Effects of Spermidine Pretreatment through the Roots on Growth and Photosynthesis of Chilled Cucumber Plants (*Cucumis sativus* L.)

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**Summary**

Effects of endogenous polyamine levels on the growth of chilled cucumber plants (*Cucumis sativus* L.) were investigated, with a focus on susceptibility of photosynthesis to photoinhibition. Plants, at the second leaf stage, were administered 0.5 mM spermidine (Spd) through the roots before they were exposed to mild chilling stress at 10/7 °C (day/night) for 8 days under a photosynthetically active photon flux density of 240 μmol·m⁻²·sec⁻¹. The Spd-pre-treated plants had a high Spd content in both leaves and thylakoid membranes, compared to the control. The pre-treated plants had higher growth rates and leaf chlorophyll content than had the control during chilling as well as after being transferred to 28/22 °C. The control plants showed a marked decline in photosynthetic rates during chilling, which was less pronounced in Spd-pre-treated plants. Spd pretreatment did not affect stomatal conductance of chilled leaves, but alleviated the decline of the chlorophyll fluorescence yield (Fv/Fm), photosynthetic electron transport activity of thylakoids, and activity of enzymes in carbon metabolism, as well as chill-induced increase of lipid peroxidation in the thylakoid membranes. In addition, preloading the isolated thylakoids with Spd resulted in decreased susceptibility to low-temperature photoinhibition of photochemistry. The results indicate that the high levels of Spd in leaves and/or chloroplasts contribute to the improvement of chilling tolerance of the photosynthetic apparatus in cucumber leaves.

**Key Words:** chilling stress, *Cucumis sativus*, photoinhibition, photosynthesis, polyamine, spermidine.

**Introduction**

Chilling-sensitive plants of tropical or subtropical origin, including cucumber, may incur irreversible damage to the photosynthetic apparatus during growth at or below 13 °C (Öquist, 1983; Allen and Ort, 2001). This low-temperature photoinhibition of photosynthesis is caused primarily by an excess of absorbed light energy beyond that utilized in photosynthetic CO₂ reduction (Krause, 1994). Plants have evolved elaborate photoprotective mechanisms to cope with the potential danger of excess excitation energy in thylakoids. One of such a mechanism is associated with avoiding excess accumulation of excitation energy in thylakoids by decreasing light absorption (Haldimann, 1998), increasing the enzyme activity in carbon metabolism (Holaday et al., 1992), or enhancing the xanthophyll cycle-dependent energy dissipation (Demmig-Adams and Adams, 1996). Increased scavenging activity for reactive oxygen species (ROS) in response to low temperature has also been demonstrated (Schöner et al., 1990). Increases in lipid unsaturation of thylakoid membranes have been correlated with the ability to repair the damaged photosynthetic systems rather than the ability to tolerate the photoinhibition per se (Gombos et al., 1997). However, there could be other important components that are responsible for the tolerance of the photosynthetic apparatus to low-temperature photoinhibition.

Polyamines are polybasic aliphatic amines that occur in all plant cells, among which the diamine putrescine (Put), the triamine spermidine (Spd), and the tetraamine spermine (Spm) are most common. These polyamines are implicated in plant growth, morphogenesis, senescence, and response to environmental stresses (Galston and Kaur-Sawhney, 1995; Bouchereau et al., 1999). There was a close correlation between the chilling tolerance of rice cultivars and the extent to which Put accumulates in leaves in response to chilling (Lee et al., 1995). The growth of excised rice roots at 5 °C was promoted by the addition of Put to the growth medium (Lee, 1997). Recently, we found that the chilling tolerance of different cucumber cultivars was correlated with the ability to enhance the accumulation of Spd in leaves during exposure to 3 °C in the dark (Shen et al., 2000). Increased Spd suppressed the increase of NADPH oxidase-mediated ROS generation in chilled leaves. These results indicate an important role of polyamines in plant defense to chilling injury. However, little is known about whether high endogenous polyamine levels in
plants can alleviate the adverse effect of long-term chilling stress on growth and photosynthesis. In this study, we investigated the effects of polyamine levels in plants on the growth and photosynthesis of cucumber plants (Cucumis sativus L.) at low temperatures, with a focus on susceptibility of photosynthesis to low-temperature photoinhibition.

**Materials and Methods**

**Plant materials, spermidine pretreatment and chilling treatment**

Seeds of cucumber (Cucumis sativus L., cv. Suyo) were sown in vermiculite in the glasshouse. When the seedlings had fully expanded cotyledons, they were transplanted to plastic pots (7.5 cm in diam. at the top) filled with gravel and placed in a tray containing Hoagland’s nutrient solution at a depth of about 2 cm. The plants were grown in a growth chamber under a 14-hr photoperiod at 28/22°C (day/night) and 70/85% relative humidity. Light was provided by fluorescent lamps with a photosynthetically active photon flux density (PPFD) of 240 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1} \) at the plant level. When the second leaves were fully expanded, one set of plants was provided with nutrient solution containing 0.5 mM Spd (triethylenechlordie salt). Another set of plants, administered the same nutrient solution but lacking Spd, served as the control. The addition of Spd to the nutrient solution did not alter its pH. After 2 days, Spd-pretreated and control plants were transferred to fresh nutrient solution without Spd. Then, the temperature in the chamber was lowered to 10/7°C for 8 days, after which it was returned to 28/22°C for another 3 days. The following measurements and sample preparations were made during the middle of the light period using the second leaves from the base. The experiments were duplicated and each replicate consisted of three plants.

**Measurement of chlorophyll fluorescence and photosynthetic activity**

Chlorophyll fluorescence, photosynthesis, and chlorophyll content of leaves were measured immediately after the plants were moved to a chamber kept at 28°C and at 240 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1} \) PPFD. Photosystem (PS) II chlorophyll fluorescence yields (Fv/Fm) were determined with a fluorometer (Mini PAM, H. Walz, Germany) after a 30-min dark adaptation. Photochemical (qP) and non-photochemical (qN) fluorescence quenching coefficients were estimated after about 5 min under actinic light when the leaves had reached steady-state of fluorescence. Gross and net photosynthetic rates and stomatal conductance were measured with an infrared gas analyzer (SPD-H4, Shimadzu, Japan) at a leaf temperature of 28°C with an ambient concentration of \( \text{CO}_2 \). A chlorophyll-meter (SPAD-502, Minolta, Japan) was used to determine the chlorophyll content. The readings were converted to chlorophyll content using the equation, \( Y=1.176X - 14.402 \left( r^2=0.935 \right) \), where \( Y \) is the chlorophyll content in \( \mu \text{g} \cdot \text{cm}^{-2} \) and \( X \) is the meter reading. Chlorophyll contents are the average of several measurements on different parts of a leaf. Measurements of chlorophyll fluorescence and photosynthetic rates were discontinued after the plants were transferred to 28°C, because the leaves of the control became unevenly chlorotic.

**Isolation of thylakoid membranes**

Immediately after sampling, leaves were homogenized in chilled isolation buffer containing 50 mM Hepes-KOH (pH 7.5), 0.3 M sorbitol, 1 mM NaCl, 1 mM EDTA, 5 mM MgCl₂, 5 mM isoascorbate, and 0.1% (w/v) bovine serum albumin. The homogenate was filtered through two layers of Miracloth (Calbiochem, USA), and the filtrate centrifuged at 5,000 \( \times \) g for 5 min. The pellet was suspended in a buffer consisting of 50 mM Hepes-KOH (pH 7.5), 0.3 M sorbitol, 10 mM NaCl and 5 mM MgCl₂. After centrifugation at 500 \( \times \) g for 30 sec, the supernatant was re-centrifuged at 5,000 \( \times \) g for 5 min. The pellet was resuspended in a buffer at a chlorophyll concentration of 250 \( \mu \text{g} \cdot \text{ml}^{-1} \). The membrane preparations were stored at -80°C until use. This procedure did not affect the electron transport activity of the membranes for at least 12 days of storage. Chlorophyll was quantified by the method of Arnon (1949).

**Determination of electron transport activity of thylakoids**

Activities of PS II and PS I electron transport were measured polarographically in a Clark-type oxygen electrode at 25°C at a PPFD of 1.1 \( \text{mmol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1} \). PS II activity was based on \( \text{O}_2 \) evolution rates in a reaction mixture containing 25 mM Mes-NaOH (pH 6.5), 0.3 M sorbitol, 10 mM NaCl, 0.5 mM 2,6-dichlorophenol indophenol (DCIPI), 0.05% (w/v) bovine serum albumin, and thylakoid membranes (25 \( \mu \text{g} \) chlorophyll) in a final volume of 2 ml. PS I activity was based on ascorbate/DCIP-methylviologen dependent \( \text{O}_2 \) uptake rates. The reaction mixture consisted of 50 mM Tricine (pH 8.3), 0.3 mM DCIP, 0.1 mM methylviologen, 0.08 mM 3-(3,4-dichlorophenyl)-1,1-dimethyleurea, 5 mM ascorbic acid, 2 mM KCN, 10 mM methylamine and thylakoid membranes (25 \( \mu \text{g} \) chlorophyll) in a final volume of 2 ml.

**Polyamine analysis**

One-g (FW) samples of leaf blades and roots were homogenized in 5% (w/v) perchloric acid (PCA). After centrifugation, the supernatant was preserved; the pellet was resuspended in 5% PCA after several washes with the same solution. Aliquots of the PCA-soluble fractions containing free and conjugated polyamines and PCA-insoluble fractions containing bound polyamines
were hydrolyzed in 6 N HCl at 110 °C for 18 hr to convert conjugated and bound forms to free forms. The hydrolyzate was dried in an aluminum bath at 70 °C; the residue was dissolved in a small amount of 5% PCA. Following dansylation, polyamines in acid extracts and hydrolyzates were quantified by HPLC using a μ-Bondapack C18 reverse phase column (Waters, USA) with a μ-Bondapack C18 guard column (Burtin et al., 1989). Conjugated polyamine contents were calculated by subtracting the free polyamines from the acid-soluble polyamine contents. 1,6-Hexanediamine was used as an internal standard. Polyamines in thylakoid membranes were assayed as above after the membrane suspensions were subjected to acid hydrolysis. Thus, the total polyamine (the sum of free, conjugated, and bound forms) is available in thylakoid membranes.

**Enzyme analysis**

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was extracted in 100 mM Tricine-KOH (pH 7.8), containing 5 mM dithiothreitol, 5 mM MgCl₂, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 2% (w/v) polyvinylpyrrolidone. After centrifugation at 3,500 × g, the initial activity of Rubisco in the supernatant was determined as described by Makino et al. (1994). Fructose-1,6-bisphosphatase (FBPase) was extracted in 0.1 M Tris-HCl (pH 7.8) and dialyzed overnight against the extraction buffer. Assay of FBPase activity was based on enzyme-linked formation of NADPH (Kelly et al., 1976). Both enzymes were assayed at 30 °C. Proteins were determined by the method of Bradford (1976), using bovine serum albumin as a standard.

**Assay of hydrogen peroxide and malondialdehyde**

Hydrogen peroxide (H₂O₂) was extracted from leaves by homogenizing with 100 mM sodium phosphate (pH 6.8). After centrifugation, H₂O₂ in the supernatant was quantified spectrophotometrically according to Lee and Lee (2000). Thiobarbituric acid-reacting substances in thylakoid membranes were extracted in 100 mM trichloroacetic acid containing 0.05% (w/v) butylated hydroxytoluene, and malondialdehyde in the extracts was quantified according to Albrecht and Wiedenroth (1994). Malondialdehyde has been used as an index of membrane lipid peroxidation (Heath and Packer, 1968).

**Photo inhibitory treatment to detached leaves and isolated thylakoid membranes**

Effects of Spd levels in leaves or isolated thylakoids on their susceptibility to photoinhibitory treatment were examined. Leaf discs (1.5 cm diam.); taken from unchilled leaves of Spd-pretreated and control plants 2 days after Spd treatment, were placed on wet filter paper in a plastic dish, and exposed to a light of 240 μmol·m⁻²·sec⁻¹ PPFD at temperatures ranging from 5 to 25 °C. After 5 hr, the rate of photosynthetic O₂ evolution was determined at 25 °C in a Clark-type oxygen electrode under illumination at 1.1 mmol·m⁻²·sec⁻¹ PPFD. The reaction mixture consisted of 25 mM Tricine-KOH (pH 7.8), 100 mM sucrose, 5 mM EDTA, 1 mM KH₂PO₄, 5 mM KCl, 1 mM MgCl₂, and 15 mM NaHCO₃.

One aliquot of the membrane suspensions, isolated as above from leaves of the control plants growing at 28/22 °C, was mixed with Spd at a final concentration of 0.5 mM and another aliquot with isolation buffer. Subsamples of each (0.3 ml, 60 μg chlorophyll·m⁻¹) were placed in small vials and exposed to 5 °C in a light of 240 μmol·m⁻²·sec⁻¹ PPFD. During this photoinhibitory treatment, electron transport activities of thylakoids were determined as above at 1-hr intervals for 5 hr.

**Results**

**Polyamine content in leaves, roots and thylakoid membranes**

Contents of total (free + conjugated + bound forms) Spd and Spm in leaves before chilling (2 days after Spd pretreatment) were about 2-fold higher in Spd-pretreated plants than in the control (Fig. 1A). The contents of these polyamines decreased gradually during chilling in both treatments; after 7 days of chilling, the difference between the treatments was no longer statistically significant.

**Fig. 1.** Changes in the content of putrescine (Put), spermidine (Spd) and spermine (Spm) in leaves (A) and thylakoid membranes (B) of Spd-pretreated cucumber plants and the control during the chilling treatment. T and C represent Spd-pretreated plants and the controls, respectively. Asterisks indicate that the content of the marked polyamines is higher in T than in C with statistical significance by the 5% level student’s t-test.
significant. Spd contents in the roots were also higher in Spd-pretreated plants than in the control (52.3 ± 5.9
and 90.3 ± 9.3 nmol·g⁻¹ FW before chilling, and 34.6 ± 4.5 and 53.3 ± 6.8 nmol·g⁻¹ FW after 7 days of chilling in the pretreated plants and the control, respectively). Total Spd content in thylakoid membranes before chilling was also 3-fold higher in the pretreated plants than in the control (Fig. 1B). Spd content decreased substantially during chilling in both treatments, but was still higher in Spd-pretreated plants as was the Spm content after 4 days of chilling than in the control.

**Plant growth and leaf chlorophyll content**

The control plants stopped growing after 2 days of chilling; they did not resume growth even after being transferred to warm conditions (Fig. 2A). In contrast, Spd-pretreated plants grew constantly during the chilling periods as well as after being returned to warm conditions. Consequently, the pretreated plants had a 3-fold larger dry mass than the control had at the end of the experiment. Root growth did not differ between the treatments during chilling, but it was greater in the pretreated plants after 3 days in the warm conditions (Fig. 2A). However, the growth rate of the chilled, Spd-pretreated plants was much slower than that of the heated control plants. Specifically, the pretreated plants showed a 2.5-fold increase in dry weights during

![Figure 2](image-url)

**Fig. 2.** Effects of Spd pretreatment on growth (A) and leaf chlorophyll content (B) of cucumber plants during chilling and after a return to warm conditions. Means with different letters are significantly different at \( P \leq 0.05 \).

![Figure 3](image-url)

**Fig. 3.** Effects of Spd pretreatment on PS II chlorophyll fluorescence yields (Fv/Fm) (A), photochemical (B) and non-photochemical (C) chlorophyll fluorescence quenching coefficients (qP and qN, respectively), gross (D) and net (E) photosynthetic rates, and stomatal conductance (F) in chilled leaves of cucumber. Measurements were made immediately after the plants were transferred to warm conditions. Means with different letters are significantly different at \( P \leq 0.05 \).
growth at 10/7 °C for 8 days, while the control did a 5.4-fold increase during growth at 28/22 °C for the same duration.

Leaf chlorophyll content decreased gradually after 2 days of chilling, but the decrease was less pronounced in Spd-pretreated plants than in the control (Fig. 2B). After being returned to the warm conditions, leaves of the control continued to lose chlorophyll, while those of the pretreated plants were restored to a full green color. Leaf water content was about 89% (w/w) before chilling, which decreased to about 83% at the end of the chilling periods in both treatments.

Chlorophyll fluorescence and photosynthesis

Fv/Fm declined markedly after 5 days of chilling in the control but not in Spd-pretreated plants (Fig. 3A). This decline of Fv/Fm was mainly attributable to a reduction in the maximum fluorescence yield (Fm). In the control plants, photochemical fluorescence quenching coefficients (qP) also decreased markedly parallel with Fv/Fm, whereas the non-photochemical fluorescence quenching coefficients (qN) increased substantially after 5 days of chilling (Fig. 3B, C). Spd-pretreated plants made fewer changes in both of these parameters.

Gross photosynthetic rates declined markedly during chilling in both treatments (Fig. 3D), but the Spd-pretreated plants exhibited significantly higher photosynthetic rates than did the control throughout the chilling period. The rates of net photosynthesis in the control plants became negative after 5 days of chilling, while the pretreated plants showed positive rates throughout the entire chilling period (Fig. 3E). Dark respiration rates of leaves were unaffected by Spd pretreatment (data not shown). Chilled leaves had very low stomatal conductance in both treatments (Fig. 3F).

Photosynthetic electron transport activity of thylakoids

The control plants had thylakoids with impaired photosynthetic electron transport activity through both PS II and PS I (Fig. 4). Apparently, PS I was more severely damaged than PS II, based on the relative activities of chilled and unchilled leaves. Leaves of Spd-pretreated plants had thylakoids with significantly less damaged photochemical activities as compared to the control.

Activities of ribulose-1,5-bisphosphate carboxylase/oxygenase and fructose-1,6-bisphosphatase

Chilling treatment brought about a substantial decrease in the initial activity of Rubisco, which was significantly less pronounced in the Spd-pretreated plants than in the control (Fig. 5A). The enzyme activity, 6 days after chilling, was 57% of that before chilling in the control, whereas it was 82% in the pretreated plants. FBPase activity, which likewise decreased markedly in the chilled control particularly after 4 days (Fig. 5B), was also alleviated by the Spd pretreatment.

Concentrations of hydrogen peroxide in leaves and malondialdehyde in thylakoid membranes

The concentrations of H₂O₂ in the leaves and malondialdehyde in the thylakoid membranes steadily increased during chilling in both treatments (Fig. 6A, B), but those in the Spd-pretreated plants were significantly lower than in the control.
Fig. 6. Effect of Spd pretreatment on the concentrations of H$_2$O$_2$ in leaves and malondialdehyde in thylakoid membranes in chilled cucumber plants. Means with different letters are significantly different at $P \leq 0.05$.

Effect of Spd on the susceptibility of detached leaves and isolated thylakoids to low-temperature photoinhibition

Leaf discs exposed to 15 °C in the light for 5 hr exhibited similar O$_2$ evolution activity as those exposed to 25 °C in the Spd-pretreated plants and the control (Fig. 7). However, when the incubation temperature was lowered below 10 °C, those of the control plants lost their activity to evolve O$_2$ to a much greater extent than did those of the pretreated plants. The difference between the treatments became larger as the temperature was lowered. Similarly, when isolated thylakoid membranes were preloaded with Spd before the photoinhibitory treatment, loss of electron transport activity of the membranes was substantially alleviated in both PS II and PS I (Fig. 8).

Discussion

Spd pretreatment substantially alleviated the low-temperature growth inhibition of cucumber plants (Fig. 2A). The sensitivity of cucumber to low temperature is not only confined to the aerial parts but also to the roots, with severe growth inhibition occurring in the roots exposed to 15 °C or below (Tachibana, 1982). Lee (1997) indicated that high Spd content in the roots could facilitate growth of whole plants at low root-zone temperatures because of increased root growth. In our study, Spd-pretreated plants maintained higher Spd content in the roots than did the control, but this did not increase root growth over the control during the chilling period (Fig. 2A) nor the water content of the chilled leaves. Therefore, the contribution of the roots to the increased growth rate of whole plants, attributable to the Spd pretreatment, could be negligible.

The control plants showed negative rates of net photosynthesis after 5 days of chilling when they also stopped growing (Fig. 3E). In contrast, Spd-pretreated plants had positive rates of net photosynthesis and accumulated dry matter throughout the chilling period. Thus, this difference in the net photosynthetic rates in
chilled leaves may largely account for the higher growth rates of the Spd-pretreated plants than in the control. Stomatal conductance of chilled leaves may not be related to the difference in the photosynthetic rates between the treatments, because it decreased to very low levels similarly in both treatments (Fig. 3). Generally, the chill-induced stomatal closure is common to chilling-sensitive plants, but its contribution to limitations of photosynthesis in chilled leaves remains to be clarified (Allen and Ort, 2001). Thus, the higher photosynthetic rate in Spd-pretreated plants than in the control may well be attributable to a lesser degree of chill-induced damage to the photosynthetic apparatus.

Cucumber is highly sensitive to low-temperature photoinhibition of photosynthesis (Smillie et al., 1988), especially below 13 °C (Hodgson et al., 1987; Terasshima et al., 1994). The chilled leaves of control plants showed significant reductions in chlorophyll contents (Fig. 2B), Fv/Fm and qP (Fig. 3 A, B), photosynthetic electron transport activities of thylakoids (Fig. 4), and Rubisco and FBPase activities (Fig. 5). Reductions in these parameters are all characteristic of photoinhibited leaves (Öquist, 1983; Andrews et al., 1995). It is most likely, therefore, that the photosynthetic apparatus in the control plants suffered severe photoinhibition during growth under our chilling conditions. Marked increases of H$_2$O$_2$ and malondialdehyde in chilled leaves (Fig. 6) indicate an oxidative damage to the photosynthetic apparatus (Asada, 1994). Because Spd-pretreated plants exhibited much smaller reductions in all of the above parameters in comparison to the control, it is inferred that Spd in leaves and/or chloroplasts somehow prevents low-temperature photoinhibition of photosynthesis. That the loss of photosynthetic O$_2$ evolution activity in detached leaves due to photoinhibitory treatment was much smaller in the Spd pretreated plants than in the control (Fig. 7) may support this view.

Szalai et al. (1997) observed a marked increase of Put and Spd contents together with a significant decline of Fv/Fm in maize leaves during chilling at 5 °C in the light. However, chilling in the dark resulted in a less marked increase in Put and a significant decrease in Spd. Based on these observations, they postulated that the increase of Spd content might be important in plant defense to low-temperature photoinhibition. Our results may have substantiated their view. Thus, we conclude that the high levels of Spd in cucumber leaves and/or chloroplasts protect the photosynthetic apparatus against the low-temperature photoinhibition.

Because of the polycationic nature at a physiological pH, polyamines can bind strongly to negatively charged functional groups in nucleic acids, proteins, and membrane lipids. It has been shown that polyamines in chloroplasts are covalently bound to chlorophyll-protein complexes in thylakoids and Rubisco in the stroma (Del Duca et al., 1994). These ionic interactions are considered to increase stabilization of the subcellular compounds and membranes under stress (Roberts et al., 1986). Indeed, Besford et al. (1993) found that exogenously supplied Spd prevented the destruction of chlorophyll, Rubisco, and molecular complexes of thylakoids in osmotically stressed oat leaves. In our study, preloading the isolated thylakoid membranes with Spd resulted in a significant reduction of loss of electron transport activity during the photoinhibitory treatment (Fig. 8), which suggests that Spd can protect thylakoid protein complexes from chill-induced photodamage. However, polyamines are known to have multiple functions in plant cells including the scavenging of ROS (Bors et al., 1989; Kurepa et al., 1998). Therefore, the mechanism by which Spd counteracts the potential danger of photoinhibition in chilled leaves of cucumber requires further study.

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低温下でのキュウリの生育と光合成に及ぼす根へのスペルミシン前処理の影響

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要 約

低温下でのキュウリの生育と光合成に及ぼす内生ポリアミンレベルの影響を調べた。人工気象室（昼/夜温度 28/22 ℃，光強度 240 μmol・m⁻²・sec⁻¹，日長 14時間）で、第 2 本葉展開時の苗を 0.5 mM スペルミシン (Spd) を含む水耕培養液で 2 日間栽培した後、温度を 10/7 ℃に下げ、無処理対照植物とともに Spd を含まない培養液で 8 日間栽培し、その後 28/22 ℃に 3 日間戻した。その結果、Spd 前処理により、葉やチラコイド膜の Spd 含量が高まり、低温による生育および光合成の抑制や葉緑素の減少の程度が小さくなった。また、葉緑素蛻光 (Fv/Fm)，チラコイドの電子伝達活性，炭素代謝酵素活性の低温による低下やチラコイド膜の脂質過酸化が軽減された。さらに、低温非遭遇葉から単離したチラコイド膜に Spd を前処理することにより，その後の低温処理による光化学系の障害が軽減された。これらの結果は，Spd には低温障害から光合成器官を保護する機能があることを示唆する。