifuged at 5000 × g for 20 min at 4°C and the supernatant dialyzed at 4°C against 2 l extraction buffer for 24 h in darkness. Dialyzed extract (50 μl) was used in the enzyme assay. Enzyme activity was determined by measuring CO_2 evolution. The reaction buffers for ADC, ODC and SAMDC assays were 0.1 ml of 200 mM Tris–HCl (pH 8.5) buffer, 0.1 ml of 200 mM Tris–HCl (pH 8.0) buffer and 0.1 ml of 200 mM potassium-phosphate (pH 7.5) buffer, respectively. After reincubation of enzyme extract and reaction buffer at 0°C for 5 min, 10 (l of the respective substrate solution, 3.66 mM arginine (containing 185 KBq/ml L-[U-^{14}C]arginine), 21.55 mM ornithine (containing 185 KBq/ml L-[1-^{14}C]ornithine) and 0.57 mM *S*-adenosylmethionine (containing 37 KBq/ml *S*-adenosyl-L-[carboxyl-^{14}C]methionine) were added to the reaction mixture. The 10-ml reaction tubes were sealed with silicone rubber caps and incubated at 40°C for 120 min with shaking. The released $^{14}CO_2$ was trapped by two 2 M KOH-impregnated filter paper discs. The reaction was stopped by injecting 0.2 ml 10% (w/v) trichloroacetic acid with a syringe and then trapping for a further 60 min. The paper discs were allowed to dry, put in 5 ml scintillation liquid and their radioactivity was measured in a Beckman LS-1801 liquid scintillation spectrometer (Beckman Instruments, Irvine, CA). Enzyme activity was expressed as nanomoles of $^{14}CO_2$ released per milligramme of protein per hour. Protein content was determined according to Bradford [22] with bovine serum albumin as standard.

2.5. Extraction and determination of free ABA

Tissues (about 1.5 g dry weight) were frozen in liquid nitrogen, ground to a fine powder and extracted with 4 ml of extraction solution (80% methanol, 1% (v/v) acetic acid, 100 mg/l of butylated hydroxytoluene) by shaking at 4°C for 36 h in darkness. DL-*cis*, *trans*-[G-3H]-ABA (0.1461 pmol, 166.5 Bq) was added as an internal standard for estimating extraction efficiency. Average recovery in free ABA extraction ranged from 59 to 75%. The isolation of *cis*-ABA in samples by HPLC and the determination of levels of *cis*-(+)-ABA by an indirect enzyme-linked immuno-sorbent assay were according to Lee et al. [11].

2.6. Chemicals

(±)-ABA, putrescine, spermidine and spermine were purchased from Sigma (St. Louis, MO). DL-*cis*, *trans*-[G-3H]-ABA (1.91 TBq/mmol), L-[U-^{14}C] arginine mono-chloride (1.85 GBq/mmol), L-[1-^{14}C]ornithine monochloride (1.85 GBq/mmol), and *S*-adenosyl-[carboxyl-^{14}C]methionine (1.85 GBq/mmol) were obtained from Amersham (Buckinghamshire, UK). Monoclonal antibody for (*S*)-ABA was purchased from Idetek (San Bruna, CA). DFMA and DFMO were kindly provided by Dr P.P. McCann (Merrill-Dow Research Center, Cincinnati, OH).

3. Results

3.1. Evaluation of chilling tolerance of TNG.67 and TN.1 seedlings

The degree of chilling tolerance of TNG.67 and TN.1 was evaluated by survival, electrolyte leakage and ability to reduce TTC. As compared with the chilled TN.1, the chilled TNG.67 had high survival (ca. 100%) (Fig. 1B) and low electrolyte leakage (Fig. 1C). The chilled TNG.67 had a greater ability to reduce TTC than the chilled TN.1 (Fig. 2). Fig. 1A shows that chilled TN.1 seedlings had a serious bleaching damage after re-warming at 30°C/25°C while chilled TNG.67 seedlings remained healthy and green, only a slight bleaching on leaf tips was observed.

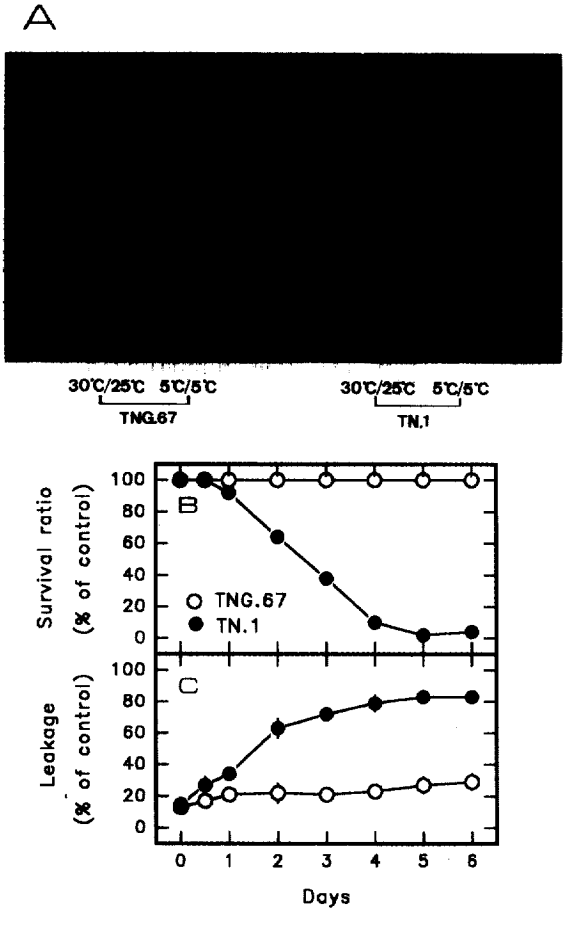

Fig. 1. Effects of chilling (5°C/5°C, 96 h) on the appearance of rice seedlings after re-warming at 30°C/25°C for 6 days, and evaluation of chilling tolerance by survival ratio ($n = 100$) and electrolyte leakage ($n = 4$). Means ± S.E. are indicated. A, appearance; B, survival ratio; C, electrolyte leakage.

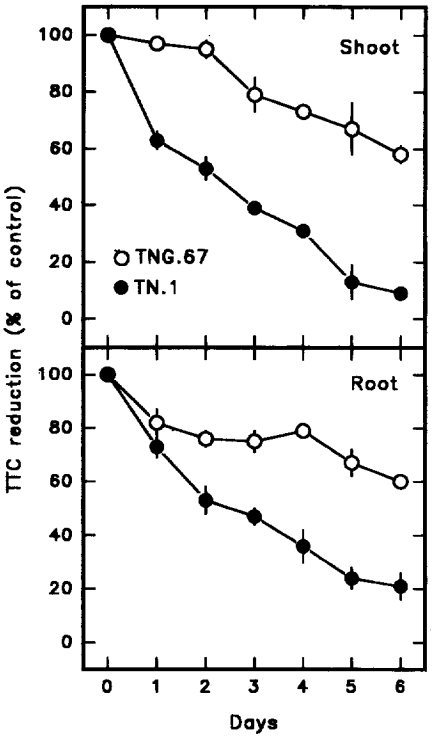

Fig. 2. TTC reduction ability in shoots and roots of rice seedlings. The reduction of TTC of chilled shoots or roots was presented as a percentage of 30°C/25°C controls. Means ± S.E. ($n = 4$) are indicated.

3.2. Levels of free polyamines and activities of ADC, ODC and SAMDC

In TNG.67 shoots, free putrescine levels increased after chilling and reached a maximum at day 2, while free spermidine/spermine levels increased, with a peak at day 4 (Fig. 3). In TN.1 shoots, free putrescine levels started to increase at day 3 after chilling and then decreased, while free spermidine/spermine levels were similar to the controls at 30°C/25°C (Fig. 3).

In chilled roots, free putrescine levels increased in TNG.67 but decreased in TN.1 (Fig. 4). Free spermidine/spermine in roots of both cultivars

remained stable with time after exposure to chilling (Fig. 4). The chilling-induced changes in free polyamine levels in TNG.67 were superior to those in TN.1.

Changes of ADC, ODC and SAMDC activities in response to chilling are shown in Figs. 5 and 6. In chilled TNG.67, ADC activities in both shoots (Fig. 5) and roots (Fig. 6) increased rapidly and reached the maximum at day 1. In chilled TN.1, ADC activity in shoots increased slightly at day 2 (Fig. 5), and that in roots decreased below the 30°C/25°C control after 4 days (Fig. 6). In both TNG.67 and TN.1, as compared to the control at 30°C/25°C, ODC activities in shoots (Fig. 5) or roots (Fig. 6) remained almost unchanged after chilling.

In TNG.67, SAMDC activity in shoots increased after 1 day of chilling (Fig. 5) but that in roots remained similar as the 30°C/25°C controls (Fig. 6). In TN.1, SAMDC activity in shoots remained unchanged after chilling but that in roots decreased after 3 days (Fig. 6).

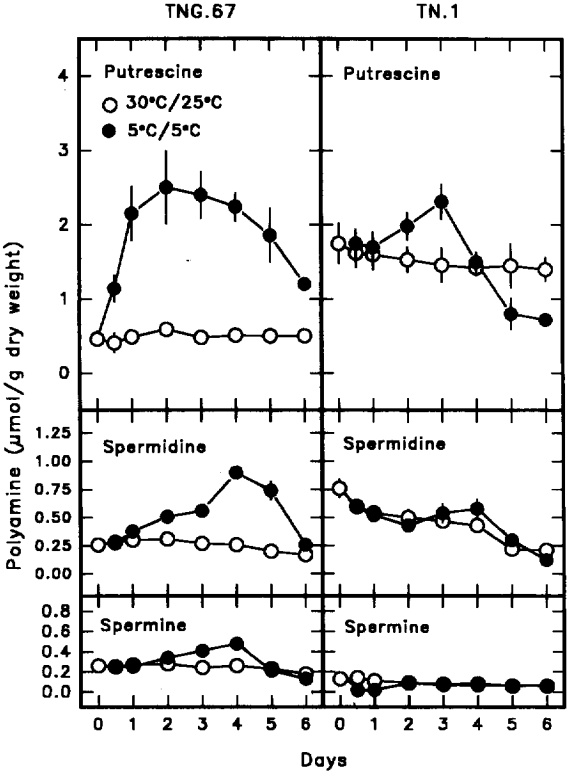

Fig. 3. Levels of free polyamines in shoots of TNG.67 and TN.1. Means ± S.E. ($n = 4$) are indicated.

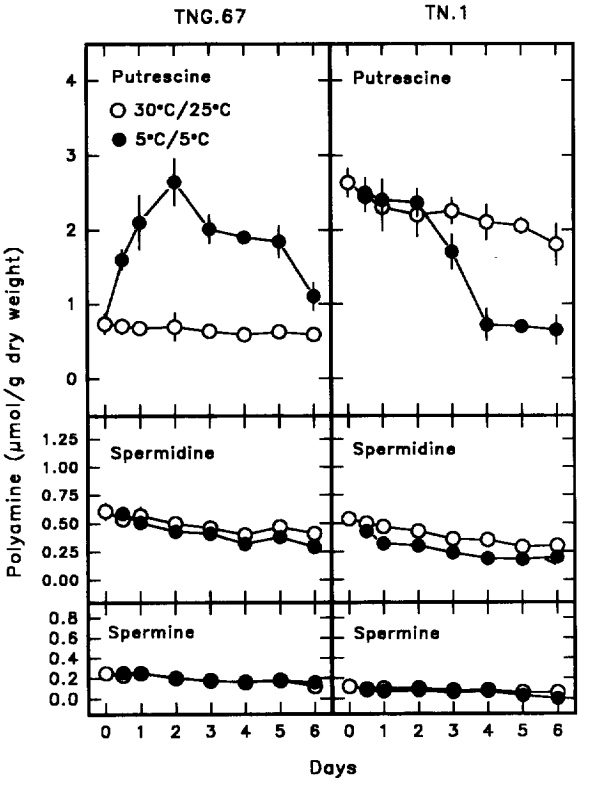

Fig. 4. Levels of free polyamines in roots of TNG.67 and TN.1. Means ± S.E. ($n = 4$) are indicated.

3.3. Effects of DFMA and DFMO on chilling tolerance and free polyamine levels of chilled TNG.67

The role of putrescine in the tolerance of TNG.67 to chilling stress was further determined by applying α-difluoromethylarginine (DFMA), an irreversible inhibitor of ADC, or α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC. As shown in Table 1, DFMA, at 0.5 mM, inhibited free putrescine accumulation in chilled TNG.67, and resulted in a decrease in chilling tolerance; a decrease in survival and an increase in electrolyte leakage. The effects of DFMA could be partially reversed by the addition of 0.5 mM putrescine (Table 1). DFMO, at 0.5 mM, had little effects on chilling tolerance and free polyamine levels (Table 1).

3.4. Effects of fluridone on free ABA levels, free putrescine levels and chilling tolerance of chilled TNG.67

In chilled TNG.67, free ABA levels in whole plants increased at around 2 h while ADC activity started to increase at 4 h and free putrescine levels started to increase at 6 h (Fig. 7).

Since fluridone, an inhibitor of ABA biosynthesis [19], could inhibit the chilling-induced ABA accumulation in TNG.67 [9], the effects of fluridone only or fluridone plus ABA on chilling tolerance and levels of free ABA and free putrescine were compared to elucidate the role of ABA in chilling-induced putrescine accumulation.

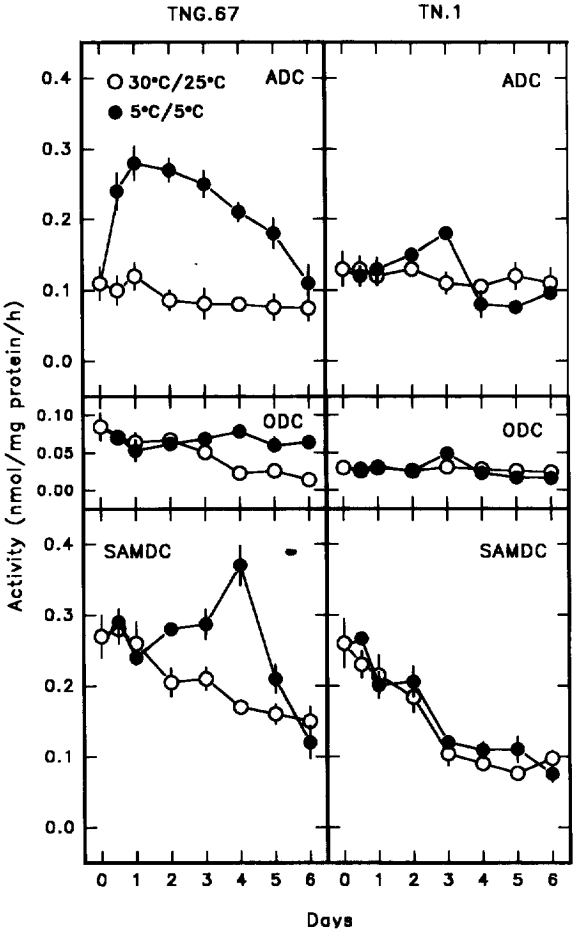

Fig. 5. Activities of ADC, ODC and SAMDC in shoots of TNG.67 and TN.1. Means ± S.E. ($n = 4$) are indicated.

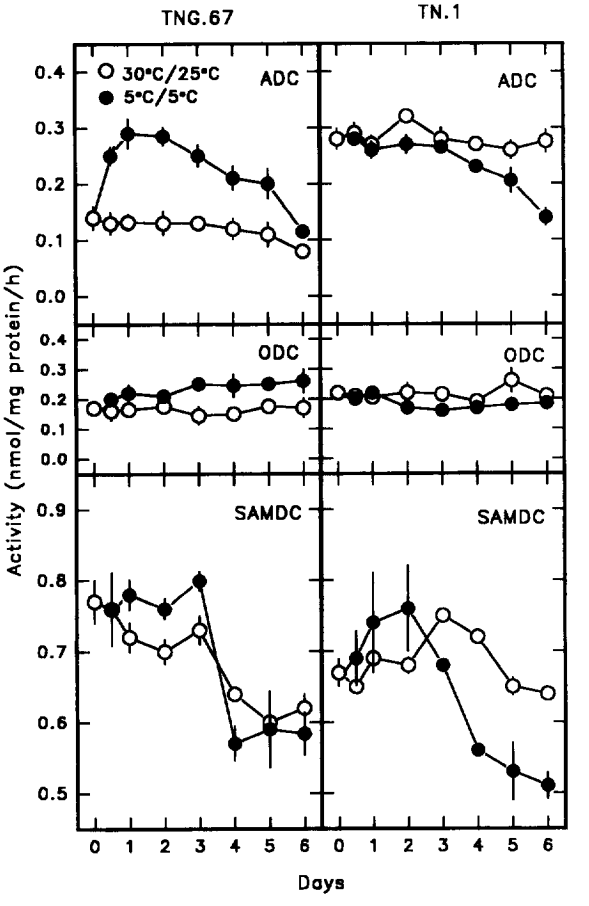

Fig. 6. Activities of ADC, ODC and SAMDC in roots of TNG.67 and TN.1. Means ± S.E. ($n = 4$) are indicated.

It was found that fluridone, at 0.5 mM, inhibited the chilling-induced accumulation in both free ABA and free putrescine (Table 2). As shown in Table 2, the fluridone-treated seedlings exhibited less tolerance to chilling; a decrease in survival and an increase in electrolyte leakage. The effects of fluridone could be partially reversed by the application of 10 μM ABA on hydroponically grown roots for 48 h before chilling (Table 2).

3.5. Levels of free polyamines in ABA-pretreated TN.1 seedlings under chilling conditions

Application of 10 μM ABA in hydroponical roots for 48 h before chilling resulted in an enhancement of chilling tolerance of TN.1. As com-

Table 1
Effects of 0.5 mM DFMA and 0.5 mM DFMO on polyaine levels ($n = 4$), electrolyte leakage ($n = 4$) and survival ratio ($n = 100$) of TNG.67 rice (*Oryza sativa* L.) seedlings after 96 h at 5°C/5°C

Treatments	Tree polyamine levels (μmol/g dry weight)			Leakage (%)	Survival ratio (%)
	Putrescine	Spermidine	Spermine		
30°C/25°C	1.52 ± 0.13	0.87 ± 0.06	0.21 ± 0.03	12.06 ± 3.50	—
5°C/5°C	5.06 ± 0.18	1.51 ± 0.04	0.34 ± 0.03	14.04 ± 2.51	100
5°C/5°C + DFMA	0.81 ± 0.07	0.52 ± 0.10	0.14 ± 0.04	52.57 ± 9.14	48
5°C/5°C + DFMO	4.59 ± 0.27	1.73 ± 0.06	0.30 ± 0.09	20.81 ± 3.01	93
5°C/5°C + DFMA + putrescine	8.97 ± 2.01	2.11 ± 0.59	0.95 ± 0.21	37.05 ± 4.14	67

DFMA (0.5 mM) or DFMO (0.5 mM) containing 0.01% Tween 20 was applied in whole plants at the start of chilling. In the '5/5°C + DFMA + putrescine' treatment, putrescine at 0.5 mM containing 0.01% Tween 20 was pretreated in roots and sprayed on shoots 12 h before chilling. Means ± S.E. are indicated.

pared with TN.1 without ABA pretreatment, ABA-pretreated seedlings had high survival (ca. 100%) and low electrolyte leakage (ca. 17%) after 4 days of chilling.

As compared with TN.1 without ABA treatment, 48 h of 10 μM ABA treatment resulted in a decrease in levels of both free putrescine and spermidine, but an increase in levels of free spermine. When transferred to chilling conditions, the levels of free putrescine in shoots of ABA-pretreated seedlings increased and reached the maximum at day 2, and those in roots also rose rapidly with a peak at 3 h after chilling and then dropped thereafter (Fig. 8). Levels of free spermidine in both shoots and roots of ABA-pretreated seedlings also increased after chilling (Fig. 8). Levels of free spermine in ABA-pretreated shoots remained unchanged after chilling while those in ABA-pretreated roots increased with a peak at 12 h after chilling (Fig. 8). As shown in Fig. 8, the increase of polyamine levels in ABA-pretreated TN.1 seedlings was more remarkable in roots than in shoots.

4. Discussion

Levels of free polyamines, especially putrescine, are greatly different between two tested rice cultivars grown at 30°C/25°C; free putrescine levels in both shoots and roots of TN.1 are about 2.5-times those in TNG.67. However, when transferred to chilling conditions, free polyamines, especially putrescine, significantly accumulated in TNG.67 (a chilling-tolerant cultivar) instead of TN.1 (a chilling-sensitive cultivar). A change in the relative amount, rather than the absolute amount, of free putrescine is positively associated with chilling tolerance of rice seedlings. A similar result has been observed in *Phaseolus* [5]. Current data from a decrease of chilling tolerance by DFMA and its partial reversion by putrescine in TNG.67 indicate that putrescine might be related to the ability of seedlings of TNG.67 to tolerate chilling stress. However, more work is needed to elucidate the correlation between putrescine and chilling tolerance in rice seedlings.

In addition to cultivar difference, the responses of polyamine metabolism to chilling are also different among tissues in TNG.67. In chilled shoots, free putrescine, spermidine and spermine accumulated, but only free putrescine accumulated in roots.

The result which ADC activity rose before free putrescine accumulation in chilled TNG.67 indicates that ADC, but not ODC, responds to the chilling-induced free putrescine accumulation. The inhibition of putrescine accumulation in chilled TNG.67 by DFMA instead of DFMO is a further support. This is consistent with the notion that most stress-induced putrescine increase is derived from ADC [1,2]. A parallelism in the increase between free spermidine/spermine levels and SAMDC activity suggests that SAMDC may con-

Table 2
Effects of 0.5 mM fluridone on acitivity of ADC and levels of free ABA and polyamines and electrolyte leakage (means ± S.E., $n = 4$) in TNG.67 rice (*Oryza sativa* L.) seedlings after 48 h of chilling treatment

Treatments	Free ABA level (nmol/g dry weight)	ADC activity (nmol/mg protein/h)	Free polyamine levels (μmol/g dry weight)			Leakage (%)	Survival ratio (%)
			Putrescine	Spermidine	Spermine		
30°C/25°C	1.04 ± 0.19	0.12 ± 0.03	1.52 ± 0.09	0.74 ± 0.12	0.49 ± 0.04	11.75 ± 2.65	—
5°C/5°C	3.59 ± 0.08	0.28 ± 0.07	5.19 ± 0.27	0.73 ± 0.09	0.39 ± 0.05	18.94 ± 5.07	100
5°C/5°C	0.99 ± 0.27	0.15 ± 0.04	0.93 ± 0.11	0.37 ± 0.05	0.54 ± 0.01	56.17 ± 5.13	6
+ fluridone							
5°C/5°C	6.91 ± 0.53	0.35 ± 0.06	4.38 ± 0.57	0.69 ± 0.07	0.12 ± 0.08	12.9 ± 4.56	89
+ fluridone							
+ ABA							

Fluridone was added in culture solution and sprayed on shoots at the start of the chilling treatment. ANA at 10 μM containing 0.01% Tween 20 was applied on both shoots and roots 46 h before chilling. Tween 20 at 0.01% had no effects on activity of ADC and levels of free ANA and polyamines and electrolyte leakage.

tribute to the accumulation of free spermidine/spermine in shoots of chilled TNG.67.

Several lines of evidence suggest that ABA is one of factors involving in the regulation of putrescine synthesis in chilling-tolerant rice cultivars exposed to chilling. In the chilling-tolerant cultivar (cv. TNG.67), ABA rose before ADC activity increase and free putrescine accumulation. Fluridone inhibited the accumulation of both free ABA and putrescine in chilled TNG.67. However, the inhibitory effects of fluridone on an increase of ADC activity and free polyamine levels were only partially reversed by ABA; indicating that ABA might not be the only factor in the regulation of polyamine metabolism in chilled rice seedlings.

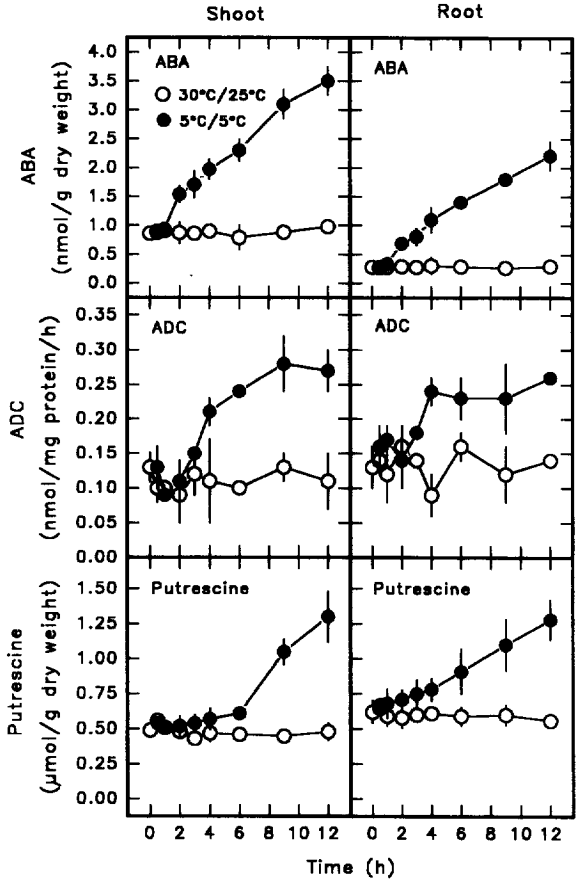

Fig. 7. Changes in free ABA levels, ADC activity and free putrescine levels in whole plants of TNG.67. Means ± S.E. ($n = 4$) are indicated.

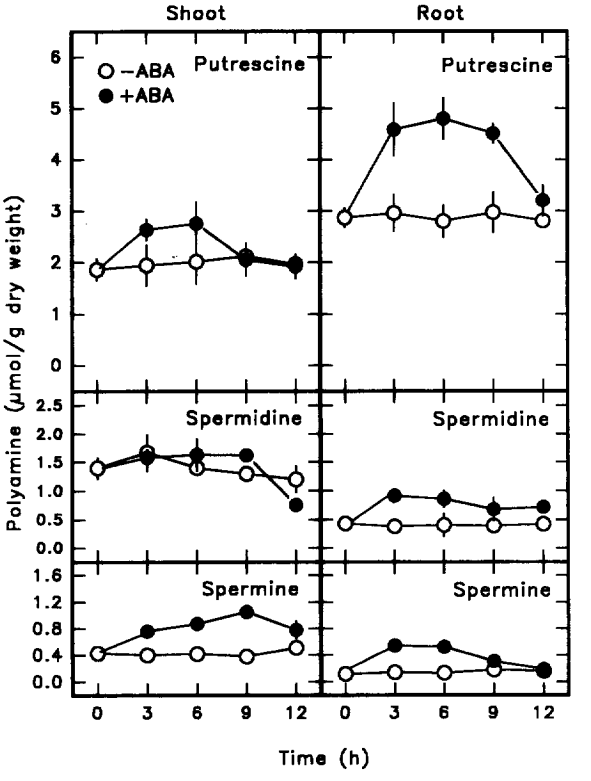

Fig. 8. Effects of chilling on free polyamine levels in 10 μM ABA-pretreated TN.1 seedlings. After 48 h of ABA pretreatment in hydroponical roots, seedlings were transferred to 5°C/5°C for various periods. Means ± S.E. ($n = 4$) are indicated.

Apparently, one of the mechanisms in chilling-tolerant rice cultivars to resist chilling stress is to raise free ABA levels and, in turn, one of ABA's functions is to enhance the ADC-mediated putrescine synthesis. If the above review sustains, the acclimation of a chilling-sensitive cultivar (cv. TN.1) by ABA pretreatment should induce a change in the pattern of polyamine metabolism. Data from Fig. 8 show that ABA (10 μM) pretreatment, except inducing tolerance to TN.1, also rendered polyamine accumulation under chilling as TNG.67 did. It seems that, in chilling-sensitive rice seedlings, ABA acclimation also causes a change in polyamine metabolism.

In conclusion, a link between ABA levels, ADC activity, putrescine levels and chilling tolerance was observed in the materials (cv. TNG.67 and TN.1) used here. However, whether this is a gen-

eral phenomenon is still waited to be tested in seedlings of other rice cultivars. Besides, even it is demonstrated that polyamines, especially putrescine, may mediate one of ABA's effects in acclimation or tolerant related processes to chilling, more questions still need to be clarified. The rationale of relationships among ABA, polyamines and other chilling-related physiological characteristics has been now undertaken in hoping to unveil the mechanisms conferring chilling tolerance of rice seedlings.

Acknowledgements

The authors thank Dr P.P. McCann (Merrill-Dow Research Center, Cincinnati, OH) for providing DFMA and DFMO. This paper is based upon work supported by the National Science Council, Executive Yuan, Republic of China, under Grant No. NSC 86–2611-B-110–009.

References

[1] A.W. Galston, R. Kaur-Sawhney, Polyamines as endogenous growth regulator, in: P.J. Davies (Ed.), Plant Hormones and Their Role in Plant Growth and Development, Martinus Nijhoff, Boston, 1987, pp. 208–295.

[2] P.T. Evans, R.L. Malmberg, Do polyamines have roles in plant development?, Annu. Rev. Plant Physiol. Plant Mol. Biol. 40 (1989) 235–269.

[3] R.D. Slocum, R. Kaur-Sawhney, A.W. Galston, The physiology and biochemistry of polyamines in plants, Arch. Bioch. Biophys. 235 (1900) 283–303.

[4] T.A. Smith, Polyamines, Ann. Rev. Plant Physiol. 36 (1900) 117–143.

[5] M.G. Guye, L. Vigh, J.M. Wilson, Polyamine titre in relation to chill-sensitivity in *Phaseolus* sp, J. Exp. Bot. 37 (1986) 1036–1043.

[6] T.M. Lee, H.S. Lur, C. Chu, Abscisic acid and putrescine accumulation in chilling-tolerant rice cultivars, Crop Sci. 35 (1995) 502–508.

[7] F.G. Kramer, C.Y. Wang, Correlation of reduced chilling injury with increased spermine and spermidine levels in zucchini squash, Physiol. Plant. 76 (1989) 479–484.

[8] F.G. Kramer, C.Y. Wang, Effects of chilling and temperature preconditioning on the activity of polyamine biosynthetic enzymes zucchini squash, J. Plant Physiol. 136 (1990) 115–119.

[9] K. Tajima, N. Kabaki, Effects of sugars and several growth regulators on the chilling injury of rice seedlings, Jpn. J. Crop Sci. 50 (1981) 411–412.

[10] T.M. Lee, Polyamine regulation of growth and chilling tolerance of rice (*Oryza sativa* L.) roots cultured in vitro, Plant Sci. 122 (1997) 111–117.

[11] T.M. Lee, H.S. Lur, C. Chu, Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings. I. Endogenous ABA levels, Plant Cell Environ. 16 (1993) 481–490.

[12] R.C. Pan, The role of abscisic acid in chilling resistance, in: R.P. Pharis, S.B. Rood (Eds.), Plant Growth Substances, Springer-Verlag, New York, 1988, pp. 391–399.

[13] M.R. Suresh, S. Ramakrishna, P.R. Adiga, Regulation of arginine decarboxylase and putrescine levels in *Cucumis sativa* cotyledons, Phytochemistry 17 (1978) 57–63.

[14] M.M. Choudhuri, B. Ghosh, Purification and partial characterization of arginine decarboxylase from rice (*Oryza sativa* L.) embryo, Agric. Biol. Chem. 46 (1982) 739–743.

[15] A. Mukhopadhyay, M.M. Choudhuri, K. Sen, B. Ghosh, Changes in polyamines and related enzymes with loss of viability in rice seeds, Phytochemistry 22 (1983) 1547–1551.

[16] P.P.C. Lin, Effects of methylglyoxal-bis-(guanylhydrazone) and abscisic acid on polyamine metabolism in embryonectomized barley seeds, Plant Growth Regul. 3 (1985) 257–268.

[17] T.C. Chen, C.H. Kao, Senescence of rice leaves XXXII. Effects of abscisic acid and benzyladenine on polyamines and ethylene production during senescence, J. Plant Physiol. 139 (1992) 617–620.

[18] N. Aurisano, A. Bertani, M. Mattana, R. Reggiani, Abscisic acid induced stress-like polyamine pattern in wheat seedlings, and its reversal by potassium ions, Physiol. Plant. 89 (1993) 687–692.

[19] R. Moore, J.D. Smith, Growth, graviresponsiveness and abscisic acid content of *Zea mays* seedlings treated with fluridone, Planta 162 (1984) 342–344.

[20] P.L. Steponkus, O.F. Lanphear, Refinement of the triphenyl tetrazolium chloride method of determining cold injury, Plant Physiol. 42 (1967) 1423–1426.

[21] T.M. Lee, C. Chu, Ethylene-induced polyamine accumulation in rice (*Oryza sativa* L.) coleoptiles, Plant Physiol. 100 (1992) 238–245.

[22] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.