Japan. Jour. Crop Sci. 56(4) : 632–640 (1987)

Studies on Ultrastructure and Function of Photosynthetic Apparatus in Rice Cells

II. Effect of low temperature on early development of rice plastids

Taiji IKEDA* and Susumu TOYAMA**

(*Department of Biology, College of General Education, Niigata University, Niigata 950-21, **Department of Biology, Faculty of Science, Ochanomizu University, Tokyo 112)

Received April 10, 1987

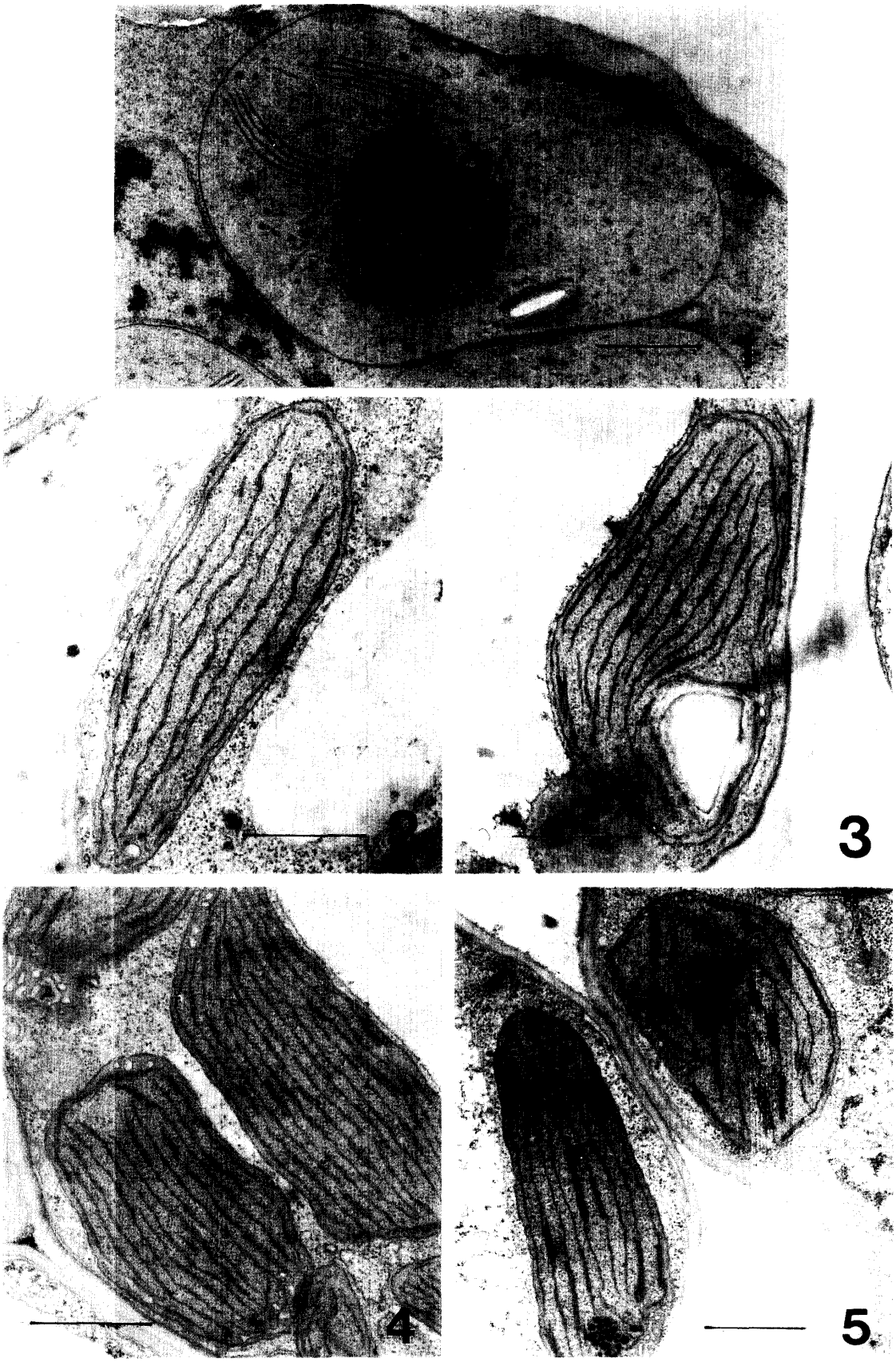
As the previous paper[15], for the development of rice plastids, light and temperature are important environmental factors. Effect of light on the ultrastructural development of plastids have been studied in detail[2,12,26]. On the other hand, only a few information is known about the effects of temperature, above all low temperature on the plastid development[16,27,28]., although a number of studies have been reported about the effects of temperature on the photosynthesis of many higher plants including rice[1,5,22,25]. Most of studies on the effects of low temperature on plastids deal with the alterations of thylakoid membrane lipids and proteins during cold-hardening[6,14,24], cold acclimation[3,7,9,10], and freeze-thaw cycle[8] of young or mature chloroplasts. Low temperature is able to inhibit the chloroplast development in chilling-sensitive plants such as maize[23] and sorghum[28]. HUNER et al. showed that rye at cold-hardening temperature had a significant effect on thylakoid membrane ultrastructure and pigment content[14]. Rye chloroplasts grown at low temperature also accumulated more plastoquinone and plastoglobuli than warm-grown rye chloroplasts[11]. However, nothing is known about the effect of low temperature on the early stage of plastid development. In the present paper, the effect of low temperature on the early development of rice plastids was comparatively studied using three rice cultivars of different chilling resistance with special reference to the formation of weak-light prolamellar bodies (w-PLBs).

Materials and Methods

Seeds of three rice cultivars, *Oryza sativa* L. cv. Bouzu, Honenwase and IR 8 were soaked in distilled water for 3h, sown on the sheets of moistend cotton and grown in the dark at 20°C. Cold resistance of Bouzu, Honenwase and IR 8 is high, medium and low respectively. It took 10 days for Bouzu, 11 days for Honenwase and 15 days for IR 8 to grow up to 7 cm of third leaf length. And then the plants were greened under fluorescent lamp of light intensity 5.5 or 4.3 W/m^2 at 20°C. The greening level of leaves was classified as in the previous paper[15], expressing with the term of Color Index (CI) from 0 (greenless) to 8 (deep green). To greening up to CI-8, it took 4 days for Bouzu and 5 days for Honenwase and IR 8. Upper parts than one third of third leaf were used as materials. The green leaves of CI-2, 4, 6, and 8 were prepared for electron microscopy by the conventional methods used in the previous paper, and examined with the H-300 electron microscope. More than 100 plastids on each sample were observed to calculate the rate of plastids having Prolamellar bodies (PLBs) or w-PLBs.

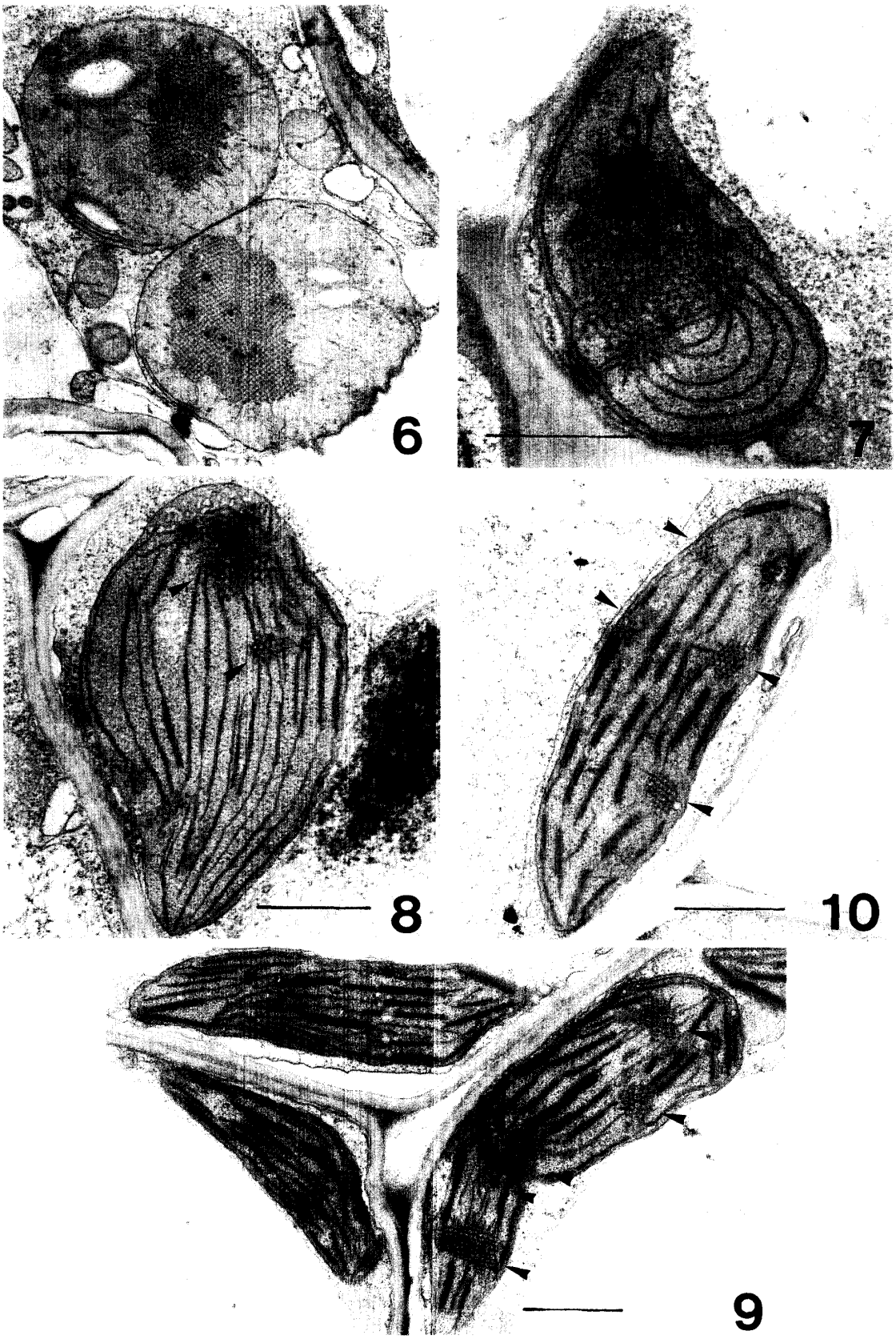
Results

Fig. 1 shows a etioplast in dark-grown IR 8 leaf for 15 days at 20°C, in which a prolamellar body (PLB) is visible as the paracrystalline structure and prothylakoids are going to extend from it. Starch grains are also contained in it. Figs. 2–5 show young plastids in IR 8 leaves on the way of greening under 5.5 W/m^2 light at 20°C. In plastids of CI-2 leaf, primary thylakoids with small grana thylakoids are formed, but PLB was already disappeared (Fig. 2). At this stage of greening, PLBs were observed in only 7% of plastids examined (Table 1). Plastids in CI-4 leaf include grana thylakoids consisting of 2–3 layers, but no plastids containing PLB (Fig. 3). Figs. 4 and 5 show the plastids in leaves of CI-6 and 8 respec-



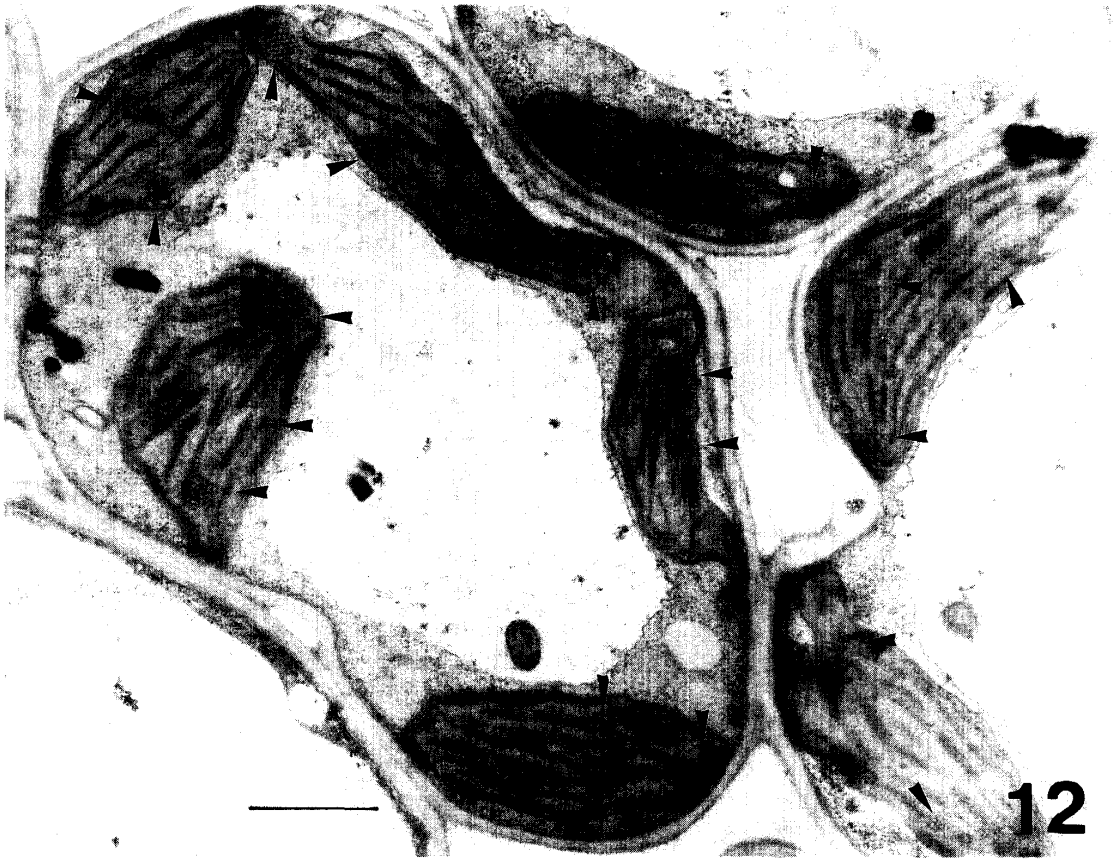
Explanation of Figures (Figs. 1–5).

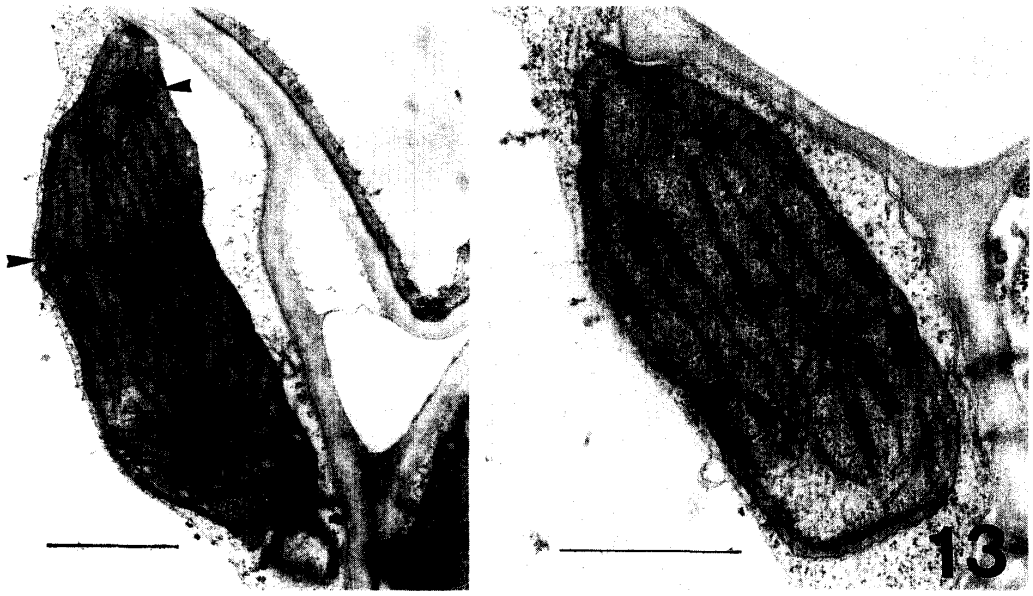
Fig. 1. A etioplast in third leaf of dark-grown IR 8 at 20°C for 15 days. Figs. 2–5. Plastids in IR-8 leaves greened under 5.5W/m² light at 20°C. The greening levels of Figs. 2, 3, 4 and 5 correspond to CI-2, 4, 6 and 8 respectively. Length of all bars shows 1μm.



Explanation of Figures (Figs. 6-10).

Fig. 6. Etioplasts in third leaf of dark-grown Honenwase at 20°C for 11 days. Figs. 7-10. Plastids in Honenwase leaves greened under 5.5 W/m^2 light at 20°C. The greening levels of Figs. 7, 8, 9 and 10 correspond to CI-2, 4, 6 and 8 respectively. Length of all bars shows 1 μm.





Explanation of Figures (Figs. 11-13).

Figs. 11 and 12. Plastids in Bouzu leaves greened up to CI-4 and 8 respectively under 5.5 W/m² light at 20°C. Fig. 13. A plastid in IR 8 leaf greened up to CI-6 under 5.5 W/m² at 26°C. Length of all bars shows 1μm.

tively. Grana thylakoids were composed of the stacks of 4-5 layers, but no PLBs were observed and starch grains were lost now. The structural changes of plastids in IR 8 leaves under 4.3 W/m² light at 20°C were closely similar to them under 5.5 W/m² light at 20°C. Characteristic was to contain the aggregates of osmiophilic plastoglobuli in the plastids of IR 8 leaves. There were very small number of plastoglobuli or no one on the early development of plastids. Thus, PLBs in plastids of

IR-8 leaves disappeared at the early stage of plastid development and were not reformed through the subsequent development under both light intensity 5.5 and 4.3 W/m² (Table 1). The peripheral reticula are observable with increasing clearness with the progress of plastid development.

Fig. 6 shows the etioplasts in dark-grown Honenwase leaf for 11 days at 20°C. The etioplasts include PLBs of paracrystalline structure as similar as those of IR 8 and starch grains. However, starch grains were rapidly disappeared at the early stage of plastid development. Figs. 7-10 show the young plastids in Honenwase leaves in process of greening under 5.5 W/m² light at 20°C. Fig. 7 shows a plastid of CI-2 leaf, in which the flabby structure of PLB and the perforated thylakoids protruding from it are observed. At this stage of plastid development, PLBs lie in 72% of plastids examined (Table 1). In the plastids of CI-4 leaf, grana thylakoid stacks of 2-3 layers and w-PLBs also were observed (Fig. 8, arrow). Fig. 9 shows plastids in CI-6 leaf in which w-PLBs are clearly observable. As the greening level makes progress, w-PLBs increased in number still more, and were observed in 55% of plastids examined (Table 1). Fig. 10 shows a plastid in CI-8 leaf. The plastid includes the stacks of grana thylakoid consisting of 5-6 layers and several w-PLBs (arrow) which lie in 57% of plastids observed. Thus, when the etiolated leaves of Honenwase were placed under light intensity 5.5 or 4.3 W/m² at 20°C, w-PLBs were formed in the plastids with the progress of leaf greening. A small number of plastoglobuli was visible at the area of PLBs. There were the peripheral reticula though the plastid development.

Fig. 11 shows a plastid in CI-4 leaf of Bouzu under 5.5 W/m² light at 20°C. The w-PLBs were observed in 41% of plastids examined. In CI-8 green leaf of Bouzu, w-PLBs were included in 96% of plastids examined (Fig. 12). Whereas, during the greening of IR 8 etiolated leaf under 5.5 W/m² at 26°C, w-PLBs in CI-6 (Fig. 13) and CI-8 leaves were observed in 52% and 63% of plastids examined respectively. These values are similar to the rate of w-PLBs in Honenwase plastids under 5.5 W/m² light at 20°C. A small number of plastoglobuli and peripheral reticula were observed in the vicinity of PLBs in Bouzu plastids.

Discussion

The present study dealt with the effects of cold acclimation on the early stage of rice plastid development. Most of studies on the effects of chilling stress on plastids are concerned with the alterations during acclimation or hardening of young or mature chloroplasts. Concerning the effects of chilling on the early development of plastids there was no study. From the present study, it was shown that there were three common features in the effects of chilling treatment on the plastid structure regardless of the developmental stage ; the alterations of membrane system including PLBs, increase of plastoglobuli and rapid disappearance of starch grains.

Though the PLBs formation in rice cultivars was affected by chilling treatment dependng on their cold resistance, the thylakoid membranes of young or mature chloroplasts also were injured by cold treatment as indicated by the following reports. Cold resistant potato species had fewer thylakoids appeared swollen and irregular under cold temperature[4]. The acclimation to cold stress takes place over the entire temperature range in which chloroplast development is possible, although at low temperature (2-5°C) chloroplast biogenesis was inhibited[27]. The lamellar structure of chloroplasts in the C_3- and C_4- pathway plant leaves underwent ultrastructural disruption under

Table 1. Rate of plastids including PLB or w-PLBs during the plastid development at 20°C.

Cultivar	Light intensity (W/m²)	Color Index (thylakoid number in a granum) 2(1-2)	4(2-3)	6(3-4)	8(6-7)
IR 8	5.5	7%	0%	0%	0%
	4.3	33	6	0	0
Honenwase	5.5	72	6	55	57
	4.3	70	69	63	72
Bouzu	5.5	61	41	56	96
	4.3	73	70	61	92

moderate light intensity (170 W/m^2) at low temperature (10°C) for a day[29]. Rye chloroplast ultrastructure was affected by growth at low temperature as indicated by smaller grana stacks[14].

It appears that numerous alterations take place in the thylakoid membranes and stroma during acclimation to cold stress even though no morphological change of membranes is visible ; conformational changes of RuBPCase[13], one size group of particles on inner fracture face of cold acclimated thylakoids[7,14], release of plastocyanin and coupling factor from thylakoids[8], and absence of 51 Kilodalton polypeptide in cold hardened thylakoids[10] have been reported. However, there were no significant differences in the lipid composition and level of unsaturation of fatty acids between plastids from warm-and cold-grown *Pisum Sativum*[3].

To clarify the reason of PLBs formation in etioplasts is indispensable to analyse their chemical composition. There are some differences between PLBs and thylakoid membranes on both the major components and minor materials[18]. It is plausible that to transform PLBs to thylakoid membranes are required the essential components which their biosynthesis are accelerated by a trigger of high light intensity.

The lipid composition of etioplasts is not very different from that of the thylakoid membrannes, and the presence of non-phytylated pigments is not a prerequisite for the formation of PLBs[18]. So that, it seems that the transformation of PLBs to thylakoid membranes might be largely dependent on the presence of proteinous materials. It is well known that plastoglobuli contain verious lipidic materials. When the plants, *Hordeum*[27], *Fragaria*[24], *Solanum*[4], and winter rye[11] grown at low temperature are compared with their warm-grown counterparts, the number of plastogloguli per chloroplast in the formers increases more than in the latters. The number of plastoglobuli in the plastids of cold-resistant rice species is much lower than in a cold-susceptible cultivar. Since the major components of biomembrane are phospholipids or glycolipids, a number of plastoglobuli in plastids mentioned above may provide better sources of developmental and repairing material for the plastid membrane system, which might be inhibited or injured during cold treatment.

Plastoquinone A levels also increased in intact chloroplasts isolated from cold grown rye when compared with warm-grown rye[11]. LICHTENTHALER[21] has reported that excess plastoquinone A is stored in plastoglobuli in the chloroplasts stroma when the plants are grown at high light intensity. This suggests that some plastoquinone A may also be stored in plastoglobuli of cold-grown plants. Since in thylakoid membranes, plastoquinone A functions as a mobile electron carrier in photosynthetic electron transport, plastoglobuli might be regarded as a temporary storehouse of the essential materials to the structural and functional differentiation of plastids.

Peripheral reticulum (PR) was observed through the plastid development of three kinds of rice under low temperature without regard to cold-resistance. It has been stated that it consists of anastomosing tubules contiguous with the inner membrane of chloroplast envelope, and provides a connection between the thylakoid membrane system and chloroplast envelope[19]. PR has been observed in many C_4 plant species. It is generally accepted that PR is a characteristic of chlorplast of C_4 plant species, but reports of its occurrence in some C_3 plants has raised doubts about it being specially correlated with C_4 photosynthesis[19]. TAYLOR et al. has reported that the vesicles identified as PR occur in soybean chloroplasts of leaves given 1.5 days of chilling treatment[29]. It has been suggested that PR is involved either in rapid transport of photosynthetic precursors or end products between cytoplasm and chloroplasts, or that it represents a site for enzyme systems involved in C_4 photosynthesis. The correlation between PR formation in rice plastids at low temperature and the function remains to be proved.

The starch grains in IR 8 plastids were kept up to the CI4-5 stage, while in both Honenwase and Bouzu these disappeared at the very early stage of platid development, at CI 1-2 stage. The disappearance of starch grains in the chloroplasts of *Solanum acaule* after cold acclimation treatments indicates that starch transforms to soluble sugars during the hardening process[4]. The protective effect of soluble sugars against cold injury has been intensively reviewed by LEVITT[20]. GARBER and STEPONKUS[7] have reported that some alterations occur in the hydrophobic region of the cold-acclimated thylakoids, and that both non-acclimated and acclimated thylakoids require sucrose solution for the maximum protection during a freeze-thaw cycle. The increase of plastoglobuli and retention of infact starch grains in IR 8 plastids grown at low temperature suggest why IR 8 cannot be cold acclimated.

KIMBALL and SALISBURY[17] have reported that chloroplasts were the most sensitive organelles using three grass species exposed to low temperature. In the present study, rice plastids sensitively responded to cold acclimated treatment by the alterations of membrane system including PLBs, disappearance of starch grains and increase of plastoglobuli. And also it was suggested that the rate of w-PLBs formation may be used as a possible indicator to judge the level of chilling resistance of rice species.

Summary

Effect of low temperature on the ultrastructural development of rice plastids was observed with special reference to w-PLBs formation using three cultivars Bouzu, Honenwase and IR 8 which are highly resistant, medium and susceptible to chilling treatment respectively. During the development of rice plastids, three remarkable alterations, which are closely related to the cold resistance, were observed ; the alterations of plastid membranes including PLBs, increase of plastoglobuli and disappearance of starch grains.

In IR 8, the PLBs disappeared in the early stage of plastid development and never reformed subsequently under both light intensity 5.5 W/m^2 and 4.3 W/m^2 at 20°C. While, PLBs in Honenwase increased in number with the plastid development. In Bouzu, the rate of plastids including w-PLBs reached to about 96% under 5.5 W/m^2 light at 20° C. However, the w-PLBs formation in IR 8 at 26° C rose by the similar rate as that of Honenwase at 20°C ; the w-PLBs in IR 8 plastids were formed in higher temperature range than that of two other cultivars. Under 5.5 W/m^2 light at 20°C, the more the cultivar is resistant to low temperature, the more w-PLBs are easily formed in plastids. Accordingly, the rate of w-PLBs formation may be used as a possible indicator to judge the level of chilling resistance.

The starch grains in IR 8 plastids were retained until the late stage of plastid development, however, in two other cultivars disappeared at the early stage, meaning the protection of soluble sugar against cold injury. Plastoglobuli were more accumulated in IR 8 plastids when compared with Honenwase and Bouzu, indicating the result of inhibited thylakoid development.

References

1. BAKER, N.R., T.M. EAST and S.P. LONG 1983. Chilling damage to photosynthesis in young *Zea mays*. II Photochemical function of thylakoids *in vivo*. J. exp. Bot. **34** : 189—197.
2. BONZI, L.M. and P.LUZZI 1981. Ultrastructural modification of etioplasts, related to their position in the leaf tissue, in dark-grown seedlings of *Salvia splendens* L. upon exposure to continuous light. Caryologia **34** : 25—52.
3. CHAPMAN, D.J., J. DE-FELICE and J. BARBER 1983. Growth temperature effects on thylakoid membrane lipid and protein content of pea chloroplasts. Plant Physiol. **73** : 225—228.
4. CHEN, P., P.H. LI and W.P. CUNNINGHAM 1977. Ultrastructural diferences in leaf cells of some *Solanum* species in relation to their frost resistance. Bot Gaz. **138** : 276—285.
5. EAST T. M., S.P. LONG and N.R. BAKER 1981. Changes in *in vivo* photosynthesis of *Zea mays* induced by low temperature stress. In Photosynthesis. Prodeedings of Vth International Congress on Photosynthesis. (Ed.) G. Akoyunoglou, Balaban International Science Service, Philadelphia. Vol. VI, 369—377.
6. ELFMAN, B., N.P.A. HUNER, G. GRIFFITH, M. KROL, W.G. HOPKINS and D.B. HAYADEN 1984. Growth and development at cold-hardening temperature. Chlorophyll-protein complexes and thylakoid membrane polypeptides. Can. J. Bot. **62** : 61—67.
7. GARBER, M.P. and P.L. STEPONKUS 1976. Alterations in chloroplast thylakoids during cold acclimation. Plant Physiol. **57** : 681—686.
8. ——and——1976. Alterations in chloroplast thylakoids during an *in vitro* freeze-thaw cycle. Plant Physiol. **57** : 673—680.
9. GARBER, M.P. 1979. Low temperature response of chloroplast thylakoids. In Low Temperature Stress in Crop Plants (Eds.) J.M. Lyons, D. Graham and J.K. Raison, Academic Press, New York. 203—214.
10. GRIFFITH, M., G.N. BROWN and N.P.A. HUNER 1982. Structural changes in thylakoid proteins during cold acclimation and freezing of winter rye (*Secale cereale* L. cv. Puma). Plant Physiol. **70** : 418—423.
11. ——, B. ELFMAN and E.L. CAMM 1984. Accumulation of plastoquinone A during low temperature growth of winter rye. Plant Physiol. **74** : 727—729.
12. GUNNING, B.E.S. and M.W. STEER 1975. Plastids. In Ultra-structure and the Biology of Plant Cells (Eds.) B.E.S. Gunning and M.W. Steer, Edward Arnold, London. 97—133.
13. HUNER, N.P.A. and J.V. CARTER 1982. Differential subunit aggregation of a purfied protein from cold-hardened and unhardened Puma rye. Z. Pflanzenphysiol. **106** : 179—184.
14. HUNER, N.P.A., B. ELFMAN and M. KROL 1984.

Growth and development at cold-hardening temperature. Chloroplast ultrastructure, pigment content and composition. Can. J. Bot. **62** : 53—60.

15. ILKER, R., R.W. BREIDENBACH and J.M. LYONS 1979. Sequence of ultrastructural changes in tomato cotyledons during short periods of chilling. In Low Temperature Stress in Crop Plants (Eds.) J.M. Lyons, D. Graham and J.K. Raison, Academic Press, New York. 97—113.
16. IKEDA, T. and S. TOYAMA 1987. Studies on ultrastructure and function of photosynthetic apparatus in rice cells. I. Effects of light intensity and temperature on plastid development of *Oryza sativa* L. Japan. Jour, Crop Sci. **56** : 85—91.
17. KIMBALL, S.L., and F.B. SALISBURY 1973. Ultrastructural changes of plants exposed to low temperature. Amer. J. Bot. **60**, 1028—1033.
18. KIRK, J.T.O. and R.A.E. TILNEY-BASSETT 1978. The Plastids. Their chemistry, structure, growth and inheritance. Elsevier/North-Holland Biomedical Press, New York.
19. LAETSCH, W.M. 1974. The C_4 syndrome : A structural analysis. Ann. Rev. Plant Physiol. **25** : 27—52.
20. LEVITT, J. 1972. Responses of plants to environmental stresses. Academic Press, New York. 697.
21. LICHTENTHALER, H.K. 1977. Regulation of prenylquinone synthesis in higher plants. In Lipids and Lipid Polymers in Higher Plants. Springer-Verlag, New York. 231—258.
22. LONG, S.P., T.M. EAST and N.R. BAKER 1983. Chilling damage to photosynthesis in young *Zea mays*. I. Effects of light and temperature variation on photosynthetic CO_2 assimilation. J. exp. Bot. **34** : 177—188.
23. MCWILLIAM, JR., and A.W. NAYLOR 1967. Temperature and plant adaptation. I. Interaction of temperature and light in the synthesis of chlorophyll in corn. Plant Physiol. **42** : 1711—1715.
24. O'NEILL, S.D., D.A. PRIESTLY and B.F. CHABOT 1981. Temperature and aging effects on leaf membranes of a cold hardy perennial, *Fragaria virginiana. Plant Physiol.* **68** : 1409—1415.
25. OQUIST, G. 1983. Effects of low temperature on photosynthesis. Plant Cell Environ.**6** : 281—300.
26. 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.
27. SMILLIE, R.M., C. CRITCHLEY, J.M. BAIN and R. NOTT 1978. Effect of growth temperature on chloroplast structure and activity in barley. Plant Physiol. **62** : 191—196.
28. SLACK, C.R., P.G. ROUGHAN and H.C.M. BASSETT 1974. Selective inhibition of mesophyll chloroplast development in some C_4-pathway species by low night temperature. Planta (Berl.) **118** : 57—73.
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.

〔和 文 摘 要〕

イネの光合成器官の微細構造と機能に関する研究

第2報 イネの色素体の初期発達に及ぼす低温の影響

池 田 泰 治*・遠 山 益**
(*新潟大学教養部・**お茶の水女子大学理学部)

イネの色素体の初期発達に及ぼす低温の影響を，低温抵抗性の異なる3品種を用いて，特に“弱光プロラメラボディ（w-PLB)”の形成に注目して研究した．イネの色素体が発達する間に，低温抵抗性と密接に関連した3つの顕著な変化が認められた．すなわち，プロラメラボディ（PLBs）を含めた色素体の膜系の変化，プラスト顆粒の増加，および，デンプン粒の急速な消失である．

1. IR 8 を 20°C，光強度 5.5 w/m² の条件下で培養するとき，PLBs は色素体の発達初期に消失し，その後，PLBs が再形成されることはなかった．しかるに，ホウネンワセの色素体では，色素体の発達と共に w-PLBs の数が増加した．坊主では，w-PLBs を含む色素体が観察した色素体の 96%に達した．しかし，IR 8 を 26°C，5.5 w/m² で培養すると，20°C，5.5 w/m² で培養したホウネンワセと同程度の w-PLBs を形成した．20°C，5.5 w/m² の条件下では，低温抵抗性が大きいほど，w-PLBs の形成が容易であった．したがって，w-PLBs の形成が低温抵抗性のレベルを判定する1つの指標として用いられるかもしれない．

2. IR 8 の色素体内のデンプン粒は，色素体の発達後期まで保持された．しかし，ホウネンワセと坊主では，発達初期にデンプン粒は消失した．これは，可溶性糖の低温障害に対する保護を示唆している．

3. IR 8 の色素体では，他の2品種と比較すると，より多くのプラスト顆粒が蓄積された．これは PLBs からチラコイド膜への発達が，低温によって阻害された結果を示している．