園学雑. (J. Japan. Soc. Hort. Sci.) 67(6) : 849-855. 1998.

Effect of Various Inhibitors on Ethylene-enhanced Degreening of Radish (*Raphanus sativus* L.) Cotyledons

Masaru Adachi, Yoshinori Uchida and Keishi Shimokawa
Faculty of Agriculture, Miyazaki University, Miyazaki 889-2192

Summary

When the ethylene-enhanced degreening of radish (*Raphanus sativus* L.) cotyledons held in the dark was investigated, the rates of chlorophyll (Chl) catabolism decreased and the action of ethylene was blocked by inhibitors. Inhibition of the degreening of cotyledons occurred when cotyledons of seedlings were treated with cycloheximide (CHI), actinomycin D (ACD), silver nitrate ($AgNO_3$), silver thiosulfate (STS), and allyl-isothiocyanate (AITC), but not chloramphenicol (CAP), an inhibitor of plastid RNA synthesis. Moreover, a high negative correlation between the degree of greenness of radish cotyledons and Chl-degrading enzyme activity was obtained: $Y = -11.85X + 64.27$, $P < 0.001$ ($r^2 = 0.995$), where X and Y are Chl *a*-degrading relative activity and intensity of the green color of the cotyledons, respectively. The correlation indicates that ethylene promotes the degreening process of radish cotyledons through the *de novo* synthesis of a Chl-degrading enzyme protein.

Direct inhibition of AITC on Chl-degrading activity was not observed at the level of concentration that could completely inhibit the ethylene-enhanced degreening of the cotyledons. This finding suggests that AITC is to act as an inhibitor of Chl-degrading enzyme induction in radish cotyledons.

Key Words: allyl-isothiocyanate, chlorophyll, degreening, ethylene, *Raphanus sativus*.

Introduction

The promotion of Chl degradation in fruits and leaves by ethylene treatment is well-known, whereas the role of endogenous ethylene on the enzymology of the degreening process in fruits and leaves is not fully understood. In addition, other phytohormones that retard aging or senescence, such as IAA, cytokinins, and gibberellin suppress ethylene-induced Chl catabolism (Abeles et al., 1989). Jasmonic acid enhanced Chl catabolism independently of the action of ethylene (Abeles et al., 1989). Treatment of the tissues with inhibitors of ethylene action and synthesis has failed to prevent endogenous Chl catabolism (Halder-Doll and Bangerth, 1987). These reports indicate that Chl catabolism is the result of ethylene-induced aging as opposed to the direct effect of ethylene itself. Knee (1991) proposed that ethylene acts as an accessory factor or modulator than a dominant regulator of leaf senescence. Senescence is a complex process controlled by various factors.

AITC (allyl mustard oil), $CH_2=CH-CH_2-N=C=S$, one of the most important volatile pungent components of wasabi (*Wasabi japonica* Matsum), horseradish (*Armoracia rusticana*), black mustard (*Brassica nigra* L.), and other *Brassica* crops (Kawakishi, 1985) is used in the food industry as a spice or flavoring agent. Its biological activities (anticarcinogenesis, antibacterial activity, and inhibition of platelet aggregation) have been investigated. Biological formation of AITC from its immediate precursor glucosinolate (sinigrin) is catalyzed by myrosinase (β-thioglucosidase). As active electrophilic reagents, the isothiocyanate moieties of many isothiocyanates, including AITC, easily react with nucleophilic reagents such as water, amines, and alcohols to form corresponding adducts (Kawakishi, 1985). Some reports have described AITC as an inhibitor of both the action and synthesis of ethylene in various fruits and vegetables. Shimokawa (1990) reported that AITC inhibits ethylene action during fruit ripening (*e.g.* tomato and banana-fruit), floral senescence (cut carnation flowers) and epinasty. AITC also inhibits ethylene-enhanced degreening in *Citrus unshiu* fruit (Shimokawa et al., 1994). Moreover, Yano et al. (1986) and Nagata et al. (1992a; b; 1993) showed that AITC inhibits ethylene synthesis and browning of shredded cabbage.

In a previous study, we concluded that Chl-oxidative enzymes are involved in Chl catabolism in the ethylene-enhanced degreening of radish cotyledons held in the dark (Adachi et al., 1996; Adachi, 1997; Adachi and Shimokawa, 1998), and Adachi and Shimokawa (1995) confirmed the involvement of O_2^- in the degradation of Chl *a* which is catalyzed by an ethylene-enhanced enzyme.

Received; July 14, 1997. Accepted; November 5, 1997.
A part of this paper was presented at the 1997 Autumn Meeting of the Japanese Society for Horticultural Science.

In this study we intend to clarify the mechanism of ethylene-enhanced Chl catabolism by administrating various inhibitors on intact radish cotyledons kept in the dark. Furthermore, AITC as a degreening inhibitor on ethylene-enhanced Chl catabolism was investigated, and the results are discussed briefly.

Materials and Methods

Plant materials

Six-day-old intact radish (*Raphanus sativus* L.) seedlings which were obtained from a local farm were used within 2 hr.

Inhibitor and ethylene treatments

Treatments consisted of soaking intact cotyledons in water for 0 to 60 min, in an inhibitor solutions for 5 to 60 min, and then blotted dry with soft paper towels (Adachi et al., 1996). After the treatments, the seedlings were placed on a moistened sponge in a 30-l plastic box containing a beaker with 50 ml of 5% KOH to absorb CO_2. They were then exposed to 100 ppm ethylene for 12 hr. Thereafter, the seedlings were further incubated in fresh air for 36 hr. All of the incubation were carried out in the dark at 25 °C (Adachi et al., 1996). Cotyledons soaked in water for 60 min were used as the control. For AITC vapor treatment, AITC solution was dripped on a strip of paper hanging in jars containing seedlings and left to evaporate (Shimokawa, 1990). After exposure to vaporous AITC, the treated-plants were transferred to AITC-free air; the control plants were held in the jars without AITC.

Measurement of green density and determination of Chl content

After 48 hr in the dark, the green density of the cotyledons was determined non-destructively in triplicate, using a Minolta SPAD-502 Chl meter, and expressed as SPAD values (Adachi et al., 1996). The Chl was extracted with 80% aqueous acetone (Arnon, 1949) and then the concentration determined spectrophotometrically under a dim, green light at 25 °C (Adachi et al., 1996).

Preparation of enzyme extracts

The excised cotyledons were immersed in cold (−20 °C) acetone and homogenized in a Waring blender. The homogenate was filtered through a Buchner funnel and the residue dried under vacuum and pulverized. The crude enzyme extract was prepared according to Adachi et al. (1996) by dissolving 5 g of acetone powders in 400 ml of 0.02 M potassium phosphate buffer (pH 7.0, with 0.2% Triton X-100) for 30 min at 25 °C. After filtering the mixture, the filtrate was centrifuged at 12,000 × *g* for 20 min at 0 °C, and the resultant supernatant was used as the crude enzyme extract.

Assay of Chl a-degrading activity

Chl *a*-degrading activity was determined spectrophotometrically by measuring absorbance at 670 nm. The standard reaction mixture contained the following: 2.4 ml of 0.02 M potassium phosphate buffer (pH 5.6, with 0.2% Triton X-100), 65.3 μM H_2O_2, 1.0 mM DCP, 0.1 mM EDTA, 13.5 μM Chl *a*, and the crude enzyme (0.43 mg protein) in a total volume of 3 ml. Activity is expressed as the decreased absorbance at 670 nm for 3 min per mg protein. The enzymatic assay was carried out under a dim green light at 25 °C (Adachi et al., 1996).

Measurement of protein content

Protein content was determined according to the method of Lowry et al. (1951).

Reagents

Chl *a* (purity, 99.0%) from chlorella was obtained from Wako Pure Chemical Industries Co., Ltd (Osaka); actinomycin D from the Sigma Chemical Co. (St. Louis, U. S. A.); other chemicals from the Nacalai Tesque, Inc. (Kyoto).

Results

1. Effect of inhibitors of RNA and protein synthesis on the Chl content in radish cotyledons

Immediately after exposure to 100 ppm ethylene for 12 hr, there was no significant difference in the Chl levels in radish cotyledons between air-treated (control) and ethylene-treated whole seedlings. After 24 hr, however, Chl decreased in the ethylene-treated intact radish seedlings and continued for 48 hr (data not shown). The effect of various compounds on the Chl levels after a 48 hr incubation period in the dark at 25 °C is shown in Fig. 1. Ethylene treatment caused a significant loss of Chl, compared with the controls ($P < 0.001$). CHI, an inhibitor of protein synthesis on cytoplasmic ribosomes (80S), prevented ethylene-enhanced Chl catabolism but in the CHI-treated control radish seedlings Chl catabolism was affected slightly. ACD, an inhibitor of nuclear RNA synthesis, also prevented ethylene-enhanced Chl catabolism. In contrast, CAP, an inhibitor of plastid RNA synthesis, did not prevent it.

2. Effect of inhibitors of ethylene action on the ethylene-enhanced degreening

Effects of time of immersion on the green density of radish cotyledons pretreated with STS are shown in Fig. 2A. Treatment for 10 min significantly inhibited the ethylene-enhanced degreening. An increase in green density with immersion time occurred during the first 30 min. The mean green density of radish cotyledons treated with increasing concentrations of STS is shown in

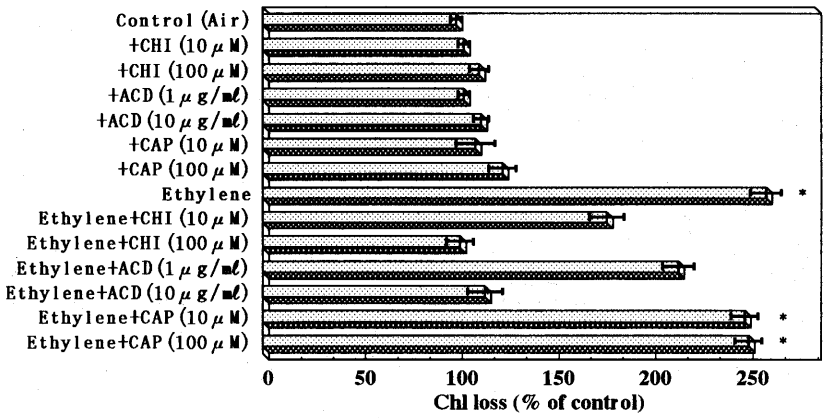

Fig. 1. Effect of inhibitors of RNA and protein synthesis on the Chl content of radish cotyledons. The horizontal bars represent S.D. of the mean. *Significantly different from the control at $P<0.001$ ($n=4$) by t-test. Data are means of triplicate experiments.

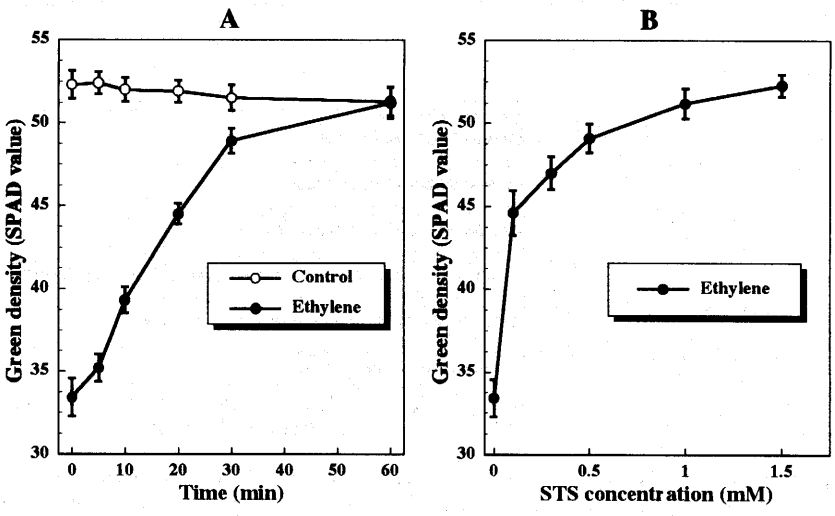

Fig. 2. Time-course study on the green density of the cotyledons pretreated with 1 mM STS followed with or without ethylene treatment (A). Relationship between the green density and STS concentration (B). The treatment time was 40 min. The vertical bars represent S.D. of the mean. Data are typical results from triplicate experiments.

Fig. 2B. Treatment with 50 μM STS did not significantly inhibit the degreening of cotyledons because it could not block ethylene-enhanced degreening (data not shown), whereas cotyledons treated with 1 mM STS completely blocked the action of ethylene. Similarly, $AgNO_3$ inhibited ethylene-enhanced degreening (Figs. 3A, B). In addition, both STS and $AgNO_3$ treatments of the control had little effect on the green density of radish cotyledons (Figs. 2A and 3A).

3. Effect of AITC on the ethylene-enhanced Chl catabolism

The effect of AITC on the Chl content after 48 hr of incubation in the dark is shown in Fig. 4. AITC (0.5 μl/l) vapor treatment significantly inhibited ethylene-enhanced Chl catabolism. Furthermore, one hour of exposure to volatilized AITC (2.5 μl/l) was sufficient to block ethylene-enhanced Chl loss in radish cotyledons. AITC treatment of the control radish seedlings enhanced the rate of Chl loss slightly in the cotyledons because AITC is a stress-inducing compound. To investigate its inhibitory actions further, AITC solution was applied to the reaction mixture of Chl *a*-degrading system but the mixture had no effect (Fig. 5).

4. Effect of various compounds on degreening and Chl a-degrading enzyme activity in radish cotyledons

The effect of various compounds on the green density

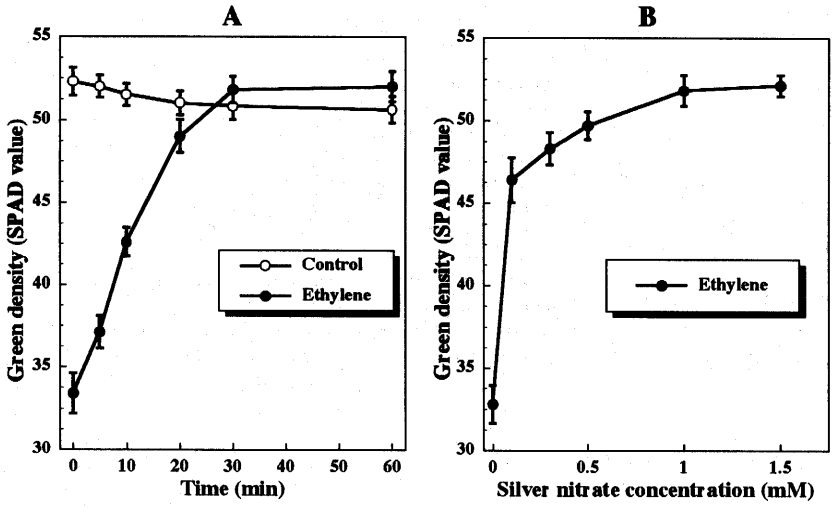

Fig. 3. Time-course study on the green density of the cotyledons pretreated with 1 mM $AgNO_3$ followed with or without ethylene treatment (A). Relationship between the green density and $AgNO_3$ concentration (B). The treatment time was 30 min. The vertical bars represent S.D. of the mean. Data are means of triplicate experiments.

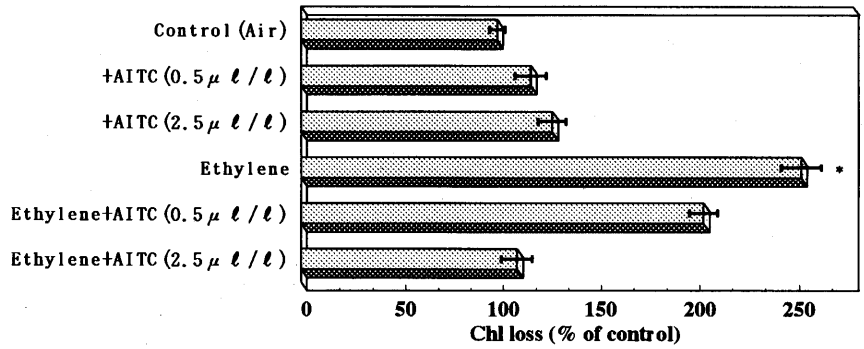

Fig. 4. Effect of AITC vapor treatments on the Chl content of radish cotyledons. The horizontal bars represent S.D. of the mean. *Significantly different from the control at $P<0.001$ (n=6) by *t*-test. Data are means of triplicate experiments.

and Chl *a*-degrading enzyme activity in the cotyledons after a 48 hr incubation period in the dark at 25 °C is shown in Table 1. The data show that ethylene caused the degreening of the cotyledons through the enhancement of the Chl *a*-degrading enzyme activity. A linear curve and a high significant negative coefficient of correlation ($r=-0.997$) express the relationship between green density and enzyme activity. The regression equation which is represented by: $Y=-11.85X+64.27$, $P<0.001$, $r=-0.997$ (Fig. 6) indicates that ethylene-induced Chl-degrading enzyme is the "key" enzyme in the degreening processes.

Discussion

CHI diminished the ethylene-enhanced Chl loss, thus a decrease in green density, and the Chl *a*-degrading enzyme activity in radish cotyledons (Fig. 1, Table 1), indicate that mRNA translation is involved in the induction of Chl-degrading enzyme(s) in the cotyledons. The inhibitory effect of CHI on chloroplast senescence under various natural (Choe and Thimann, 1975) and artificial (Shimokawa et al., 1978a; Shimokawa, 1979) conditions has been reported. It has been postulated that chloroplast senescence requires *de novo* cytoplasmic protein synthesis. However, 100 μM CHI reversed ethylene-induced Chl catabolism almost completely (Fig. 1, Table 1). Similar results were shown in *Citrus* fruits by Shimokawa et al. (1978a). These results indicate that CHI can stimulate ethylene-dependent gene expression leading to Chl catabolism, and as a consequence promote the degreening in radish cotyledons in the absence of ethylene. Recently, it was reported that CHI can stimulate the production of ethylene-inducible DNA binding proteins that interact with an ethylene-responsive region in the promoter of a tobacco gene (Ohme-Takagi and Shinshi, 1995).

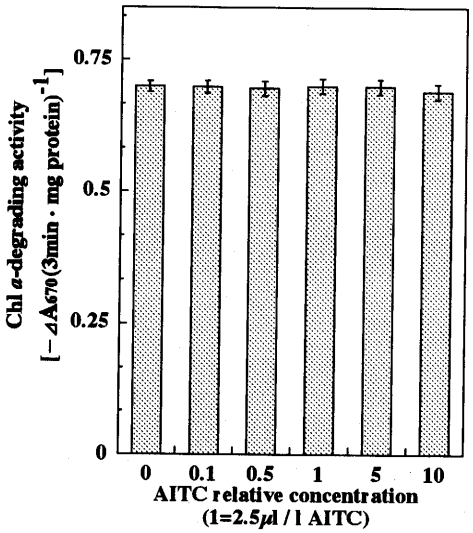

Fig. 5. Effect of AITC concentration on Chl *a*-degrading enzymatic activity. The vertical bars represent S.E. of the mean (n = 6). Data are means of three independent extracts.

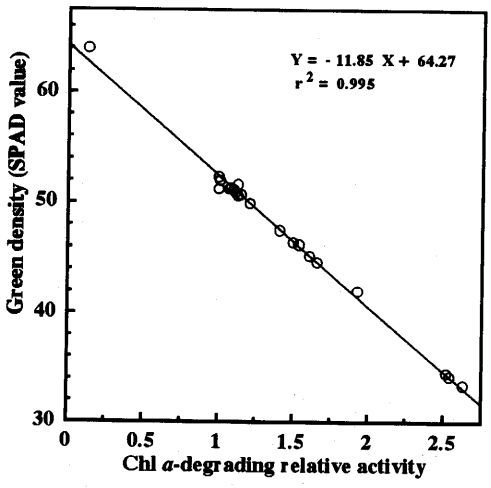

Fig. 6. A negative correlation (r = −0.997) between Chl *a*-degrading enzymatic activity and the greenness in the radish cotyledons. Data plotted from Table 1.

Table 1. Effects of various compounds on green denisity and Chl *a*-degrading activity in radish cotyledons.

Compounds	Concentration	Green density (SPAD value)	Chl *a*-degrading activity (% of control)
Initial (0h)		64.0 ± 1.6[z]	13 ± 2[y]
Control (Air, 48h)		52.3 ± 0.9	100 ± 5
+ CHI	(100 μM)	50.9 ± 0.8	111 ± 4
+ ACD	(10 μg/mℓ)	51.1 ± 0.9	110 ± 3
+ CAP	(100 μM)	49.9 ± 1.0	121 ± 4
+ STS	(1 mM)	51.3 ± 0.9	108 ± 3
+ $AgNO_3$	(0.75 mM)	50.6 ± 0.8	113 ± 3
+ AITC	(2.5 μl/l)	47.5 ± 0.9	141 ± 4
Ethylene (100 ppm, 48h)		33.4 ± 1.2	263 ± 5
Ethylene + CHI	(10 μM)	46.2 ± 0.9	154 ± 3
	(100 μM)	50.7 ± 0.9	115 ± 2
Ethylene + ACD	(1 μg/mℓ)	45.2 ± 0.8	161 ± 2
	(10 μg/mℓ)	51.6 ± 0.8	113 ± 3
Ethylene + CAP	(10 μM)	34.5 ± 0.9	252 ± 3
	(100 μM)	34.2 ± 0.9	254 ± 3
Ethylene + STS	(0.1 mM)	44.6 ± 0.8	166 ± 2
	(1 mM)	51.2 ± 0.9	107 ± 2
Ethylene + $AgNO_3$	(0.1 mM)	46.4 ± 0.8	150 ± 2
	(0.75 mM)	52.0 ± 0.9	101 ± 3
Ethylene + AITC	(0.5 μl/l)	42.0 ± 0.9	193 ± 3
	(2.5 μl/l)	51.2 ± 1.0	100 ± 2

[z] Means ± S.D. (n = 30)
[y] Means ± S.E. (n = 5)

The importance of mRNA synthesis in ethylene-mediated processes was initially tested by showing that ACD inhibited various ethylene-mediated processes (Gahagan et al., 1968; Abeles, 1969; Dilley and Klein, 1969; Kahl and Laties, 1989). ACD also suppressed ethylene-enhanced Chl catabolism in radish cotyledons (Fig. 1, Table 1), providing an indirect evidence that a factor is involved in gene expression. As previously observed by Ke and Saltveit (1989), ACD was more effective than was CHI as a protein synthesis inhibitor.

In contrast, CAP did not suppress the ethylene-enhanced Chl loss, the decrease in green density, or the activity of Chl *a*-degrading enzymes in radish cotyledons (Fig. 1, Table 1). A similar effect of CAP

on the ethylene-enhanced Chl degradation in the peel of *Citrus unshiu* fruits was observed by Shimokawa et al. (1978a). Our result offers additional evidence that the gene encoding the Chl *a*-degrading enzyme is present in the nuclei. Thus, we conclude that ethylene promotes degreening in senescing radish cotyledons by initiating *de novo* synthesis of Chl-degrading enzymes.

Shimokawa et al. (1978b) reported that the normal thylakoidal membranes in the chloroplast of ethylene-treated *Citrus* peels collapsed and resulted in the formation of macrograna causing the plastids to become filled with many plastoglobules. Eytan and Ohad (1972) found that normal lamellar systems are composed of proteins synthesized on both 80S and 70S ribosomes. Toyama (1980) reported that macrograna is seemingly composed of proteins synthesized on 80S ribosomes. Thus, the effect of protein synthesis inhibitors on the ethylene-enhanced degreening in radish cotyledons (Fig. 1, Table 1), indicate that the *de novo* cytoplasmic protein synthesis is an essential process during the degreening of cotyledons.

This agrees with other ethylene-mediated effects such as seed germination (Abeles, 1986), root growth (Abeles and Wydoski, 1987), the vase life of cut carnations (Reid et al., 1980), and opening of cut rose flowers (Reid et al., 1989), our application of STS and $AgNO_3$ retarded the aging process of the radish cotyledons induced by ethylene treatment (Figs. 2A, B and 3A, B, Table 1). Ethylene's actions are known to be inhibited by two: CO_2 and Ag^+ ion, which have been used as diagnostically. Therefore, we conclude that ethylene participates indirectly in the degreening process of cotyledons through the induction of Chl-degrading enzyme proteins.

Shimokawa (1990) made the first implication that AITC inhibits the ethylene-mediated physiological action during fruit ripening, floral senescence, and epinasty induction. In addition, AITC blocked color development and softening during tomato and banana fruit ripening. In shredded cabbage, Yano et al. (1986) showed that isothiocyanates such as ethyl-(EITC), buthyl-(BITC), phenyl-(PITC) and benzyl-isocyanates have the same effects as AITC on inhibition of the browning reaction, ethylene production and respiration; cyclohexyl isocyanates did likewise except that it did not inhibit browning.

Nagata et al. (1992b) showed that AITC has not only direct, but also indirect inhibitory effects on ethylene production by suppressing its synthesis in shredded cabbage. Furthermore, Nagata et al. (1993) showed that AITC appears to act as a protein synthesis inhibitor that is similar to CHI. In *Citrus unshiu* fruit, ethylene-enhanced chlorophyllase activity is inhibited by CHI (Shimokawa et al., 1978a) and AITC (Shimokawa et al., 1994). We obtained the same results (Figs. 1 and 4, Table 1). Direct inhibition of AITC on the Chl *a*-degrading enzyme activity did not occur at the level of concentration that blocks degreening almost completely (Fig. 5). In browning of shredded cabbage, a similar inhibitory action of AITC was obtained by Nagata et al. (1992a). From these observations, we hypothesize that the inhibitory mechanism of AITC on ethylene-induced Chl catabolism in radish cotyledons, held in the dark, is attributed to the inhibition of protein synthesis and/or of some processes upstream of ethylene-dependent protein induction, *i.e,* from the perception and the relaying of ethylene signal to gene expression.

The study of the mechanism of ethylene-induced Chl catabolism in plants, such as *Citrus unshiu* fruit, banana fruit and radish cotyledons, provides useful knowledge on how to maintain freshness in fruits and vegetables.

Literature Cited

Abeles, F. B. 1969. Abscission: role of cellulase. Plant Physiol. 44: 447-452.

Abeles, F. B. 1986. Role of ethylene in *Lactuca sativa* cv. 'Grand Rapids' seed gemination. Plant Physiol. 81: 780-787.

Abeles, F. B. and S. G. Wydoski. 1987. Inhibitors of ethylene synthesis and action: a comparison of their activities in a lettuce root growth model system. J. Amer. Soc. Hort. Sci. 112: 122-125.

Abeles, F. B., W. L. Hershberger and L. J. Dunn. 1989. Hormonal regulation, and intracellular localization of a 33-kD cationic peroxidase in excised cucumber cotyledons. Plant Physiol. 89: 664-668.

Adachi, M. 1997. The mechanism of the ethylene-induced chlorophyll metabolism in radish (*Raphanus sativus* L.) cotyledons. Ph. D. Thesis, Kagoshima Univ., Korimoto, Kagoshima.

Adachi, M. and K. Shimokawa. 1995. Evidence for the involvement of superoxide anion in the ethylen-induced chlorophyll *a* catabolism of *Raphanus sativus* cotyledons. Phytochemistry 39: 527-530.

Adachi, M., E. Tsuzuki and K. Shimokawa. 1996. Effects of ethylene on degreening of intact radish (*Raphanus sativus* L.) cotyledons. Scientia Hortic. 65: 1-9.

Adachi, M. and K. Shimokawa. 1998. Ethylene-enhanced chlorophyll catabolism in radish (*Raphanus sativus* L.) cotyledons. J. Japan. Soc. Hort. Sci. 67: 515-520.

Arnon, D. I. 1949. Copper enzymes in chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Choe, H. T. and K. V. Thimann. 1975. The metabolism of leaves during senescence. III. The senescence of isolated chloroplasts. Plant Physiol. 55: 828-834.

Dilley, D. R. and I. Klein. 1969. Protein synthesis in relation to fruit ripening. Qual. Plant Mater. Veg. 19: 55-65.

Eytan, G. and I. Ohad. 1972. Biogenesis of chloroplast membranes. VIII. J. Biol. Chem. 247: 122-129.

Gahagan, H. E., R. E. Holm and F. B. Abeles. 1968. Effect of ethylene on peroxidase activity. Physiol. Plant. 21: 1270-1279.

Halder-Doll, H. and F. Bangerth. 1987. Inhibition of autocatalytic C_2H_4-biosynthesis by AVG applications and consequences on the physiological behavior and quality of apple fruits in cool storage. Scientia Hortic. 33: 87-96.

Kahl, G. and G. G. Laties. 1989. Ethylene-induced respira-

tion in thin slices of carrot root. J. Plant Physiol. 134: 496-503.
Kawakishi, S. 1985. Glucosinolates-their enzymatic degradation, and reactivity and toxicity of degradation products. J. Japan. Soc. Food Sci. Tech. 32: 836-846 (In Japanese with English summary).
Ke, D. and M. E. Saltveit, Jr. 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. Physiol. Plant. 76: 412-418
Knee, M. 1991. Role of ethylene in chlorophyll degradation in radish cotyledons. J. Plant Growth Regul. 10: 157-162.
Lowry, O. H., N.J. Rosebrough, A. L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
Nagata, M., M. Yano and R. Saijo. 1992a. Inhibitory mechanism of allylisothiocyanate on browing of shedded cabbage. J. Japan. Soc. Food Sci. Tech. 39: 322-326 (In Japanese with English summary).
Nagata, M., M. Yano and R. Saijo. 1992b. Inhibitory mechanism of allylisothiocyanate on ethylene production of shedded cabbage. J. Japan. Soc. Food Sci. Tech. 39: 690-694 (In Japanese with English summary).
Nagata, M., Y. Tanaka, M. Hiraii and R. Saijo. 1993. Inhibitory mechanism of allylisothiocyanate on an increase in PAL activity of shedded cabbage. J. Japan. Soc. Food Sci. Tech. 40: 52-55 (In Japanese with English summary).
Ohme-Takagi, M. and H. Shinshi. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell. 7: 173-182.
Reid, M. S., J. L. Paul, M. B. Farhoomand, A. M. Kofranek and G. L. Staby. 1980. Pulse treatments with the silver thiosulfate complex extend the vase life of cut carnations. J. Amer. Soc. Hort. Sci. 105: 25-27.
Reid, M. S., Y. Evance and L. L. Dodge. 1989. Ethylene and silver thiosulfate influence opening of cut rose flowers. J. Amer. Soc. Hort. Sci. 114: 436-440.
Shimokawa, K. 1979. Preferential degradation of chlorophyll *b* in ethylene-treated fruits of 'Satsuma' mandarin. Scientia Hortic. 11: 253-256.
Shimokawa, K. 1990. Allylisothiocyanate, its role in ethylene action. Bull. Fac. Agr. Miyazaki Univ. 37: 195-201.
Shimokawa, K., S. Shimada and K. Yaeo. 1978a. Ethylene-enhanced chlorophyllase activity during degreening of *Citrus unshiu* Marc. Scientia Hortic. 8: 129-135.
Shimokawa, K., A. Sakanoshita and K. Horiba. 1978b. Ethylene-induced changes of chloroplast structure in Satsuma mandarin (*Citrus unshiu* Marc.). Plant Cell Physiol. 19: 229-236.
Shimokawa, K., H. Hashimoto, Y. Maeda, Y. Uchida and Y. Shibasaki. 1994. Inhibition of allylisothiocyanate on ethylene-enhanced chlorophyll degradation in *Citrus unshiu* fruits. J. Japan. Soc. Hort. Sci. 63: 195-201 (In Japanese with English summary).
Toyama, S. 1980. Electron microscope studies on the morphogenesis of plastids. X. Ultrastructural changes of chloroplasts in morning glory leaves exposed to ethylene. Amer. J. Bot. 67: 625-635.
Yano, M., R. Saijo and Y. Ota. 1986. Inhibition of browning and ethylene production in shedded cabbage by isothiocyanates. J. Japan. Soc. Hort. Sci. 55: 194-198 (In Japanese with English summary).

カイワレダイコン (*Raphanus sativus* L.) 子葉のエチレン誘導脱緑に及ぼす阻害剤の影響

足立　勝・内田好則・下川敬之

宮崎大学農学部　889-2192　宮崎市学園木花台西1丁目1番地

摘　要

暗所下でのカイワレダイコン子葉のエチレン誘導脱緑について，エチレン作用を阻害剤で抑制し，クロロフィル代謝率の減少を示すことで検討した．子葉の脱緑の阻害は，幼植物体の子葉をシクロヘキシミド，アクチノマイシンD，硝酸銀($AgNO_3$)，チオ硫酸銀 (STS) さらにアリルイソチオシアネート (AITC) などの処理で観察されたものの，クロラムフェニコール (クロロプラストRNA合成阻害剤) では観察されなかった．さらに，緑色の保持とクロロフィル分解酵素活性との間には，高い負の相関関係が得られた：$Y=-11.85X+64.27$，$P<0.001$ ($r^2=0.995$) (ただしXは相対的クロロフィル*a*分解活性，Yは緑色値である)．この結果は，エチレンがクロロフィルを分解する酵素タンパク質を新たに誘導することによりカイワレダイコン子葉の脱緑過程を進行させることを示している．

クロロフィル分解酵素の活性に対するAITCの直接の阻害は，子葉のエチレン誘導脱緑を完全に抑制できる濃度のレベルでは観察されなかった．これらの調査結果は，AITCはカイワレダイコン子葉中でクロロフィルを分解する酵素タンパク質誘導の阻害剤として作用していることを示唆する．