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SENESCENCE OF RICE LEAVES
XVI. Regulation by Light

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Abstract

Senescence of detached rice leaves in darkness and in light was compared. Light intensity required to retard senescence of detached leaves is quite low. The optimum light intensity was found to be 16.7 Wm^{-2}. Senescence of leaves attached to plants was also retarded by light. Light-induced protein loss in both detached and intact leaves was observed to be earlier than light-induced chlorophyll loss. The retardation of senescence by light seemed to be age and season independent. The effect of light in retarding senescence of detached rice leaves was transportable. Leaf segments under light condition had higher MACC but lower ACC levels than those under dark condition, when incubation time was more than 8 hours. The lower ethylene production in the light during this period might be partly regulated by reducing level of ACC via malonylation of ACC to MACC. Light achieved its cytokinin-like effect without actually cytokinin production. Protein band at 65 KD was found to be accumulated in leaf segments incubated in light or cytokinin under dark condition.

Key words: Leaf senescence; light; *Oryza sativa.*

Introduction

During past eight years we have extensively studied the senescence of detached rice leaves under dark condition (Cheng and Kao, 1984a, b, c, d; Cheng *et al.*, 1984; Kao, 1977, 1978, 1979, 1980, 1981, 1985; Kao and Yang, 1983; Kao and Yu, 1981; Wang and Kao, 1983; Wang *et al.*, 1982; Yu and Kao, 1980). However, leaves do senesce in light. Light has been found to retard leaf senescence by several investigators (Boissya, 1960; Haber *et al.*, 1969; Hsia and Kao, 1977; Goldthwaite and Laetsch, 1967; Lewington and Simon, 1969; Mishra and Pradhan, 1973; Misra and Biswal, 1973; Thiman *et al.*, 1977). With the object in understanding more precisely the nature of rice leaf senescence, the present study was designed to

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compare the differences between senescence in light and darkness.

In the previous work, we found that during the course of senescence ethylene production rate was lower under light condition than dark condition (Kao and Yang, 1983). The mehanism of light inhibited ethylene production during the senescence of detached rice leaves was therefore also investigated.

It has been shown that depodding of soybean plants retarded the loss of leaf chlorophyll and changed the composition of leaf proteins (Wittenbach, 1983). Therefore, it would be interesting to find out whether senescence retarding treatment such as light or BA treated under dark condition also changes the composition of soluble protein in detached rice leaves. We reported here the results of SDS gel electrophoretogram of soluble proteins from detached rice leaves of various treatments.

Materials and Methods

Plant Material and Incubation Condition

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as previously described (Cheng and Kao, 1984). Briefly, rice seedlings were grown in a greenhouse with natural light at 30°C day/25°C night and humidity 95%. 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 of distilled water or test solution. Unless otherwise indicated, incubation was carried out at 27°C under light (16.7 Wm^{-2}) provided by fluorescent tubes or in darkness.

For the experiments using intact leaves, seedlings were incubated in the light or dark. At the indicated period, the apical 3 cm of the third leaves were excised to analyze chlorophyll and protein.

For the purpose of studying whether light effect was localized or transportable, the method similar to that described by Thimann *et al.* (1977) was used. Leaf segments 4 cm long were excised. The apical or basal 2-cm halves or both were treated with light or darkness. When one or both halves were treated with darkness, they were covered with aluminum foil. The halves remained joined untill just before analysis of chlorophyll, protein and amino nitrogen.

For the experiment in which monthly light response of detached rice leaves was determined, rice seedlings were grown at the last week of each month during the period from March 1984 to March 1985 under the condition similar to that described above. Leaf segments of the third leaves excised from those seedlings grown monthly were incubated in water for 4 days at 27°C under light and dark conditions.

Determination of Chlorophyll, Amino Nitrogen and Protein

Chlorophyll, amino nitrogen, and protein were extracted and determined as

described before (Kao, 1981). Chlorophyll, amino nitrogen and protein were expressed as A_{666}, A_{570} and A_{700} per ten segments, respectively.

Determination of 1-Aminocyclopropane-1-carboxylic Acid (ACC) and 1-(Malonylamino)-cyclopropane-1-carboxylic Acid (MACC)

ACC and MACC were extracted with 80% ethanol. After evaporation an aliquot of the resulting aqueous extract was assayed for its ACC content according to the method of Lizada and Yang (1979). For determination of MACC, another aliquot of the extract was passed through a column of cation-exchange resin, Dowex 50 (H^+ form), to remove free amino acids including ACC, and the effluent was then hydrolyzed with 2N HCl at 100°C for 6 h. The resulting ACC was then assayed for its content as described before. ACC and MACC were expressed as nmoles per g fresh weight.

Gel Electrophoresis

Leaf segments were homogenized in a mortar and pestle with Tris-HCl buffer (237 mM, pH 8.5). After centrifugation of leaf extracts, a 0.3 ml aliquot was taken from the supernatant fractions and added to 0.1 ml 375 mM Tris-HCl (pH 8.5) containing 8% (w/v) SDS, 20% (w/v) mercaptoethanol, 40% (w/v) sucrose and 1% bromophenol blue. The proteins were completely dissociated by immersing the sample for 1.5 min in boiling water. Electrophoresis was carried out at a constant current of 30 mamp for 4-4.5 h on 10% acrylamide slab gels overlaid with a 4% acrylamide stacking gel. Gels were stained for 1.5 h in a solution containing 0.25% (w/v) Coomassie blue R-250, 50% (v/v) methanol and 7% (v/v) glacial acetic acid and were destained in 30% (v/v) methanol and 7% (v/v) acetic acid. Photographs were taken after the gels were dried.

Molecular weight of the major polypeptide bands was estimated by comparing R_f values with those of reference proteins: albumin (66,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (20,100).

Results

The intensity dependence of the light response is shown in Fig. 1. Leaf segments were incubated under light of different intensities and assayed after 4 days for chlorophyll, protein or amino nitrogen content. The light-induced retardation of chlorophyll loss increased from zero intensity, reached a maximum around 5 Wm^{-2} and then decreased slightly. The light-induced retardation of protein loss increased from zero intensity and reached a plateau in the region around 16.7 Wm^{-2}. Low light intensity was also effective in retarding amino nitrogen accumulation and

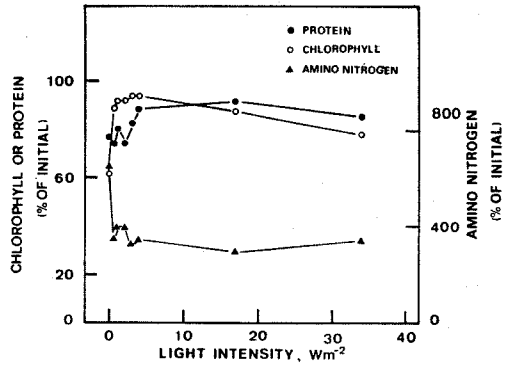

Fig. 1. Effect of different light intensities on chlorophyll, amino nitrogen or protein content of detached rice leaves. Leaf segments were incubated under light of different intensities and assayed after 4 days for chlorophyll, protein and amino nitrogen. Four experiments were conducted. Since the trends were identical, only one set of the results was presented.

the optimum light intensity for the retardation of amino nitrogen accumulation was also at 16.7 Wm^{-2}. When chlorophyll, protein and amino nitrogen contents were considered, it seems that that the optimum light intensity for the retardation of senescence was 16.7 Wm^{-2}, which was the light intensity chosen for following experiments.

Changes of chlorophyll, protein, amino nttrogen and total sugars for both detached and intact leaves under light and dark conditions were examined. Only the results of chlorophyll and protein are reported here (Fig. 2). The noticeable

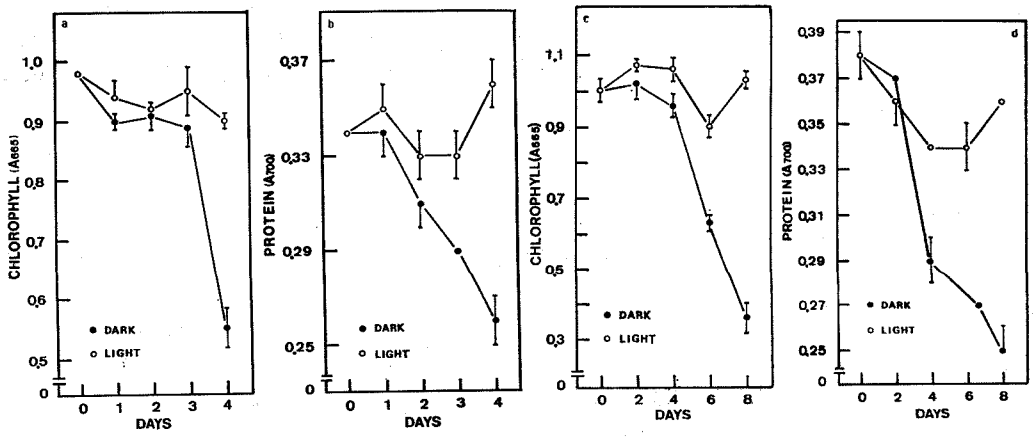

Fig. 2. Changes of chlorophyll and protein in detached leaves (a and b) and intact leaves (c and d) of rice under light and dark conditions.

effect of light on the retardation of chlorophyll loss in detached leaves was evident at 4th day (Fig. 2a). However, retardation of protein loss by light was observed at 3 days after incubation (Fig. 2b).

Figures 2c and 2d show the time courses of chlorophyll and protein levels in intact leaves under light and dark conditions. It clearly demonstrated that light acted similarly as in detached leaves in retarding senescence. It is also interesting to note that the light-induced retardation of protein loss was observed to be earlier than the light-induced retardation of chlorophyll loss.

Light also exerted its retardation effect on the amino nitrogen accumulation and sugar loss for both detached and intact leaves of rice (data not shown).

Figure 3 shows that light retarded senescence of leaf segments detached from seedlings grown every month from March 1984 to March 1985, though light response varied from month to month. It is obvious that the senescence retardation effect of light is season independent response.

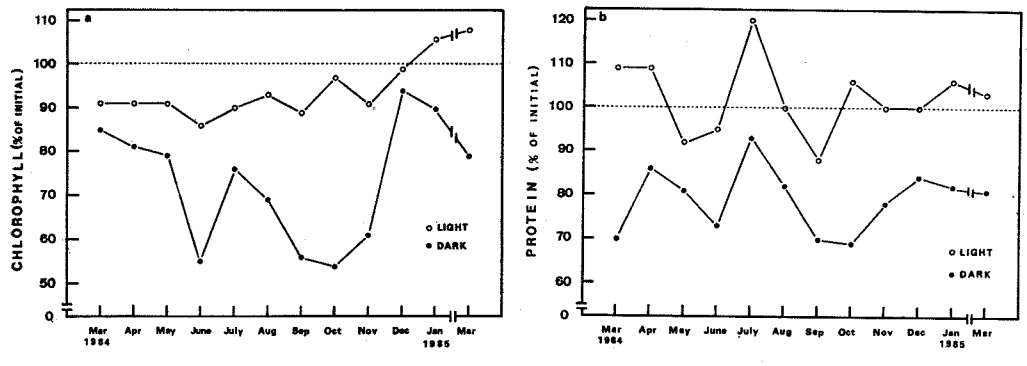

Fig. 3. Response to light of leaf segment excised from seedlings grown every month from March 1984 to March 1985.

The response of leaf segments of various ages to light is shown in Fig. 4. The relative rates of chlorophyll loss in darkness as well as in light increased with increasing leaf age. However, the retardation of the loss of chlorophyll induced by light was observed in leaves of all ages tested. It seems that light-induced retardation of chlorophyll is age independent response.

An experiment was conducted to explore whether the light effect was localized or transportable. When the basal part was illuminated, chlorophyll or protein content in the apical part (darkness) was higher and amino nitrogen content was lower than that of the apical part of the leaf segments with both halves in darkness (Fig. 5). Similarly, when the apical part was illuminated, chlorophyll or protein content in the basal part (darkness) of the leaf segments was also higher than that of the basal part of the leaf segments with both halves in darkness.

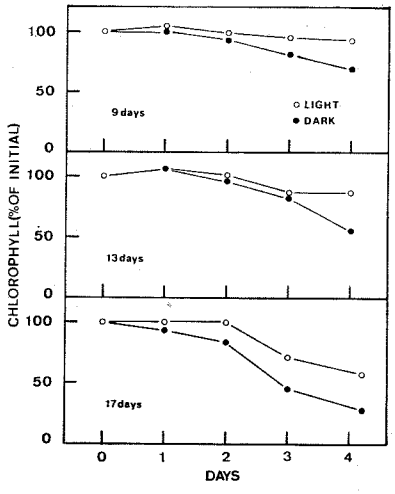

Fig. 4. Time course of chlorophyll loss of leaf segments incubated in darkness or light. Days of incubation indicated on abscissa. Leaf segments taken from the third leaves of 9-, 13- and 17-day-old seedlings.

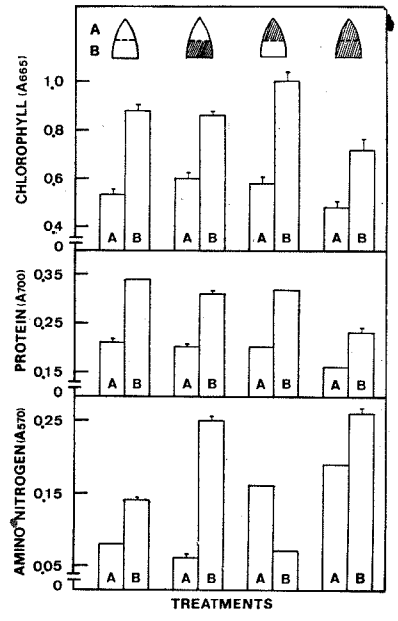

Fig. 5. Effect of light and darkness on apical (A) and basal (B) halves of 4 cm leaf segments at 27°C. The halves remained joined until just before analysis.

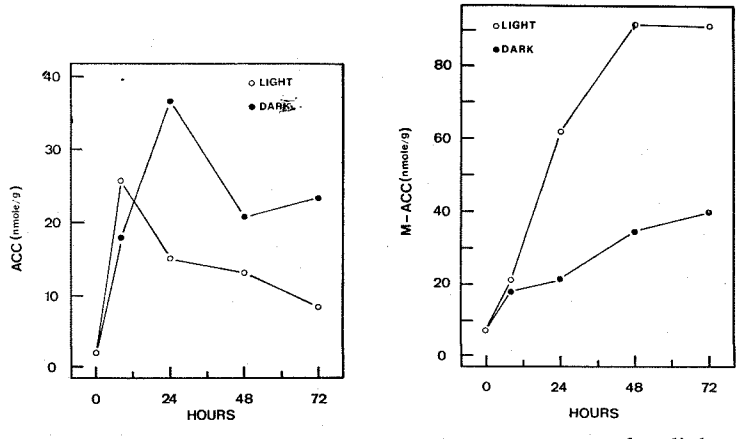

Fig. 6. Changes of ACC and MACC contents in leaf segments under light and dark conditions. Two experiments were conducted. Since the trends were the same, only one set of the results was presented.

However, amino nitrogen content in the basal part (darkness) of the leaf segments with apical part illuminated was almost the same as that in the basal part of the leaf segments with both halves in darkness. This result might be due to the fact that amino nitrogen is mainly polarly transported from apical part to basal one (Kao, 1977; Thimann *et al.*, 1976).

Figure 6 shows the time courses of ACC and MACC contents in rice leaf segments floating on water in light or darkness. ACC content increased immediately after excision and reached maximum at 8 and 24 h in the light and dark, respectively, and subsequently declined. MACC level in the leaf segments incubated in the light was found to be higher than that incubated in darkness.

The effects of benzyladenine (BA) and abscisic acid (ABA) in regulating senescences of rice leaf segments incubated in the light and dark are shown in Figs. 7 and 8, respectively. Results indicated that BA was more effective in retarding senescence in the dark than in the light, whereas ABA was more effective in promoting senescence in the light than in the dark. It is clear that light effect in retarding senescence is quite similar to cytokinin effect under dark condition.

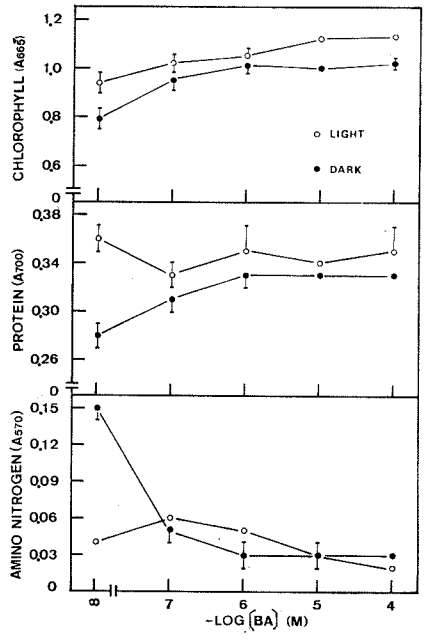

Fig. 7. Effects of BA on chlorophyll, protein and amino nitrogen contents in leaf segments under light and dark conditions. Leaf segments were floated on BA solution of various concentrations or distilled water for 4 days.

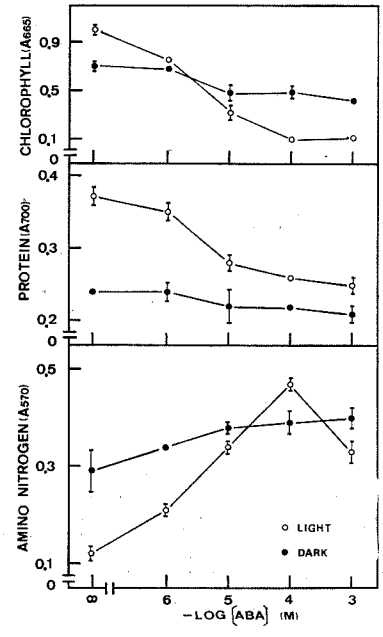

Fig. 8. Effects of ABA on chlorophyll, protein and amino nitrogen contents in leaf segments under light and dark conditions. Leaf segments were floated on ABA solution of various concentrations or distilled water for 4 days.

The protein patterns of detached rice leaves were analyzed by SDS polyacrylamide gel electrophoresis. Most of the major protein bands (denoted by arrows) were decreasing in amount or disappeared in leaf segments of those treatments promoting senescence such as dark (D) or ABA treated under light condition (LA) (Fig. 9). Afrer 4 days of light (L) or BA treated in darkness (DB) there was evidence of an increase in the amount of at least one protein band (65 KD). It is also interesting to point out that protein band near 51 KD disappeared after leaf excision.

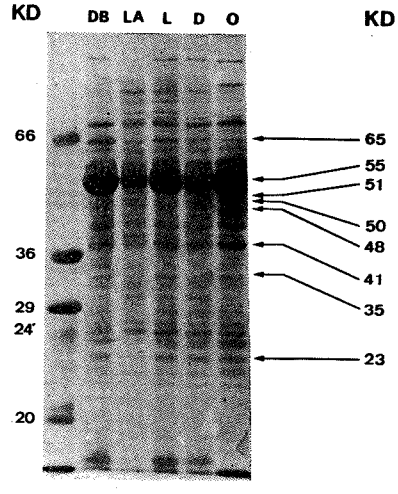

Fig. 9. SDS gel electrophoretogram of soluble proteins from leaf segments of initial (O), 4 days in light (L), in darkness (D), in BA under dark condition (BD), and in ABA under light condition (LA).

Discussion

Light has been reported to retard senescence of leaves, detached and intact (Boissya, 1960; Haber *et al.*, 1969; Hsia and Kao, 1977; Goldthwaite and Laetsch. 1967; Lewington and Simon, 1969; Mishra and Pradhan, 1973; Misra and Biswal, 1973; Thimann *et al.*, 1977). The present work also demonstrates that light retards senescence of both detached and intact rice leaves. Senescence retardation by light of detached rice leaves seems to be age and season independent. Furthermore, light-induced retardation of protein loss by light was found to be earlier than light-induced retardation of chlorophyll loss.

Our results suggest that light effect is transportable in rice leaf system, which is different from that found in oat leaves by Thimann *et al.* (1977) who reported that light effect was localized. This shows that light may act by producing

a diffusiable product. Since cytokinin was found to be effective in retarding senescence of detached rice leaves under dark condition (Fig. 7). Cytokinin seems to be the likely candidate that produced by light. However, this suggestion does not seem to be valid, because light promotes but cytokinin inhibits respiration rate during senescence of detached leaves (Kao, 1964). Therefore, we can only conclude that light has cytokinin-like effect without actually cytokinin production as suggested by Thimann *et al.* (1977).

Ethylene is a plant hormone regulating many aspects of plant growth and development. It has been shown that ethylene is biosynthesized in plant tissues via the following sequence: methionine→S-adenosylmethionine→1-aminocyclopropane-1-carboxylic acid (ACC)→ethylene (Adams and Yang, 1979; Lurssen *et al.*, 1979). The formation of ACC was considered to be the rate-limiting step in ethylene biosynthesis (Yang and Hoffman, 1984). In the previous work, we have shown that, during the course of detached rice leaf senescence, ethylene production rate was lower under light condition than dark condition (Kao and Yang, 1983). Light inhibited ethylene production during early period of rice leaf senescence was found to he mediated via inhibition of the conversion of ACC to ethylene (Kao and Yang, 1982). This could explain why leaf segments that under light condition at 8 h after detachment had higher ACC content but produced lower ethylene (Kao and Yang, 1983).

In addition to the metabolism of ACC to ethylene, Amrhein *et al.* (1981) and Hoffman *et al.* (1982) have shown that plant tissues are capable of metabolizing ACC to MACC. Ethylene production could, therefore, also be regulated by controlling the level of free ACC level via malonylation of ACC to MACC (Amrhein *et al.*, 1982; Yang *et al.*, 1982). When incubation time was more than 8 h, leaf segments treated with light had lower ACC but higher MACC levels than those treated with darkness (Fig. 6). It seems that the lower ethylene production rate uner light condition during this period (leaf segments incubated for more than 8 h) could be, at least partly, regulated by reducing level of ACC via malonylation of ACC to MACC.

Senescence of leaves is normally characterized by the loss of leaf protein and chlorophyll (Fig. 2). Wittenbach (1983) showed that depodding of soybean plants retarded the loss of leaf chlorophyll and changed the composition of leaf proteins. Our results also showed that light or BA treated under dark condition changed the patterns of leaf proteins in detached rice leaves. There was evidence of an increase in the amount of at least one protein band with molecular weight of 65 KD in leaf segments treated with light or BA under dark condition. At present, we do not know the identity and function of this protein. However, further work seems worthwhile to pursue the nature of this protein. It is also interesting to point out that protein band near 51 KD disappeared after excision, suggesting this

protein band is seneitive to wounding and specific for intact leaves.

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Literature Cited

Adams, D. O. and S. F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. USA **76**: 170-174.

Amrhein, N., D. Schneebeck, H. Skorupka, S. Tophof, and J. Stockight. 1981. Identification of a major metabolite of ethylene precursor 1-aminocyclopropane-1-carboxylic acid in higher plants. Naturwissenschaften **68**: 619-620.

Amrhein, N., F. Breuing, J. Eberle, H. Skorupka, and S. Tophof. 1982. The metabolism of 1-amino-cyclopropane-1-carboxylic acid. *In* P. F. Wareng (ed.), Plant Growth Substances 1982. Academic Press, London, pp. 249-258.

Boissya, C. L. 1960. The effect of kinetin and sucrose on the preservation of chlorophyll a and b in detached ***Phaseolus vulgaris*** leaves. J. Assam. Sci. Soc. **13**: 35-50.

Cheng, S. H. and C. H. Kao. 1984a. Senescence of rice leaves. IX. Action of straight-chain alcohols on chlorophyll loss. Proc. Natl. Sci. Counc. ROC **8**: 99-103.

Cheng, S. H. and C. H. Kao. 1984b. Senescence of rice leaves. X. The effects of metal chelators. Bot. Bull. Academia Sinica **25**: 87-93.

Cheng, S. H. and C. H. Kao. 1984c. Senescence of rice leaves. XI. Inhibition of protease activity by α, α'-dipridyl. Bot. Bull. Academia Sinica **25**: 95-101.

Cheng, S. H. and C. H. Kao. 1984d. The role of proteolytic enzymes in protein degradation during senescence of rice leaves. Physiol. Plant. **62**: 231-237.

Cheng, S. H., Y. Y. Shyr, and C. H. Kao. 1984. Senescence of rice leaves. XII. Effects of 1, 3-diaminopropane, spermidine and spermine. Bot. Bull. Academia Sinica **25**: 191-196.

Goldthwaite, J. J. and W. J. Laetsch. 1967. Regulation of senescence in bean leaf discs by light and chemical growth regulators. Plant Physiol. **42**: 1757-1762.

Haber, A. H., P. J. Thompson, P. L. Walne, and L. L. Triplett. 1969. Non-photosynthetic retardation of chloroplast senescence by light. Plant Physiol. **44**: 1619-1628.

Hoffman, N. E., S. F. Yang, and T. McKeon. 1982. Identification of 1-(malonylamino) cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher plants. Biochem. Biophys. Res. Commun. **104**: 765-770.

Hsia, C. P. and C. H. Kao. 1977. Soybean leaf disc senescence: Regulation by light. Agri. Assoc. China (New Series) **100**: 98-102.

Kao, C. H. 1977. Senescence of rice leaves. I. Comparison between detached and intact leaves. Mem. Coll. Agric. Natl. Taiwan Univ. **17** (1): 12-18.

Kao, C. H. 1978. Senesence of rice leaves. II. Antisenescent action of cytokinins. Proc. Natl. Sci. Counc. ROC **2**: 391-398.

Kao, C. H. 1979. Senescence of rice leaves. III. The inter-organ control of leaf senescence of rice seedlings. Proc. Natl. Sci. Counc. ROC **3**: 199-204.

Kao, C. H. 1980. Senescence of rice leaves. IV. Influence of benzyladenine on chlorophyll degradation. Plant Cell Physiol. **21**: 1255-1262.

Kao, C. H. 1981. Senescence of rice leaves. VI. Comparative study of the metabolic changes of senescing turgid and water-stressed excised leaves. Plant Cell Physiol. **22**: 683-688.

Kao, C. H. 1985. Senescence of rice leaves. XIV. Changes of respiration during senescence of detached leaves. Bot. Bull. Academia Sinica **26**: 21-30.

Kao, C. H. and S. F. Yang. 1982. Light inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in leaves in mediated through carbon dioxide. Planta **155**: 261-266.

Kao, C. H. and S. F. Yang. 1983. Role of ethylene in the senescence of detached rice leaves. Plant Physiol. **73**: 881-885.

Kao, C. H. and S. M. Yu. 1981. Senescence of rice leaves. V. Retardation of senescence by nickelous or cobaltous ions. Proc. Natl. Sci. Counc. ROC **5**: 45-48.

Lewington, R. J. and E. W. Simon. 1969. The effect of light on the senescence of detached cucumber cotyledons. J. Exp. Bot. **20**: 138-144.

Lizada, M. C. C. and S. F. Yang. 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem. **100**: 140-145.

Lurssen, K., K. Naumann, and R. Schroder. 1979. 1-Aminocyclopropane-1-carboxylic acid-an intermediate of the ethylene biosynthesis in higher plants. Z. Pflanzenphysiol. **92**: 285-294.

Mishra, D. and P. K. Pradhan. 1973. Regulation of senescence in detached rice leaves by light, benzimidazole and kinetin. Exp. Gerontol. **8**: 153-155.

Misra, G. and U. C. Biswal. 1973. Factors concerned in leaf senescence. 1. Effects of age, chemicals, petioles and photoperiod on senescence in detached leaves of ***Hibiscus rosasinensis.*** L. Bot. Gaz. **134**: 5-11.

Thimann, K. V., R. M. Tetley, and T. V. Thanh. 1976. The metabolism of oat leaves during senescence. II. Senescence in leaves attached to the plant. Plant Physiol. **54**: 859-862.

Thimann, K. V., R. M. Tetley, and B. M. Krivak. 1977. Metabolism of oat leaves during senescence. V. Senescence in light. Plant Physiol. **59**: 448-454.

Wang, C. Y. and C. H. Kao. 1983. Senescence of rice leaves. VIII. The regulation of proline accumulation during senescence. Bot. Bull. Academia Sinica **24**: 145-154.

Wang, C. Y., S. H. Cheng, and C. H. Kao. 1982. Senescence of rice leaves. VII. Proline accumulation in senescing excised leaves. Plant Physiol. **69**: 1348-1349.

Wittenbach, V. A. 1982. Effect of pod removal on leaf senescence in soybean. Plant Physiol. **70**: 1544-1548.

Yang, S. F., N. E. Hoffman, T. McKeon, J. Riov, C. H. Kao, and K. H. Yung. 1982. Mechanism and regulation of ethylene biosynthesis. *In* P. F. Wareing (ed.), Plant Growth Substance 1983. Academic Press, London, pp. 239-248.

Yang, S. F. and N. E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. **35**: 155-189.

Yu, S. M. and C. H. Kao. 1980. Antisenescent activity of silver ion. Proc. Natl. Sci. Counc. ROC **4**: 335-337.

水稻葉片老化之研究

(十六) 光線之控制

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本實驗比較光處理與黑暗處理對離體水稻葉片老化之影響。光線於極低的光强度下卽可延緩水稻切離葉片的老化，最適光强度爲 16.7 Wm^{-2}。光線亦能延緩植株完整葉片之老化。光線對不同月份種植之水稻幼苗所選取的切離葉片，均有延緩老化之效果。黑暗處理者葉綠素下降之速率隨葉齡之增加而增加。光線延遲葉綠素下降之效果於各葉齡均有效。光線延緩水稻切離葉片老化的效果具有運移性。ACC 是高等植物荷爾蒙乙烯的立卽前驅物，光處理之葉片較暗處理者含有較多的 MACC，但有較少的 ACC 量，此顯示於老化過程中光處理產生較少乙烯的原因可能是由於其 ACC 多轉變成 MACC，因而降低 ACC 量所致。光線具有類似 Cytokinin 的效果而實際並無 Cytokinin 之產生。葉片於光處理或有 Cytokinin 存在之黑暗處理下有 65 KD 蛋白質帶的累積。