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Senescence of rice leaves
XIX. Ultrastructural changes of chloroplasts

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Abstract. The effects of dark, light, benzyladenine (0.01 mM, in darkness) and abscisic acid (0.1 mM, in the light) on ultrastructural changes of the chloroplast of detached rice seedling leaves were investigated. When rice leaf segments were incubated in water for 4 days under dark condition, the unstacking of thylakoid membranes and the increase of the number of plastoglobuli were clearly observed. Light and benzyladenine, which are known to retard leaf senescence, were found to inhibit these changes. However, light resulted in an accumulation of starch grains. Abscisic acid, which is known to accelerate leaf senescence, resulted in the unstacking of thylakoid membranes, the increase of the number and size of plastoglobuli, the decrease of chloroplast size and the rupture of chloroplast envelop. The shape of the chloroplasts in light-treated leaf segments (in the presence or absence of abscisic acid) changed from elliptical to spherical. However, the shape of the chloroplast in dark-treated leaf segments (in the presence or absence of benzyladenine) was similar to that in freshly excised leaf segments, which is elliptical.

Key words: Abscisic acid; Benzyladenine; Chloroplast ultrastructure; Leaf senescence; Light; *Oryza sativa*.

Introduction

The first detectable symptoms of leaf senescence are usually expressed in the chloroplast (Dalling and Nettleton, 1986). There been many studies of chloroplast ultrastructure during the course of leaf senescence (Barton, 1966; Chonan *et al.*, 1977; Hurkman, 1979; Peoples *et al.*, 1980; Shaw and Manocha, 1965; Thomas, 1977; Wittenbach *et al.*, 1980). Cytokinins and abscisic acid (ABA) are known to retard and accelerate leaf senescence, respectively (Thimann, 1980). The ultrastructural effects of cytokinins and ABA on senescing leaf tissue have been examined (Mittelheuser and Van Steveninck, 1971 a, b; Naito *et al.*, 1981; Shaw and Manocha, 1965).

Chonan *et al.* (1977) studied the ultrastructural changes of chloroplasts of rice flag leaves during the course of natural senescence. However, this

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is so far the only report relating chloroplast ultrastructure to rice leaf senescence. In the present investigation, we examined the effects, if any, of dark, light, benzyladenine (BA) in darkness and ABA in the light on ultrastructural changes of chloroplasts of detached rice seedling leaves.

Materials and Methods

Plant Material and Incubation Condition

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as previously described (Kao, 1980). The apical 3 cm of the third leaves of 11-day-old seedlings were used for experiments. A group of 10 segments was floated in a 50-ml flask containing 10 ml distilled water, ABA or BA solution. Incubation was carried out at 27°C under light (16.7 Wm^{-2}) provided by fluorescent tubes or in darkness for desired period.

Determination of Chlorophyll and Amino Nitrogen

Chlorophyll and amino nitrogen were extracted and determined as described before (Kao, 1981). Chlorophyll and amino nitrogen were expressed in terms of A_{665} and A_{570} per 10 segments, respectively.

Electron Microscopy

Small pieces of leaf segment were fixed in 0.1 M cacodylate buffer pH 7.0 containing 3% glutaraldehyde. After 2 h at room temperature and two rinses with buffer, the tissue was post-fixed with buffered 1% osmium tetraoxide for 2 h. Tissue was dehydrated in an ethanol series and 100% acetone, and embeded in Spur resin (Spurr, 1969). Thin sections were stained with uranyl acetate and lead citrate (Reynold, 1963) and examined in a Hitachi H-600 electron microscope operated at 75 kV.

Results

The senescence of rice leaf segments was followed by measuring the decrease of chlorophyll

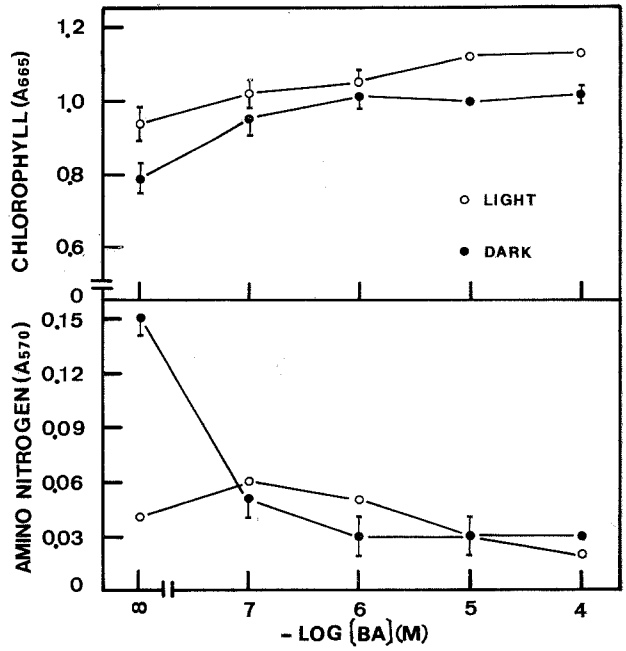

Fig. 1. Effects of BA on chlorophyll and amino nitrogen contents in leaf segments under light and dark conditions. Leaf segments were floated on BA solution of various concentrations or distilled water for 4 days.

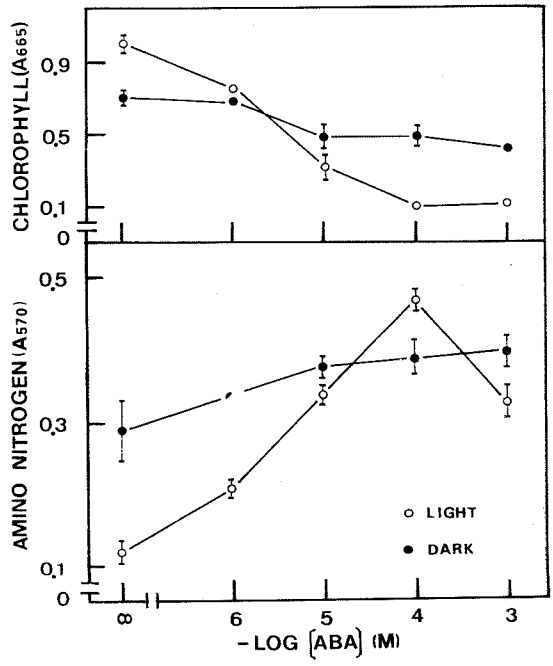

Fig. 2. Effects of ABA on chlorophyll and amino nitrogen contents in leaf segments under light and dark conditions. Leaf segments were floated on ABA solution of various concentrations or distilled water for 4 days.

and the increase of amino nitrogen. Figures 1 and 2 show the effect of BA and ABA in regulating the senescence of rice leaf segments. It is clear from these results that senescence of leaf segments was retarded by light. Results also indicated that ABA was more effective in promoting senescence in the light than in the dark and BA was more effective in retarding senescence in darkness than in light. Accordingly, the effects of dark, light, and BA under dark condition and ABA under light condition on chloroplast ultrastructure were examined in the present investigation.

Chloroplasts of freshly excised leaf segment were elliptical (Fig. 3A). The shape of the chloroplast remained unchanged when leaf segments were senesced under dark condition (in the presence or absence of BA) (Figs. 3D, 3E). However, chloroplasts of light-treated leaf segments

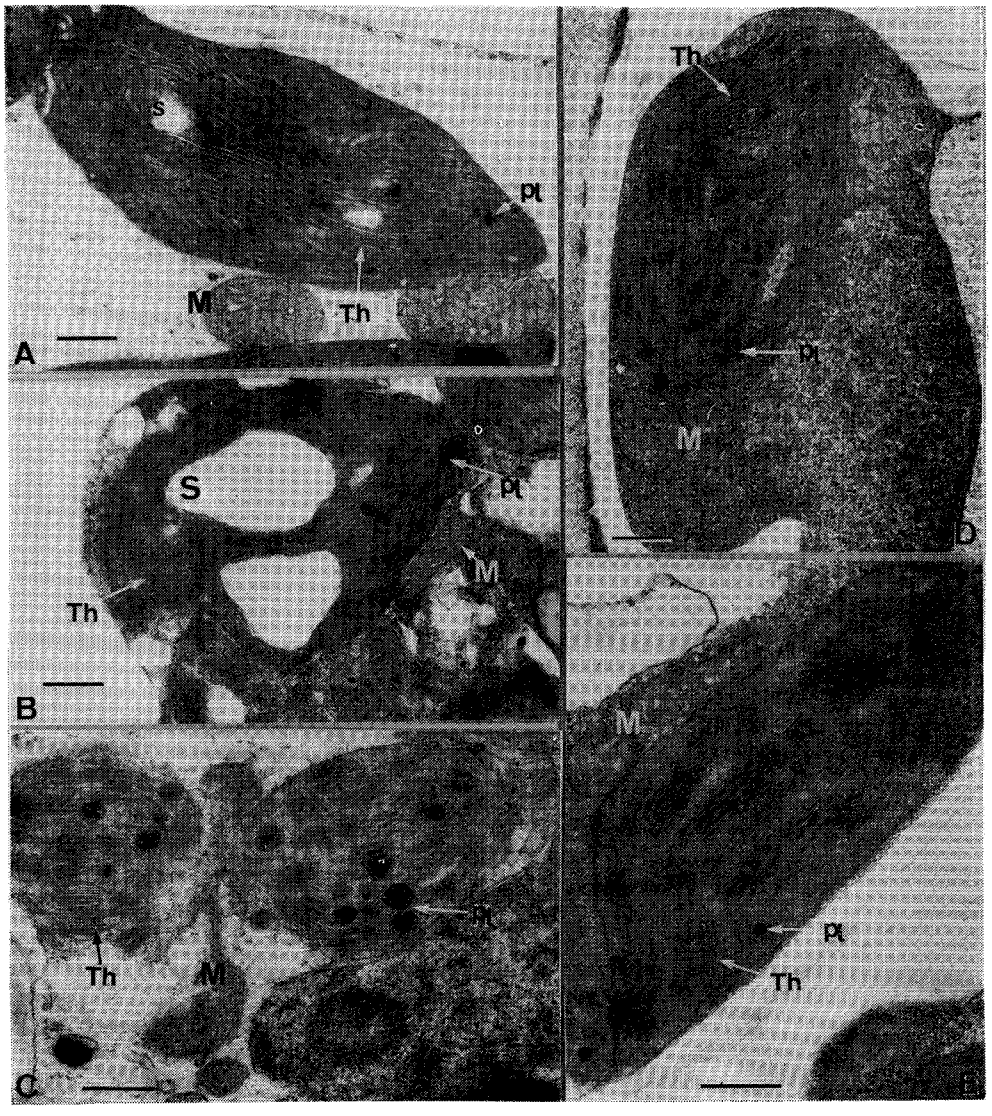

Fig. 3. Chloroplasts in leaf segment of initial (A), 4 days in light (B), in ABA (0.1 mM) under light condition (C), 4 days in darkness (D) and in BA (0.1 mM) under dark condition (E). M, mitochondria; Pl, plastoglobuli; S, starch grains; Th, thylakoids. Bars represent 0.5 μm.

(in the presence or absence of ABA) changed from elliptical to spherical (Figs. 3B, 3C).

When rice leaf segments were incubated in water under light condition, the major change of chloroplasts to occur was an accumulation of starch grains (Fig. 3B). No significant increase of the number of plastoglobuli in chloroplasts was detected.

Dramatic changes of chloroplasts in rice leaf segments incubated in ABA under light condition were observed. It is clear from Fig. 3C that chloroplast envelop was ruptured. The number and size of plastoglobuli increased significantly. The unstacking of thylakoid membranes was evident. Starch grains clearly disappeared. Chloroplast size seemed to decrease.

The unstacking of thylakoid membranes and the increase of the number of plastoglobuli were also observed in the chloroplast of rice leaf segments incubated in darkness for 4 days (Fig. 3D). The size of plastoglobuli, however, did not seem to increase. Starch grains were not observed in the chloroplast. No significant changes, except the disappearance of starch grains, were found in the chloroplast of rice leaf segments incubated in BA under dark condition (Fig. 3E).

Discussion

Light, which is known to retard the senescence of detached leaves (Figs. 1 and 2; Hurng *et al.*, 1986), was found to inhibit the separation of thylakoid membranes and the increase of the number of plastoglobuli which occurred in the chloroplast of detached leaves incubated in darkness. Other major differences of chloroplast ultrastructure between light and dark treatments were the starch grains and the shape of chloroplasts. Light, but not dark, treatment resulted in an accumulation of starch grains and the chloroplast having spherical shape.

Since the export of photosynthate is limited in detached leaves. It seems very likely that the accumulation of starch grains in the chloroplast of light-treated leaf segments is resulted from a net accumulation of photosynthate as suggested by Mitteheuser and Van Steveninck (1971b).

At the present time, we offer no explanation for the fact that the shape of chloroplasts is spherical when leaf segments were incubated under light condition. Using naturally senescing primary wheat leaves, Hurkman (1979) obtained the similar observation.

We previously suggested that the effect of light in retarding senescence of detached leaves is not via the production of cytokinins (Hurng *et al.*, 1986). This suggestion is further supported by the fact that BA, a synthetic cytokinin, resulted in the disappearance of starch grains in the chloroplast of detached leaves incubated in darkness.

The present study also indicates that BA markedly delayed the changes of chloroplast ultrastructure and ABA dramatically changed the ultrastructures of chloroplasts. These, in general, are in agreement with the results reported by other investigators (Naito *et al.*, 1981; Mittelheuser and Van Stevenick, 1971a, b; Shaw and Manocha, 1965).

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水稻葉片老化之研究

(十九) 葉綠體微細構造之變化

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本研究主要探討黑暗、光線、benzyladenine (BA, 0.01 mM，黑暗下) 與 abscisic acid (ABA, 0.1 mM，光線下) 處理水稻幼苗切離葉片 4 天後，對葉綠體微細構造之影響。切離葉片經黑暗處理 4 天後，類囊體膜明顯分離，同時親鐵性球狀體數目顯著增加。光線與處理均可延緩葉片老化，同時可抑制這些變化之發生。然而，光線處理使葉綠體內之澱粉粒聚積增大。ABA 處理加速葉片老化，同時使類囊體膜分離，親鐵性球狀體數目增加與增大、葉綠體變小、葉綠體膜破裂。不論處理 ABA 與否) 只要在光線下，葉綠體之形狀均由橢圓形轉變爲球狀形。而在黑暗下 (不論處理 ABA 與否) 則與剛切離的葉片相同，葉綠體之形狀爲橢圓形。