Improved Methods for Isolating Active Chloroplast from Chilling-Sensitive Melon, Bell Pepper and Eggplant

Kazuo Ichimura, Shin-ichi Watanabe* and Masayuki Oda

National Research Institute of Vegetables, Ornamental Plants and Tea. Ano, Mie 514-23

Summary

When chilling sensitive plants are exposed to low temperature, photosynthetic activity is rapidly reduced. To investigate the biochemical mechanisms involved in chilling-induced reduction in photosynthetic activity of the chloroplast, it is necessary to isolate active chloroplasts in a coupled state. When chloroplasts were isolated from chilling-sensitive melon (*Cucumis melo* L.), bell pepper (*Capsicum annuum* L.), and eggplant (*Solanum melongena* L.) by the current, conventional method, active, coupled chloroplasts were not obtained from melon and bell pepper leaves. To improve the method for isolating active chloroplasts from these plants, sucrose as an osmoticant was replaced with NaCl and sorbitol in the grinding and suspension media, respectively; isoascorbate and EDTA were also added in the grinding medium. With these media, isolated, chilled chloroplasts had measurable electron transport rates, and sufficient coupling to investigate the site of inhibition of photochemical processes. Melon leaves exposed to 5 °C for 24 hr under $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ showed a much reduced photosynthetic rate at 25 °C, compared to unchilled leaves. Measurements of activity of chloroplasts isolated from chilled and unchilled leaves by the improved method revealed that the whole-chain electron transport rates were unaffected by chilling, whereas the extent of coupling was decreased. This result suggests that photophosphorylation would be involved in the inhibition of photosynthesis in chilled melon leaves. Thus, the improved method for isolating chloroplasts is applicable to study the inhibition site of photochemical reactions in chilling-sensitive melon, bell pepper, and eggplant.

Introduction

When plants of tropical or subtropical origin are exposed to low temperatures from 0 °C to about 15 °C, they suffer a severe damage, because photosynthetic activity is rapidly reduced (Aoki et al., 1989; Hodgson et al., 1987; Hodgson and Raison, 1989; Long et al., 1983). Many studies have been conducted on the inhibition site of photochemical reactions in chloroplasts of chilling-sensitive plants, such as cucumber (Garber, 1977; Hodgson and Raison, 1989; Peeler and Naylor, 1988; Shen et al., 1990; Terashima et al., 1989,

1991a, b); tomato (Kaniuga et al., 1978; Martin and Ort, 1982; Walker et al., 1991); pumpkin (Tyystjärvi et al., 1989); maize (Baker et al., 1983; Long et al., 1983; Ortiz-Lopez et al., 1990); and rice (Terashima et al., 1989). These studies have shown that the site of inhibition of photochemical processes in chloroplasts depends on the incident light intensity. Exposure to $1,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ severely inhibits photosystem II (Baker et al., 1983; Ortiz-Lopez et al., 1990; Tyystjärvi et al., 1989); the inhibition is ascribed to the inactivation and disappearance of D1 protein, which binds plastoquinone, the secondary acceptor in photosystem II (Kyle et al., 1984; Ohad et al., 1984). Under dark conditions, the oxygen evolution system is selectively inhibited (Kaniuga et al., 1978; Margulies and Jagendorf, 1960; Shen et al., 1990; Terashima et al., 1989). The inhibition has been attributed to dissociation of 14- and 20-k Da pro-

Received for publication 24 October 1994.

A part of this work was supported by Grants-in-Aid for STA fellowship from the Science and Technology Agency, Japan.

*Present address : Hokuriku Natl. Agr. Exp. Stn. Joetsu, Niigata 943-01

teins in thylakoid membrane, which play a regulatory role in the oxygen evolution (Shen et al., 1990). On the other hand, from 100 to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, photophosphorylation (Garber, 1977; Terashima et al., 1989, 1991 a, b) and photosystem I are inhibited (Terashima et al., 1994).

Melon, bell pepper and eggplant are chilling-sensitive vegetables whose cultivation period in the open field is limited by low temperature, whereas cultivation under artificially heated greenhouse is limited by cost. Because of these problems, studies on the biochemical mechanism of chilling injury in these vegetables were undertaken, specifically to clarify the inhibition site of photochemical reactions because chloroplasts are sensitive to chilling. One of the most available methods for investigating photochemical reactions is to isolate active chloroplasts in a coupled state, and to measure their electron transport capacity. Isolation of active chloroplasts is usually difficult, except in plants such as spinach (Kato, 1976 a). To our knowledge, isolation of active chloroplasts in a coupled state from melon, bell pepper and eggplant has not yet been reported.

In this study, we improved a method of chloroplast-isolation from melon, bell pepper, and eggplant and studied the inhibition site of photochemical reactions in chloroplasts in chilled melon.

Materials and Methods

Plant material

Seeds of melon (*Cucumis melo* L. cv. Andes), bell pepper (*Capsicum annuum* L. cv. Kyomidori), and eggplant (*Solanum melongena* L. cv. Senryo No. 2) were sown in pots and grown in a temperature controlled chamber, 25 °C, 14 hr light period/20 °C, and 10 hr dark period. Light intensity at plant height was about 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. After 3 weeks fully grown primary or secondary true leaves were used for the subsequent experiments.

Improved method for the isolation of active chloroplasts

Five g of fresh leaves of fresh weight were excised, cut into 1 cm strips, and the strips ground for 10 sec in 25 ml of ice-cold 50 mM Hepes-KOH (pH 7.5) that contained 0.4 M NaCl, 5 mM MgCl_2 , 1 mM Na_2EDTA , 5 mM sodium isoascorbate and 0.1% bovine serum albumin with an Ace

homogenizer (Nihonseiki Kaisha Ltd, Tokyo). The homogenate was passed through 8 layers of gauze and the filtrate centrifuged at 500 $\times g$ for 3 min. The resulting pellet was resuspended in 5 ml of 50 mM Hepes-KOH (pH 7.5) that contained 0.3 M sorbitol, 10 mM NaCl, and 5 mM MgCl_2 and re-centrifuged at 500 $\times g$ for 3 min. The pellet was resuspended in 1 ml of the second medium. These procedures were performed below 4 °C. The final suspensions were stored on ice but they were used for the determination of electron transport rates within 1 hr.

The difference between the improved method as described above and the conventional method (Kato, 1976 a, 1981) was that the latter consisted of : 50 mM Hepes-NaOH (pH 7.5), 0.4 M sucrose, 10 mM NaCl, 5 mM MgCl_2 and 0.1% bovine serum albumin for the grinding and first resuspending medium; whereas the second resuspending medium consisted of 50 mM Hepes-NaOH (pH 7.5), 0.4 M sucrose, 10 mM NaCl, and 5 mM MgCl_2 .

Measurement of electron transport rates in chloroplasts

Electron transport rates were measured polarographically, using a Clark type oxygen electrode at an irradiance of 1,000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 25 °C. A basal reaction medium that contained 10 mM NaCl, 2 mM MgCl_2 , and 50 mM Tricine-KOH (pH 7.8) was supplemented with the following reagents: 0.1 mM methyl viologen and 1 mM NaN_3 for the whole-chain electron transport rate from H_2O to methyl viologen; 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ for the whole-chain electron transport rate from H_2O to $\text{K}_3\text{Fe}(\text{CN})_6$; 2 mM ascorbate, 50 μM 2, 6-dichlorophenol indophenol, 10 μM 3 (3, 4-dichlorophenyl)-1, 1-dimethylurea, 0.1 mM methyl viologen, and 1 mM NaN_3 for photosystem I electron transport rate; 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ and 0.5 mM p-phenylene diamine for photosystem II electron transport rate. Ammonium chloride at 5 mM or methylamine hydrochloride at 20 mM was added to the reaction medium to uncouple the photophosphorylation.

Determination of chlorophyll content in chloroplasts

The isolated chloroplasts were suspended in 80% acetone. The suspensions were left in the dark for 30 min and then centrifuged. Chlorophyll concentrations in the supernatant were determined with the equation by Arnon (1949), using the

absorbance of the supernatant at 645 and 663 nm.

Determination of photosynthetic rate in melon leaves

Rates of photosynthetic oxygen evolution in leaf discs were measured at 25 °C with a leaf-disc oxygen-electrode system (Hansatech, Kings Lynn, U. K.) as reported by Delieu and Walker (1981) under $1,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and at a saturating concentration of CO_2 (about 5%).

Chilling treatment of melon leaves

Experimental leaves were detached from the plant and floated on water at 5 °C with the adaxial surfaces facing upwards under $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 24 hr, whereas the control leaves were floated on water at 26 °C under the same light intensity.

Results

Improvement of methods for isolation of active chloroplasts from melon, bell pepper, and eggplant leaves

In general, to investigate the activity of photochemical reactions, the electron transport rates higher than $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ are desirable (Kato, 1976 b; Okayama, 1983). Furthermore, it is recognized that the coupling state of chloro-

plasts becomes higher as the ratio of the electron transport rate without uncouplers to that with uncouplers decreases (Peeler and Naylor, 1988). Tables 1 and 2 show the electron transport rates of isolated chloroplasts obtained by the conventional method. In eggplant, the electron transport rates were higher than $100 \mu\text{mol O}_2\cdot\text{mg chlorophyll}^{-1}\cdot\text{hr}^{-1}$. In addition, rates of photosystem I and II without uncouplers were about half of those with uncouplers. These results suggest that active, coupled chloroplasts were obtained from eggplant by the conventional method. However, in melon, all rates except that of photosystem I were lower than $50 \mu\text{mol O}_2\cdot\text{mg chlorophyll}^{-1}\cdot\text{hr}^{-1}$. In particular, the rate of the whole-chain electron transport from H_2O to $\text{K}_3\text{Fe}(\text{CN})_6$ was not detected. Furthermore, the extent of coupling was very low, indicating that isolated chloroplasts were uncoupled. In bell pepper, the measured rates, except those for photosystem II, were lower than $50 \mu\text{mol O}_2\cdot\text{mg chlorophyll}^{-1}\cdot\text{hr}^{-1}$ and the extent of coupling was very low. These results indicate that active chloroplasts with coupled state were not obtained from the melon and bell pepper leaves with the conventional isolation method.

To improve the chloroplast isolation method, we modified the conventional method by adding to the

Table 1. Whole-chain electron transport rates of chloroplasts isolated from melon, bell pepper and eggplant leaves by the conventional method.

Species	$\text{H}_2\text{O}\rightarrow\text{Methyl viologen}$			$\text{H}_2\text{O}\rightarrow\text{K}_3\text{Fe}(\text{CN})_6$		
	Uncoupler ^z		-/+	Uncoupler ^z		-/+
	+	-		+	-	
	$(\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1})$		(%)	$(\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1})$		(%)
Melon	22	21	95	0	0	—
Bell pepper	3	6	200	43	46	107
Eggplant	201	116	58	168	73	43

^z Ammonium chloride.

Table 2. Electron transport rates of photosystem I and II of chloroplasts isolated by the conventional method from melon, bell pepper and eggplant leaves.

Species	Asc/DPIP $\rightarrow$ Methyl viologen (Photosystem I)	$\text{H}_2\text{O}\rightarrow\text{p-Phenylenediamine}$ (Photosystem II)
	$(\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1})$	
Melon	116	23
Bell pepper	43	100
Eggplant	254	118

grinding medium, ascorbate, dithiothreitol, and polyvinylpyrrolidone (Garber, 1977; Hodgson and Raison, 1989; Kaniuga et al., 1978; Martin and Ort, 1982; Nolan, 1980). However, the chloroplasts isolated from melon and bell pepper leaves by using this modified grinding medium proved to be inactive. Peeler and Naylor (1988) showed with cucumber leaves that NaCl and Na₂EDTA are effective for isolating active chloroplasts. Terashima (1989) reported that adding NaCl to the grinding medium and sorbitol to the resuspending medium is effective for isolating chloroplasts from cucumber leaves. Thus, we added NaCl and Na₂EDTA to the grinding medium and sorbitol to the resuspending medium and determined the electron transport rates of chloroplasts. Tables 3 and 4 show that electron transport rates of chloroplasts isolated by the improved method were higher than 50 $\mu\text{mol O}_2 \cdot \text{mg chlorophyll}^{-1} \cdot \text{hr}^{-1}$ in all vegetable species tested and that the extent of cou-

pling of electron transport was also high in all leaves tested. These results reveal that the improved method is preferable for isolating active, coupled chloroplasts from chilling-sensitive melon and bell pepper.

Photosynthetic and electron transport rates of chilled leaves of melon

We tested if the improved method to isolate active chloroplasts is applicable to target the inhibition site of photochemical reactions in chilled melon leaves.

Leaf discs of melon taken from leaves chilled at 5 °C for 24 hr showed 59% lower photosynthetic rate at 25 °C than did unchilled control leaf discs (Table 5).

A determination of electron transport rates of chloroplasts isolated by the improved method revealed that the whole-chain electron transport rates from H₂O to both methyl viologen and potas-

Table 3. Whole-chain electron transport rates of chloroplasts isolated from melon, bell pepper and eggplant leaves by the improved method.

Species	H ₂ O→Methyl viologen			H ₂ O→K ₃ Fe(CN) ₆		
	Uncoupler ^z		-/+	Uncoupler ^z		-/+
	+	-		+	-	
	($\mu\text{mol O}_2 \cdot \text{mg chl}^{-1} \cdot \text{hr}^{-1}$)	(%)		($\mu\text{mol O}_2 \cdot \text{mg chl}^{-1} \cdot \text{hr}^{-1}$)	(%)	
Exp.1 Melon	153	44	29	154	44	29
Bell pepper	109	34	31	74	36	49
Eggplant	150	64	43	158	69	44
Exp.2 Melon	194	40	21	188	35	19
Bell pepper	167	52	31	71	40	56
Eggplant	255	78	31	258	48	19

^z Methylamine hydrochloride.

Table 4. Electron transport rates of photosystem I and II of chloroplasts isolated from melon, bell pepper and eggplant leaves by the improved method.

Species		Asc/DPIP→Methyl viologen (Photosystem I) ($\mu\text{mol O}_2 \cdot \text{mg chl}^{-1} \cdot \text{hr}^{-1}$)	H ₂ O→p-Phenylenediamine (Photosystem II)
Exp.1	Melon	362	146
	Bell pepper	270	105
	Eggplant	377	142
Exp.2	Melon	454	153
	Bell pepper	242	122
	Eggplant	321	140

sium ferricyanide were unaffected by the chilling treatment, when the uncoupler was absent in the reaction medium (Table 6). However, when the uncoupler was present, chloroplasts from chilled leaves showed about 2-fold higher rates than did those from unchilled leaves. These results indicate that the chilling treatment affected the chloroplasts, reducing the degree of coupling of the electron transport system (Table 6).

Discussion

To obtain active chloroplasts with coupled state from melon and bell pepper leaves, NaCl and sorbitol are necessary in the grinding and resuspending medium, respectively. Peeler and Naylor (1988) also observed with cucumber that NaCl is needed to obtain active and coupled chloroplasts. Why these compounds are effective in isolating active chloroplasts remains unsolved.

Peeler and Naylor (1988) found that with cucumber leaves 25 °C was optimal to isolate chloroplasts in the coupled state. On the other hand, Terashima et al. (1989, 1991 a, b) obtained active, coupled chloroplasts from cucumber by isolating them at 4 °C or lower. This difference seems

to be caused by the different resuspending medium composition : Peeler and Naylor (1988) used NaCl as an osmoticant whereas Terashima et al. (1989) used sorbitol. In this study, we also were able to isolate active, coupled chloroplasts from melon and bell pepper leaves under cold conditions by using sorbitol in the resuspending medium.

Chloroplast is known to be very sensitive to various stresses such as freezing, heat, high light, and water stress. When plants were subjected to these, photosynthetic activity was greatly decreased (Aoki et al., 1988, 1989; Ludlow and Björkman, 1984; Smillie and Hetherington, 1983). The modified chloroplast isolation method in this study may be applicable to melon, bell pepper, and eggplant subjected to various environmental stresses other than cold, and may serve to obtain a better understanding of chloroplast reactions to stresses.

It has been reported that photosynthesis is rapidly inactivated when chilling-sensitive plants are exposed to a low temperature (Aoki et al., 1989; Hodgson et al., 1987; Hodgson and Raison, 1989; Long et al., 1983). In this study, the photosynthetic rate of melon leaf discs was reduced greatly by keeping discs at 5 °C for 24 hr prior to rate determination at 25 °C.

Studies at the molecular level of photosynthesis inhibition by chilling have revealed that the inhibition site of a photochemical reaction varies with light intensities to which chloroplasts are exposed before extraction. Photophosphorylation is inhibited in cucumber leaves exposed from 100 to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Garber, 1977; Peeler and Naylor, 1988; Terashima et al., 1989, 1991 a, b). Terashima et al. (1989, 1991 a, b), who had investigated the mechanism of inhibition of photo-

Table 5. Effect of chilling on photosynthetic rate of melon leaves^z.

Treatment	Photosynthetic rate ($\mu\text{mol O}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
Control	34.4
Chilled	14.0

Values show the mean of duplicate experiments.

^z Detached leaves were floated on water at 5°C (chilled) or 26°C (control) under $100\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 24 hr, after which their photosynthetic rates were determined at 25°C.

Table 6. Effect of chilling on whole-chain electron rates isolated from melon leaves by the improved method.

Treatment	$\text{H}_2\text{O}\rightarrow\text{Methyl viologen}$			$\text{H}_2\text{O}\rightarrow\text{K}_3\text{Fe}(\text{CN})_6$		
	Uncoupler ^z		-/+	Uncoupler ^z		-/+
	+	-		+	-	
	($\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1}$)	($\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1}$)	(%)	($\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1}$)	($\mu\text{mol O}_2\cdot\text{mg chl}^{-1}\cdot\text{hr}^{-1}$)	(%)
Control	107	28	26	107	34	32
Chilled	108	53	49	114	74	65

Values were obtained by single experiment.

^z Methylamine hydrochloride.

phosphorylation in chilled cucumber leaves under moderate light intensity, found that CF_1 , a coupling factor between the electron transport and photophosphorylation and a component of ATPase proteins, became dissociated from thylakoid membranes, whereas in unchilled chloroplasts, CF_1 is located on the stromal surface of the thylakoid membrane and connected to another coupling factor CF_0 which is tightly associated to the membrane. In this study, the extent of coupling of chloroplasts was reduced by chilling the leaves under $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This result and the above findings suggest that photophosphorylation could be involved in the inhibition of photosynthesis in chilled melon leaves.

Literature Cited

- Aoki, S., M. Oda and K. Hosino. 1989. Varietal differences in chilling-induced depression of photosynthesis and leaf growth in cucumber seedlings. *J. Japan. Soc. Hort. Sci.* 58 : 173-179.
- Aoki, S., M. Oda and M. Nagaoka. 1988. Chilling and heat sensitivities in cucumber seedlings measured by chlorophyll fluorescence. *Bull. Natl. Res. Inst. Veg., Ornam. Plants & Tea, Japan A* 2 : 81-92.
- Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts : polyphenol oxidases in *Beta vulgaris*. *Plant Physiol.* 24 : 1-15.
- Baker, N. R., T. M. East and S. P. Long. 1983. Chilling damage to photosynthesis in young *Zea mays*. 2. Photochemical function of thylakoids *in vivo*. *J. Exp. Bot.* 34 : 189-197.
- Delieu, T. and D. A. Walker. 1981. Polarographic measurement of photosynthetic O_2 evolution by leaf discs. *New Phytol.* 89 : 165-175.
- Garber, M. P. 1977. Effect of light and chilling temperatures on chilling-sensitive and chilling-resistant plants. *Plant Physiol.* 59 : 981-985.
- Hodgson, R. A. J., G. R. Orr and J. K. Raison. 1987. Inhibition of photosynthesis by chilling in the light. *Plant Sci.* 49 : 75-79.
- Hodgson, R. A. J. and J. K. Raison. 1989. Inhibition of photosynthesis by chilling in moderate light : a comparison of plants sensitive and insensitive to chilling. *Planta* 178 : 545-552.
- Kaniuga, Z., B. Sochanowicz, J. Zabek and K. Krzysztyniak. 1978. Photosynthetic apparatus in chilling-sensitive plants. I. Reactivation of Hill reaction activity inhibited on the cold and dark storage of detached leaves and intact plants. *Planta* 140 : 121-128.
- Kato, S. 1976a. Chloroplast. p. 316-318. In : Japanese society of biochemistry (ed.). *Methods in biochemistry*. vol. 12. Energy metabolism and biological oxidation vol. 1. Tokyo Kagaku Dojin, Tokyo. (In Japanese).
- Kato, S. 1976 b. Electron transport. p. 368-379. In : Japanese society of biochemistry (ed.). *Methods in biochemistry*. vol. 12. Energy metabolism and biological oxidation vol. 1. Tokyo Kagaku Dojin, Tokyo. (In Japanese).
- Kato, S. 1981. Chloroplast. p. 251-255. In : S. Kato, S. Miyachi and Y. Murata (eds.). *Methods in photosynthesis research*, Kyoritu Shuppan, Tokyo. (In Japanese).
- Kyle, D. J., I. Ohad and C. J. Arntzen. 1984. Membrane protein damage and repair : Selective loss of a quinone-protein function in chloroplast membranes. *Proc. Natl. Acad. Sci. USA* 81 : 4070-4074.
- Long, S. P., T. M. East and N. R. Baker. 1983. Chilling damage to photosynthesis in young *Zea mays*. 1. Effects of light and temperature variation on photosynthetic CO_2 assimilation. *J. Exp. Bot.* 34 : 177-188.
- Ludlow, M. M. and O. Björkman. 1984. Paraheliotropic leaf movement in *Siratro* as a protective mechanism against drought-induced damage to primary photosynthetic reactions : damage by excessive light and heat. *Planta* 161 : 505-518.
- Margulies, M. M. and A. T. Jagendorf. 1960. Effect of bean leaves on photosynthetic reaction of isolated chloroplasts. *Arch. Biochem. Biophys.* 90 : 176-183.
- Martin, B. and D. R. Ort. 1982. Insensitivity of water-oxidation and photosystem II activity in tomato to chilling temperatures. *Plant Physiol.* 70 : 689-694.
- Nolan, W. G. 1980. Effect of temperature on electron transport activities of isolated chloroplasts. *Plant Physiol.* 66 : 234-237.
- Ohad, I., D. J. Kyle and C. J. Arntzen. 1984. Membrane protein damage and repair : Removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. *J. Cell Biol.* 99 : 481-485.
- Okayama, S. 1983. Hill reaction. p. 109-122. In : Committee of methods in experimental biology (ed.). *Methods in experimental biology*. vol. 17. *Plant Physiology (III)*. Maruzen, Tokyo. (In Japanese).
- Ortiz-Lopez, A., G. Y. Nie, D. R. Ort and N. R. Baker. 1990. The involvement of the photoinhibition of photosystem II and impaired membrane energization in the reduced quantum yield of carbon assimilation in chilled maize. *Planta* 181 : 78-84.
- Peeler, T. C. and A. W. Naylor. 1988. A comparison of the effects of chilling on thylakoid electron transfer in pea (*Pisum sativum* L.) and cucumber (*Cucumis sativus* L.). *Plant Physiol.* 86 : 147-151.
- Shen, J. R., I. Terashima and S. Kato. 1990. Cause for

- dark, chilling-induced inactivation of photosynthetic oxygen-evolving system in cucumber leaves. *Plant Physiol.* 93 : 1354-1357.
- Smillie, R. and S. E. Hetherington. 1983. Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence *in vivo*. Chilling, freezing, ice cover, heat, and high light. *Plant Physiol.* 72 : 1043-1050.
- Terashima, I., S. Funayama and K. Sonoike. 1994. The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperature is photosystem I, not photosystem II. *Planta* 193 : 300-306.
- Terashima, I., L. K. Huang and C. B. Osmond. 1989. Effects of leaf chilling on thylakoid functions, measured at room temperature, in *Cucumis sativus* L. and *Oryza sativa* L. *Plant Cell Physiol.* 30 : 841-850.
- Terashima, I., Y. Kashino and S. Katoh. 1991 a. Exposure of leaves of *Cucumis sativus* L. to low temperatures in the light causes uncoupling of thylakoids. I. Studies with isolated thylakoids. *Plant Cell Physiol.* 32 : 1267-1274.
- Terashima, I., K. Sonoike, T. Kawazu and S. Katoh. 1991 b. Exposure of leaves of *Cucumis sativus* L. to low temperatures in the light causes uncoupling of thylakoids. II. Non-destructive measurements with intact leaves. *Plant Cell Physiol.* 32 : 1275-1283.
- Tyystjärvi, E., J. Ovaska, P. Karunen and E. M. Aro. 1989. The nature of light-induced inhibition of photosystem II in pumpkin (*Cucurbita pepo* L.) leaves depends on temperature. *Plant Physiol.* 91 : 1069-1074.
- Walker, M. A., B. D. McKersie and K. P. Pauls. 1991. Effects of chilling on the biochemical and functional properties of thylakoid membranes. *Plant Physiol.* 97 : 663-669.

メロン、ピーマンおよびナスからの葉緑体単離法の改良

市村一雄・渡邊慎一*・小田雅行

野菜・茶業試験場 514-23 三重県安芸郡安濃町草生

摘 要

低温感受性植物では、低温による光合成活性の阻害が低温障害の重要な要因の一つであると考えられている。低温による光合成活性の阻害機構を解明するためには、活性が高く、共役した葉緑体を単離することが必要である。代表的な低温感受性野菜であるメロン、ピーマンおよびナスから常法により葉緑体を単離したが、メロンおよびピーマンからは電子伝達系活性が高く共役した葉緑体を得ることができなかった。そこで、これらの植物から、葉緑体を単離する方法の改良を行った。磨砕液および懸濁液の浸透圧調節剤としてNaClおよびソルビトールをそれぞれ用い、磨砕液にはイソアスコルビン酸とEDTAを加えて葉緑体を単

離した。その結果、いずれの植物においても、活性が高く共役した葉緑体を単離することができた。

メロン葉を5℃、100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ の条件下で24時間低温処理した後、常温における光合成活性と上述の改良法により単離した葉緑体の電子伝達系活性を調べた。対照区は処理温度が26℃であること以外は低温処理区と同じ条件とした。光合成活性は低温処理により対照の41%まで減少した。電子伝達系活性に差はみられなかったが、共役能は著しく減少した。これらの結果から、低温によるメロンの光合成活性阻害部位は光リン酸化である可能性が示唆された。

*現在：北陸農業試験場