Studies on Ultrastructure and Function of Photosynthetic Apparatus in Rice Cells

III. Photooxidative damages of chloroplasts in rice seedlings*

Susumu TOYAMA and Sachiko HIBI
(Department of Biology, Faculty of Science,
Ochanomizu University, Otsuka, Tokyo 112, Japan)
Received July 8, 1987

Abstract: Studies on the photooxidation of chloroplasts have been performed using carotenoid-deficient seedlings induced by the treatment with herbicides. The present study was made in association with the changes caused in the photosynthetic pigment content and ultrastructure when the chloroplasts in normal rice seedlings were subjected to photooxidation. When the seedlings grown under $16.2\text{W}/\text{m}^2$ at 23°C were continuously exposed to the light intensity of $162\text{W}/\text{m}^2$ at 23°C , the chlorophyll content went on decreasing. The carotenoid content, however, did not show a significant change. Under the cycle of light and dark, the photooxidative destruction of chlorophyll went on decreasing with passage of the cycle, but no photooxidation was observed during the third cycle. On the other hand, the photooxidative damages of chloroplast structure were observed by electron microscopy. The appearance of RuBP Case crystal, the swelling of thylakoid membranes and the formation of plastoglobules were characteristic alterations. Especially, the electron density of grana thylakoids increased abnormally according to the lysis and release of membrane components. It appeared that the released materials transferred among the thylakoid interspaces to form the plastoglobules. When the seedlings were exposed to the sun light in the field, grana thylakoids were injured and plastoglobules become temporally prominent. However, the chloroplasts seemed to be recovered from the injury in the night.

Key words: Carotenoid, Chlorophyll, Chloroplast, *Oryza sativa*, Photooxidation, Plastoglobule, Thylakoid membrane.

イネの光合成器官の微細構造と機能に関する研究 第3報 イネの幼植物における葉緑体の光酸化による障害：遠山 益・日比佐知子（お茶の水女子大学理学部）

要 旨：葉緑体の光酸化に関する研究は、ある種の除草剤処理によって誘発されたカロチノイド欠損幼植物を用いて行なわれてきた。本研究は、正常なイネの幼植物の葉緑体が光酸化を受けるとき、光合成色素量と微細構造との変化に注目して行なわれた。 $16.2\text{W}/\text{m}^2$, 23°C で18日間育てたイネの幼植物を $162\text{W}/\text{m}^2$, 23°C に連続暴露すると、カロチノイド色素が正常に含まれていても、クロロフィル含有量は減少を続け、5日後には当初の量の半分に減少した。しかし、カロチノイド含有量はこの強光暴露を通して有意な変化を示さなかった。12hの明暗周期の下で、同じイネの幼植物を $162\text{W}/\text{m}^2$ に暴露すると、第1日目は光酸化によってクロロフィル量がかなり減少したが、第2日目の減少量はわずかで、第3日目ではクロロフィルの光酸化は認められなかった。このように、明暗周期下におくと、イネの幼植物は強光暴露に対して次第に馴化するようにみえる。他方、光酸化による葉緑体の微細構造的障害が電子顕微鏡によって観察された。RuBP カルボキシラーゼの結晶の出現、チラコイド膜の膨潤、およびグラナチラコイド膜 (GM) の障害とプラスト顆粒 (PG) の生成が特徴的な変化であった。とくに、GM の構成成分が分解遊離するためグラナ部の電子密度が異常に増大する。これらの物質は膜間を移動して PG に結集するように見え、そのため光酸化の進行とともに PG は数・量ともに増大すると思われる。幼植物を朝から野外で太陽光にさらすと、GM は光酸化によって一時的に上記のような障害を受け、PG は増加する。しかし、夜間にはその障害が癒えるようにみえた。

キーワード：イネの幼植物、カロチノイド、クロロフィル、光酸化、チラコイド、プラスト顆粒、葉緑体。

Studies on the photooxidation of plastids have been performed in reference to the transformation of etioplasts to chloroplasts or to young chloroplasts in the carotenoid deficient

seedlings which are induced by the treatment with some herbicides^{6-9,22,28}). Herbicides, San 6706 and difunon, induced a severe carotenoid deficiency and intense photooxidative damages of treated leaves in the light, including the degeneration of chlorophyll, 70s ribosomes, chloroplast enzymes and even the inactivation of many peroxisomal enzymes.

* A part of this study was presented at the 178th Meeting of the Crop Science Society of Japan, Oct. 16-17, 1984.

On the other hand, photooxidative damages by the treatment with other herbicides, aminotriazol and haloxidine, were far less serious⁶⁾.

Leaf plastids of these carotenoid deficient seedlings show little or no development of internal membrane structure^{1,10)}. These plastids are deficient in plastid ribosomes and lack chloroplastic proteins^{9,13)}. Cytoplasmic protein synthesis and ribosome accumulation are largely unaffected⁴⁾. Although the structural and functional roles of carotenoids are not well understood, their importance in protecting chlorophyll from photooxidation has been well demonstrated³⁾. Under either very low-fluence-rate light or far-red light, even though the inhibition of synthesis of one set of plastid membrane component (carotenoid) took place by the treatment with herbicides, development of plastid ultrastructure, synthesis of RuBPCase and chlorophyll are not affected^{2,9,30)}. In the presence of herbicides, however, white light or red light at high-fluence-rates causes photo-destruction of chlorophyll, RuBPCase and thylakoid membranes^{8,9,22,28)}. These effects are interpreted as resulting exclusively from self-photooxidation and photosensitizing action of chlorophyll once the protection of chlorophyll by carotenoids against self- and sensitized photooxidation is lost⁹⁾.

The present study deals with the photooxidative effects on leaf chloroplasts in rice seedlings. Proceeding from a differing view point from those mentioned above, the photooxidative damages of photosynthetic pigments in normal rice seedlings grown in low frequency light which were then transferred to high frequency light (herbicides were not applied) were examined.

Materials and Methods

Seeds of rice, *Oryza sativa* L. cv. Honenwase, were sowed on soil in the tall skirted dishes containing plenty of water, and cultured under light intensity of 16.2W/m^2 (daylight fluorescent lamp), day length 12 h at 23°C for 18 days. Eighteen-day-old seedlings were divided into three groups; one was set under the continuous illumination of 162W/m^2 (daylight fluorescent lamp) for 5 days at 23°C and the other was put under the day length 12 h of 162W/m^2 at 23°C for 3 days. In all cases, the third leaves were used for observation of

chloroplast ultrastructure and measurement of pigment contents. For the measurement of chlorophyll and carotenoids, leaf materials were sampled timely from just before exposure to 162W/m^2 to 120 h after. On the other hand, to examine ultrastructural response of chloroplasts to natural condition in the field, the third group of 18-day-old seedlings were exposed to the sun light in the open air for a day. The exposure to the sun was started in the morning (at 8 a.m.) when the light intensity was 163.2W/m^2 . Through the subsequent 10.5 h, the light intensity ranged from 163.2W/m^2 to 6.12W/m^2 via maximum 663W/m^2 . Chlorophyll content was measured by Vernon's method³¹⁾ and carotenoids by Liaaen-Jensen and Jensen method²¹⁾ respectively. For electron microscopic observations, samples were prepared from the middle region of the third leaves from immediately before exposure to 162W/m^2 to 96 h afterwards by the conventional preparation method described in the previous paper¹⁵⁾. Ultrathin sections were examined with the H-700 electron microscope.

Results

1. Macroscopic observations

The third leaves on rice seedlings grown under 16.2W/m^2 at 23°C for 18 days were light green in color. After continuous exposure of these leaves to light intensity of 162W/m^2 for 12 h, the discolored tissues appeared at the apical region of the third leaves on a few seedlings. After 48 h of exposure the surface of the leaves were covered with irregular tessellation of yellowish-and light-green color. After 72 h, the apical region of the third leaves on all seedlings was found discolored into white. After 120 h, the decolored tissues spread to the middle region of the third leaves, and the remaining tissues also became lighter green in color. On the other hand, when the seedlings grown at 16.2W/m^2 for 18 days were cultured under the cycle of 12 h day length of 162W/m^2 for 3 days, the entire leaf blade kept its green color as before the exposure to light of high intensity, although the decolorization appeared only at the apical region of a few seedlings.

2. Changes of photosynthetic pigment content

Fig. 1 shows the progressive changes of chlorophyll content with time when the seed-

lings grown at $16.2\text{W}/\text{m}^2$ for 18 days were continuously exposed to the light of $162\text{W}/\text{m}^2$ and $16.2\text{W}/\text{m}^2$ for 120 h respectively. During 8 h from the start of exposure to $162\text{W}/\text{m}^2$, chlorophyll content was on the increase and subsequently went on decreasing with the passage of time, being reduced by about half ($1.08\mu\text{g}/\text{mg}$ f.w.) of the original content ($2.00\mu\text{g}/\text{mg}$ f.w.) after 120 h of the exposure. On the contrary, under the light of $16.2\text{W}/\text{m}^2$, chlorophyll content did not show the significant rise and fall through the exposure time.

Fig. 2 shows the changes of total carotenoid content measured under the same condition with the case of chlorophyll measurement. The total carotenoid content did not indicate a significant change regardless of the light intensity throughout the exposure time.

Fig. 3 shows the changes of chlorophyll content during the exposure of the seedlings to the light of $162\text{W}/\text{m}^2$ in the cycle of 12 h day length for 3 days. Under the cycle of 12 h light/12 h dark, chlorophyll content went on increasing for 8 h after the exposure started and thereafter was on the decrease till 12 h of exposure. This pattern resembles that of the earlier time of continuous exposure of the seedlings to $162\text{W}/\text{m}^2$ (Fig. 1). During the next light period, chlorophyll content increased with the illumination start and afterward had little or not reduction. On the third light period it kept on increasing rapidly. During dark period, chlorophyll content went on decreasing without exception.

3. Ultrastructural changes of chloroplasts

1) The chloroplasts in the third leaves on the seedlings grown under $16.2\text{W}/\text{m}^2$ for 18 days were typical in the structure and arrangement of thylakoid membranes and showed no changes in the stroma ultrastructure, except the accumulation of abundant starch grains (Figs 4 and 6). There were few osmiophilic plastoglobules in the stroma.

2) When the leaves described above were exposed to the light of $162\text{W}/\text{m}^2$ at 23°C for 4 h, the chloroplasts remained normal in both membrane system and stroma, although the slight swelling appeared on the outer side (cell wall side) of chloroplasts. After 12 h of exposure, the starch in chloroplasts was accumulated much more, and grana thylakoids also increased in number. A characteristic is the

Fig. 1. Changes of total chlorophyll content in the third leaves when the seedlings grown under light intensity of $1.62\text{W}/\text{m}^2$, day length 12 h, at 23°C for 18 days were continuously exposed to light intensity of $1.62\text{W}/\text{m}^2$ and $162\text{W}/\text{m}^2$ for 5 days respectively.

Fig. 2. Changes of total carotenoid content in third leaves under the same condition with the measurement of total chlorophyll content.

Fig. 3. Changes of total chlorophyll content in the third leaves when the seedlings grown under light intensity of $1.62\text{W}/\text{m}^2$, day length 12 h, at 23°C for 18 days were transferred to the light intensity of $162\text{W}/\text{m}^2$, day length 12 h, at 23°C for 3 days.

appearance of RuBPCase crystal in the stroma (Fig. 5). At this stage, the whole chloroplast increased in volume. After 16 h of exposure, starch grains decreased in both number and size. RuBPCase crystals disappeared in the stroma. In contrast, osmiophilic plastoglobules began to increase in both number and volume. At the same time, grana thylakoids slightly swelled. After that the swelling became conspicuous more and more, overspreading from grana thylakoids to stroma thylakoids (Fig. 7). During the exposure from 48 h to 120 h, the remarkable changes of chloroplast ultrastructure include a second accumulation of starch grains, the appearance of peripheral reticulum and the development of grana thylakoid membranes. A ultrastructural change becoming for the photooxidation of chloroplasts is the increase of electron density in the membrane systems (Fig. 8). The alteration occurs initially at the interspaces of grana thylakoids and subsequently in their loculi, resulting in the collapse of the membrane system (Fig. 9). In the neighbourhood of the dense membranes, plastoglobules seems to increase with the progress of photooxidation (Figs. 10 and 11).

3) To examine the chloroplast response to the sun light, the seedlings were put under the light of 163.2 W/m^2 in the open air at 8 a.m. After 4 h of exposure, the sun light intensity rose to 663 W/m^2 and the temperature went up to 36°C . At this time, both whole chloroplast and thylakoid membranes swelled slightly. At 2 : 30 p.m., though the sun light intensity and temperature went down to 96.7 W/m^2 and 31°C respectively, the plastoglobules markedly increased. The whole chloroplast and thylakoid membranes continued swelling slowly. At 4 : 30 p.m., even when the sun light intensity and temperature fell to 45.9 W/m^2 and 29°C respectively, the swelling of stroma- and grana-thylakoids was still observable. Plastoglobules continued to increase in both number and volume and their blackening

density. Peripheral reticula were also observed in the chloroplasts. At 6 : 30 p.m., the sun light intensity and temperature went down to 6.12 W/m^2 and 25°C respectively. The swelling of thylakoid membrane system appeared to have gradually restored the normal structure. At the same time, the density of the plastoglobules also gradually decreased.

Discussion

Photooxidation has been defined as light- and O_2 -dependent degradation of photosynthetic pigments²⁵⁾. It is reasonable to assume that chloroplasts are the primary site for photooxidative injury²⁰⁾ which includes the rapid appearance of photosynthetic dysfunction^{17,24,32)}, changes in the chloroplast ultrastructure^{29,33)} and membrane lipid degradation⁵⁾. Most of the studies on the photooxidation have been performed using carotenoid-deficient seedlings induced by the treatment of some herbicides. Herbicides, norflurazon and difunon, inhibit the synthesis of carotenoids almost completely at the concentration of $5 \times 10^{-6} \text{ M}$ and $2 \times 10^{-5} \text{ M}$ respectively without any adverse effects on the morphogenesis of the seedlings²⁸⁾. It is easily understood that carotenoid deficient seedlings are susceptible to photooxidation, because it has been demonstrated that carotenoid pigments protect chlorophyll from photooxidation^{9,22)}. In the presence of norflurazon and low fluence-rate white light, maize seedlings accumulate chlorophyll, chloroplast proteins and plastid ribosomes to wild levels, but fail to accumulate carotenoid pigments⁷⁾. Exposure of these seedlings to high fluence-rate light resulted in the photooxidation of over 90% of chlorophyll within 24 h²²⁾. When rye seedlings grown under low intensity light in the presence of herbicides were exposed to 162 W/m^2 at either 0°C or 30°C , rapid photooxidation of chlorophyll occurred both at 0°C and 30°C ⁸⁾. The photodestruction of chlorophyll in the presence of herbicides is related to

Explanation of Photographs (Figs. 4—7)

Fig. 4. A chloroplast in third leaf of the seedling grown at 1.62 W/m^2 for 18 days. s : starch grain. $\times 20,000$. Fig. 5. RuBPCase crystal produced in the stroma when the seedlings were exposed to light intensity of 162 W/m^2 . e : RuBPCase crystal. $\times 82,000$. Fig. 6. Normal arrangement and structure of grana thylakoids of chloroplast in the seedlings before the exposure to high light intensity. gt : grana thylakoids. $\times 62,000$. Fig. 7. Slight swelling of grana- and stroma-thylakoids after the exposure to high light intensity. $\times 99,000$.

the severe carotenoid deficiency induced by the chemicals, resulting in the occurrence of the reactive triplet state of chlorophyll that is normally quenched by protective carotenoids⁷⁾. The fact that the photodestruction of chlorophyll occurred at 0°C demonstrates that it mainly resulted from photodynamic reactions⁸⁾. Initially only singlet O₂ was thought to be involved in the photo-dynamic decomposition of chlorophyll; however, the photochemical formation of superoxide and hydroxyl radicals has been discussed as being responsible for the photodestruction of chlorophyll^{11,14)}.

The aim of this study was to examine the photooxidative damages caused when normal rice seedlings grown in low intensity light were exposed to high intensity light. As shown in Fig. 2, although the carotenoid content did not significantly change despite continuous exposure of the rice seedlings to high intensity light, the chlorophyll content went on decreasing with the exposure time (Fig. 1). Thus, it may be said that a role of carotenoids to protect chlorophyll from photooxidation is discharged incompletely. According to Kunst and Wrischer, with increasing light intensity there was a marked decrease in chlorophyll content in *Ligustrum* leaves, and the carotenoids yield became much higher¹⁸⁾. Under low intensity light condition the chlorophyll content is high, while the carotenoid concentration dropped significantly. As shown in Fig. 3, under the cycle of 12 h day length photodestructive chlorophyll content decreased with the passage of cycle and no photodestruction in the third cycle. This suggests the acclimation of rice seedlings to high intensity light.

Cereal seedlings are very useful in the studies of chloroplast ontogenesis, because along their leaves there are tissues of different ages. In this study, it was possible to show an effect of tissue ages on the photooxidation of

rice seedlings under the light of high intensity. Actually the treatment with high intensity light initially caused the photooxidative damages in the apical leaf region and thereafter in the middle leaf region; in older tissues the photodestruction of chlorophyll was severer. The age of leaf tissues affects both the etioplast greening and chlorophyll accumulation under high intensity light²⁷⁾; in the young tissues, high intensity light has a delaying effect on the etioplast greening and affects the duration of the lagphase preceding the chlorophyll accumulation. In contrast, in the old tissues etioplast greening was slow, and light intensity affects the total amount of chlorophyll pigments. Therefore, the young tissues appear to be better able to withstand the high intensity light, probably by means of a higher turnover of the components of chloroplasts, as suggested by Grumbach and Lichtenthaler¹²⁾. According to Reitz et al.²⁸⁾, and Wise and Naylor³⁴⁾, photodamages in herbicide treated seedlings are restricted to the plastids and to closely associated peroxisomes. This is compatible with studies on other plants^{23,29,33)}.

Exposure of the plant to high intensity light, in the presence of herbicides, led to a destruction of the internal structure of plastids. The thylakoid system had disappeared except for a few isolated thylakoids which seemed to be attached to the envelope, while the plastid envelope did not show any signs of damages. Plastid ribosomes were no longer detectable²⁸⁾. Chloroplast proteins showed varying degrees of sensitivity to photooxidation; the P-700 apoprotein of photosystem I was rapidly degraded and most stroma- and thylakoid-proteins either decreased progressively in photooxidative conditions or appeared to be unaffected²²⁾. The remarkable change of rice chloroplast ultrastructure by photooxidation was the increase of electron density in grana thylakoids. It seemed that this depended on

Explanation of Photographs (Figs. 8—11)

Fig. 8. Grana thylakoids with high electron density appeared by exposing the seedlings to high light intensity of 162 W/m² (arrows). $\times 38,500$. Fig. 9. At the ends of thylakoid membranes with high density or of collapsed membrane system, small plastoglobules are going to be formed. pg: plastoglobule. $\times 100,300$. Fig. 10. Advanced processes of plastoglobule formation, in which various stages of the formation are observable. $\times 54,500$. Fig. 11. A whole chloroplast in the seedlings exposed to high light intensity in which many osmiophilic plastoglobules are produced by the intense photooxidation. Peripheral reticula (pr) also are observed at the peripheral areas of chloroplast. $\times 24,000$.

the complex associated with osmium-tetraoxide of the membrane components--lipids and proteins released from the membranes according to the photooxidation. The increase of the density took place initially at the interspaces of grana thylakoids and subsequently in their loculi (Fig. 8). A new interesting metamorphosis of thylakoids was observed by means of electron microscopy; the photodestructive materials appeared to flow to concentrate in the plastoglobules through the interspaces and loculi of grana thylakoids (Figs. 8 and 9). This seems to suggest that the plastoglobules increase in both number and volume with the passage of exposure time. There are many reports on the plastoglobule formation by the inhibition of thylakoid development based on various reasons and by the collapse of thylakoid membranes¹⁶⁾. The plastoglobule formation reported in this study is essentially consistent with the information given in the studies cited above.

During the exposure to high intensity light, the peripheral vesicles and irregular protrusions from the chloroplast envelope were formed. Such changes have been reported previously in reference to the plants such as *Phaseolus*, *Gossypium* and *Glycine* in low temperatures and in the light^{23,33)}. The peripheral vesicles are similar in appearance to components of the chloroplast peripheral reticulum which thought to be involved in metabolite transport^{16,19)}. The role of the peripheral vesicles played in the plant under the stresses of low temperatures and high intensity light remains obscure. Likewise, the significance of the chloroplast envelope extensions is not known.

Acknowledgements

The authors wish to express their sincere thanks to Associate Professor Teizo Ajiri, Jissen Women University, for his technical assistance. A part of this study was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 59540442).

References

1. Bachmann, M.D., D.S. Robertson, C.C. Bowen, and I.C. Anderson 1967. Chloroplast development in pigment deficient mutants of maize. *J. Ultrastruct. Res.* 21 : 41—60.
2. —, —, — and — 1973. Chloroplast ultrastructure in pigment-deficient mutants of *Zea mays* under reduced light. *J. Ultrastruct. Res.* 45 : 384—406.
3. Bensasson, R.V., E.J. Land, A.L. Moore, R.L. Crouch, G. Dirks, T.A. Moore and D. Gust 1981. Mimicry of antenna and photoprotective carotenoid functions by a synthetic carotenoporphyrin. *Nature* 290 : 329—332.
4. Blume, D.E. and J. McClure 1980. Developmental effects of Sandoz 6707 on activities of enzymes of phenolic and general metabolism in barley shoots grown in the dark under low or high light intensity. *Plant Physiol.* 65 : 238—244.
5. Dekok, J.L. and P.J.C. Kuniper 1977. Glycolipid degradation in leaves of the thermophilic *Cucumis sativus* as affected by light and low-temperature treatment. *Physiol. Plant.* 39 : 177—185.
6. Feierabend, J. and B. Schubert 1978. Comparative investigation of the action of several chlorosis-inducing herbicides on the biogenesis of chloroplasts and leaf microbodies. *Plant Physiol.* 61 : 1017—1022.
7. —, U. Schulz, P. Kemmerich and T. Lowitz 1979. On the action of chlorosis-inducing herbicides in leaves. *Z. Naturforsch.* 34c : 1036—1039.
8. — and T. Winkelhusener 1982. Nature of photooxidative events in leaves treated with chlorosis-inducing herbicides. *Plant Physiol.* 70 : 1277—1282.
9. Frosch, S., M. Jabben, R. Bergfeld, H. Kleinig and H. Mohr 1979. Inhibition of carotenoid biosynthesis by the herbicide SAN 9789 and its consequences for the action of phytochrome on plastogenesis. *Planta* 145 : 497—505.
10. Faludi-Daniel, A., L. Fridvalszky, and I. Gyurjan 1968. Pigment composition and plastid structure in leaves of carotenoid mutants of maize. *Planta* 78 : 184—195.
11. Ginkel, van G., and J.K. Raison 1980. Light-induced formation of $O_2 \cdot$ oxygen radicals in system containing chlorophyll. *Photochem. Photobiol.* 32 : 793—798.
12. Grumbach, K.H. and H.K. Lichtenthaler 1982. Chloroplast pigments and their biosynthesis in relation to light intensity. *Photochem. Photobiol.* 35 : 209—212.
13. Harpster, M.H., S.P. Mayfield and W.C. Taylor 1984. Effects of Pigment-deficient mutants on the accumulation of photosynthetic proteins in maize. *Plant Molec. Biol.* 3 : 59—71.
14. Harbour J.R. and J.R. Bolton 1978. The involvement of the hydroxyl radical in the destructive photooxidation of chlorophylls *in vivo* and *in vitro*. *Photochem. Photobiol.* 28 : 231—234.
15. Ikeda, T. and S. Toyama 1987. Studies on ultrastructure and function of photosynthetic apparatus in rice cells. I. Effects of light intensity and temper-

- ature on plastid development of *Oryza sativa* L. Japan. Jour. Crop Sci. 56 : 85—91.
16. ——— and ——— 1987. Studies on ultrastructure and function of photosynthetic apparatus in rice cells. II. Effect of low temperature on early development of rice plastids. Japan. Jour. Crop Sci. 56 : 632—640.
 17. Kee, S.C., B. Martin and D. Ort 1986. The effects of chilling in the dark and in the light on photosynthesis of tomato : electron transfer reactions. Photosynth. Res. 8 : 41—51.
 18. Kunst, L. and M. Wrisher 1984. Adaptational changes of plastids in the leaves of *Ligustrum ovalifolium* Hassk. var. *aureum* at different light intensity. Protoplasma 122 : 132—137.
 19. Laetsch, W.M. 1968. Chloroplast specialization in dicotyledons possessing the C_4 -dicarboxylic acid pathway of photosynthetic CO_2 fixation. Am. J. Bot. 55 : 875—883.
 20. Larcher, W. 1980. Physiological Plant Ecology. Springer-Verlag, Berlin. 10—11.
 21. Liaaen-Jensen, S. and A. Jensen 1971. Quantitative determination of carotenoids in photosynthetic tissue. In Methods in Enzymology Vol 23 (Ed) A. San Pietro, Academic Press, New York and London. 586—602.
 22. Mayfield, S.P., T. Nelson and W.C. Taylor 1986. The fate of chloroplast proteins during photooxidation in carotenoid-deficient maize leaves. Plant Physiol. 82 : 760—764.
 23. Musser, R.L., S.A. Thomas, R.R. Wise, T.C. Peeler and A.W. Naylor 1984. Chloroplast ultrastructure, chlorophyll fluorescence and pigment composition in chilling-stressed soybeans. Plant Physiol. 74 : 749—754.
 24. Powles, S.B., J.A. Berry and O. Bjorkman 1983. Interaction between light and chilling temperatures on the inhibition of photosynthesis in chilling-sensitive plants. Plant Cell Environ. 6 : 117—124.
 25. ——— 1984. Photoinhibition of photosynthesis induced by visible light. Ann. Rev. Plant Physiol. 35 : 15—44.
 26. Peiser, G.D. and S.F. Yang 1978. Chlorophyll destruction in the presence of bisulfite and linoleic acid hydroperoxide. Phytochemistry. 17 : 79—84.
 27. Rascio, N., P. Mariani and G. Casadoro 1984. Etioplast-chloroplast transformation in maize leaves : Effects of tissue age and light intensity. Protoplasma 119 : 110—120.
 28. Reiss, T., R. Bergfeld, G. Link, W. Thien and H. Mohr 1983. Photooxidative destruction of chloroplasts and its consequences for cytosolic enzyme levels and plant development. Planta 159 : 518—528.
 29. Taylor, A.O. and A.S. Craig 1971. Plant under climatic stress. II. Low temperature, high light effects on chloroplast ultrastructure. Plant Physiol. 47 : 719—725.
 30. Troxler, R.F., R. Lester, F.O. Craft and J.T. Albright 1969. Plastid development in albescens maize. Plant Physiol. 44 : 1609—1618.
 31. Vernon, L.P. 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem. 32 : 1144—1150.
 32. Van Hasselt, P.R.A. and Hac Van Berlo 1980. Photooxidative damage to the photosynthetic apparatus during chilling. Physiol. plant. 50 : 52—56.
 33. Wise, R.R., J.R. McWilliam, and A.W. Naylor 1983. A comparative study of low temperature-induced ultrastructural alterations of three species with differing chilling sensitivities. Plant Cell Environ. 6 : 525—535.
 34. ——— and A.W. Naylor 1987. Chilling-enhanced photooxidation. The peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. Plant Physiol. 83 : 272—277.