Bot. Bull. Academia Sinica (1988) 29: 7-13

Senescence of rice leaves
XVII. Effects of various inhibitors

Weei-Pirng Hurng[1] and Ching-Huei Kao[2]
Department of Agronomy, National Taiwan University
Taipei, Taiwan, Republic of China

(*Received February 27, 1987; Accepted June 10, 1987*)

Abstract. The possible mechanism of the effect of light in retarding the senescence of detached rice leaves was studied by using various inhibitors. The effects of some inhibitors on the senescence of detached rice leaves under dark condition were also investigated. Chlorpromazine, an inhibitor of both cyclic and noncyclic photophosphorylation, significantly reduced the effect of light in retarding senescence. Methylamine and ammonium chloride, inhibitors of cyclic photophosphorylation, also reduced the effect of light. However, DCMU, which is known to inhibit noncyclic photophosphorylation, did not inhibit light effect. Vanadate, a specific inhibitor of plasma membrane ATPase, strongly accelerated senescence of detached rice leaves under light and dark conditions. Senescence of detached rice leaves under both light and dark conditions was, on the contrary, found to be retarded by the exogenous supply of ATP, though the effect is rather small. Results suggest, though not conclusively, that the effect of light on the senescence is possibly mediated by ATP formation via cyclic photophosphorylation and ATP utilization. It also seems that the senescence of detached rice leaves under dark condition is possibly due to the failure of ATP synthesis and utilization.

Cordycepin (3′-deoxyadenosine), an analogue of adenosine, is known to inhibit the polyadenylation of mRNA, significantly promoted senescence in the light and retarded senescence in darkness. A corollary of this is that the synthesis of specific protein(s) responsible for the retardation or the promotion of senescence process is possibly regulated at the level of polyadenylation of mRNA.

Key words: ATP formation; ATP utilization; Inhibitor; Leaf senescence; Light effect; *Oryza sativa* L.

Introduction

Light is known to retard leaf senescence (Goldthwaite and Laetsch, 1967, Hsia and Kao, 1977; Hurng *et al.*, 1986; Thimann *et al.*, 1977). The retarding effect of light was suggested to be mediated through photosynthetic production of sugars (Goldthwaite and Laetsch, 1967; Hopkison, 1966; Lloyd, 1980), ATP production from photosynthetic phosphorylation (Thimann *et al.*, 1977) or ATP utilization (Malik and Thimann, 1980). On the other hand, evidence for a phytochrome mediated control of leaf senescence was presented by several investigators (De Greef *et al.*, 1971; Mishra and Pradhan, 1973; Sugiura, 1963).

Recently, we demonstrated that the senescence of rice leaves was effectively retarded by light

[1] Tobacco Research Institute, Taichung, Taiwan, Republic of China.
[2] To whom reprint requests should be addressed.

and suggested that light effect was unlikely due to the production of cytokinins (Hurng *et al.*, 1986). There is a dearth of data relating to the mechanism of light effect in retarding the senescence of detached rice leaves. We, accordingly, investigated the possible mechanism of light effect by using various inhibitors which concern photophosphorylation, ATPase activity and ATP formation. Some inhibitors were also used to study their effects on the senescence of rice leaf segments under dark condition.

Materials and Methods

Plant Material and Incubation Condition

Rice (*Oryza sativa* L. 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 or test solution. Incubation was carried out at 27°C under light (16.7 Wm^{-2}) provided by fluorescent tubes or in darkness for desired period. Each experiment was repeated at least three times. Identical results were, in general, obtained. Results reported here were from single experiment.

Determination of Chlorophyll, Amino Nitrogen, Protein and Sugars

Chlorophyll, amino nitrogen and protein were extracted and determined as described before (Kao, 1981). Chlorophyll, amino nitrogen, protein and sugars contents were expressed as A_{665}, A_{570}, A_{700} and mg glucose per ten segments, respectively.

Chemicals

Inhibitors used included chlorpromazine, DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], methylamine, ammonium chloride, sodium vanadate (Na_3VO_4) and CCCP (carbonyl cyanide *m*-chlorophenylhydrazone). Adenine derivatives such as adenine, adenosine, 2′-deoxyadenosine, cordycepin (3′-deoxyadenosine) and ATP were also used in the present investigation. All chemicals used are the highest purity commercially available.

Results

Effects of Photophosphorylation Inhibitors

The senescence of detached rice leaves was followed by the decrease of chlorophyll and protein contents and the increase of amino nitrogen content. Table 1 shows the effect of chlorpromazine, which is known to inhibit both cyclic and noncyclic photophosphorylation (Avron and Shavit, 1965), on the contents of chlorophyll, protein, amino nitrogen and sugars. Results indicated that chlorpromazine at the concentrations 0.05 mM or higher decreased sugar content and also promoted the senescence under light condition. Ammonium chloride and methylamine have been reported to inhibit cyclic photophosphorylation (Arnon and Chain, 1975; Hinds and Whittinghan, 1963). Table 2 shows

Table 1. *Effects of chlorpromazine on chlorophyll, protein, amino nitrogen and sugar contents of rice leaf segments after 4 days in light*

Chlorpromazine concentration (mM)	Chlorophyll (A_{665})	Protein (A_{700})	Amino nitrogen (A_{570})	Sugars (mg)
0	1.09±0.02	0.38	0.12±0.01	1.93±0.03
0.05	1.03±0.03	0.28±0.01	0.17±0.01	1.73±0.03
0.10	0.90±0.03	0.29±0.01	0.17±0.02	1.34±0.08
0.50	0.70±0.02	0.32±0.02	0.19±0.01	1.30±0.06
1.00	0.55±0.01	0.31±0.01	0.18±0.01	1.20±0.05

Table 2. *Effects of methylamine and ammonium chloride on chlorophyll and sugar contents of rice leaf segments after 4 days in light*

Treatment	Chlorophyll (A_{665})	Sugars (mg)
Water	1.14±0.02	1.48±0.20
Methylamine		
100 mM	0.76±0.02	1.21±0.08
200 mM	0.69±0.01	1.28±0.03
Ammonium chloride		
50 mM	0.67±0.02	1.51±0.07
100 mM	0.46±0.03	0.64±0.06

that both methylamine and ammonium chloride decreased the accumulation of sugars and promoted the senescence under light condition. Though the concentrations of methylamine and ammonium chloride used were rather higher, the effect of these two chemicals was unlikely due to toxicity, because no loss of turgidity and toxic symptoms were observed throughout the experimental period. Furthermore, Malik and Thimann (1980) reported that very small amount of methylamine could be taken up by the leaf segments of oats. Although DCMU, which is well known to inhibit photosynthetic electron transport (Jagendorf and Avron, 1959), prevented the sugar accumulation, it did not affect the senescence under light condition (Table 3).

Effect of CCCP

CCCP inhibited the retardation effect of senescence by light and promoted senescence under dark condition (Fig. 1). It is believed that ATPase present in the membranes of mitochondria and chloroplasts synthesize ATP in response to proton gradient (Briskin and Poole, 1983). CCCP is known as proton ionophore (Harold, 1972), which permits protons to be conducted through the membrane and allows the difference in proton concentration across the membrane to dissipate. A corollary of this is that the formation of ATP is required for light effect in retarding senescence and the retardation of senescence under dark condition.

Effects of ATP and Adenine Derivatives

If the formation of ATP is indeed required for the retardation of senescence, then the senescene of detached leaves under light and dark conditions is expected to be retarded by the exogenous supply of ATP. When rice leaf segments were incubated in ATP solution for 4 days under light and dark conditions, ATP at optimum concentration slightly retarded senescence of rice leaf segments (Tables 4 and 5).

Cytokinins have been reported to retard the senescence of rice leaf segments under light and dark conditions, though the retardation effect under light condition is much less than that under dark condition (Hurng *et al.*, 1986). Since both ATP and purine cytokinins contain adenine moiety, it would be interesting to know whether some adenine derivatives also retard senescence of detached rice leaves. Adenine and adenosine were effective, though slightly, in retarding senescence under both light and dark conditions (Tables 4 and 5). However, senescence was found to be retarded in

Table 3. *Effects of DCMU on chlorophyll, protein, amino nitrogen and sugar contents of rice leaf segments after 4 days in light*

Treatment	Chlorophyll (A_{665})	Protein (A_{700})	Amino nitrogen (A_{570})	Sugars (mg)
Water	0.86±0.02	0.28±0.02	0.10±0.01	1.14±0.01
DCMU, 0.01 mM	0.86±0.02	0.32±0.02	0.11±0.01	0.58±0.10
DCMU, 0.1 mM	0.87±0.01	0.30±0.01	0.12±0.01	0.04±0.02

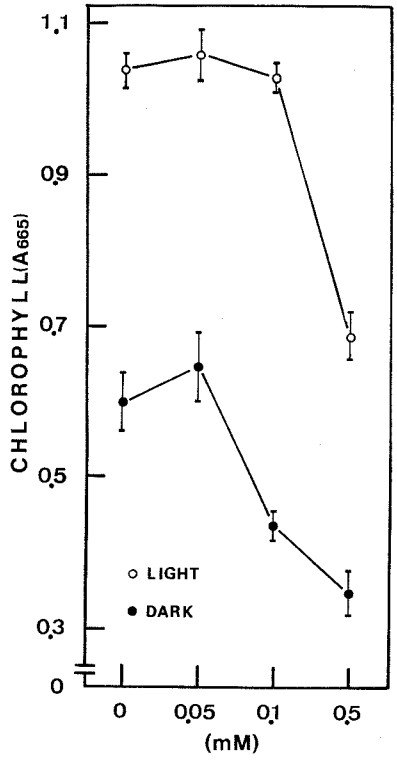

Fig. 1. Effects of CCCP on chlorophyll content of rice leaf segments after 4 days of incubation.

Table 4. *Effects of adenine derivatives on chlorophyll and protein contents of rice leaf segments after 4 days in light*

Treatment	Relative content (% of control)	
	Chlorophyll	Protein
Adenine, 1 μM	109	110
Adenosine, 1μM	103	110
2′-Deoxyadenosine, 100 μM	91	95
3′-Deoxyadenosine, 50 μg/ml	67	97
ATP, 1 μM	104	106

Table 5. *Effect of adenine derivatives on chlorophyll and protein contents of rice leaf segments after 4 days in darkness*

Treatment	Relative content (% of control)	
	Chlorophyll	Protein
Adenine, 100 μM	109	116
Adenosine, 1 μM	108	108
2′-Deoxyadenosine, 1 μM	112	114
3′-Deoxyadenosine, 10 μg/ml	153	109
ATP, 10 μM	105	104

darkness and promoted in light by 2′-deoxyadenosine and cordycepin (3′-deoxyadenosine). The retardation effect of adenine and adenosine might be due to their conversion either to ATP or cytokinins in leaf tissue.

Effect of Vanadate

Figure 2 shows that vanadate at the concentrations ranging from 0.5 to 5.0 mM promoted the senescence of detached rice leaves under both light and dark conditions. Toxic effect was not observed throughout the experimental period.

Discussion

Since sugars such as glucose have considerable effect in retarding senescence of rice leaves under dark condition (Kao, 1979). It is natural to suggest that the retarding effect of light on the senescene of rice leaves is mediated through the photosynthetic production of sugars as concluded by other investigators (Goldthwaite and Laetsch 1967; Lloyd, 1980). Light indeed caused the expected formation of sugars in detached rice leaves (Hurng *et al.*, 1986). The sugar formation in rice leaf segments under light condition was prevented by DCMU (Table 3), which is well known to inhibit photosynthetic electron transport, though not cyclic photophosphorylation (Jagendorf and Avron, 1959). However, light could still exert its effect in the presence of DCMU (Table 3). Haber *et al.* (1986) and Thimann *et al.* (1977) also noted that DCMU did not affect the retarding effect of light on senescence. It can be concluded from our data and others that the main effect of light is not exerted via the production of sugars. Since inhibitors of

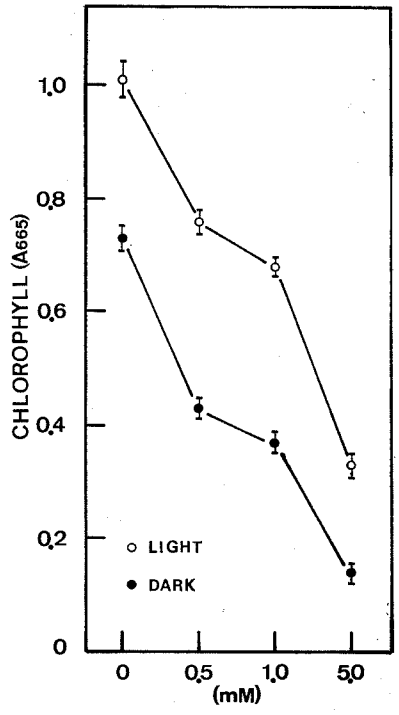

Fig. 2. Effects of vanadate on chlorophyll content of rice leaf segments after 4 days of incubation.

cyclic photophosphorylation such as chlorpromazine, methylamine and ammonium chloride all promoted the senescence under light condition (Tables 1 and 2). We therefore deduce that the effect of light in retarding the senescence of detached rice leaves might be mediated through ATP formation via cyclic photophosphorylation. Using detached oat leaves, Thimann *et al.* (1977) also reached the same conclusion. This conclusion was further supported by our data that CCCP inhibited and the exogenous supply of ATP increased the light effect (Fig. 1 and Table 4).

Vanadate is thought to be a specific inhibitor of plasma membrane ATPase (Bowman *et al.*, 1978). The senescence of detached rice leaves under light condition was promoted by vanadate (Fig. 2). Therefore, the possibility that the utilization of ATP is required for light effect can not be excluded.

Senescence under dark condition was promoted by CCCP and vanadate and retarded by the exogenous supply of ATP. It seems that the senescence of detached rice leaves under dark condition is possibly resulted from the failure of ATP formation and/or utilization.

Cordycepin, an analogue of adenosine, is known to inhibit the polyadenylation of mRNA (Darnell *et al.*, 1969) as well as nucleic acid synthesis (Siev *et al.*, 1969). Previous work in this laboratory suggested that some specific protein(s) synthesized in the light retarded senescence and in darkness promoted senescence (Chou and Kao, 1986; Hurng *et al.*, 1986). The fact that cordycepin promoted senescence in the light and retarded senescence in darkness (Tables 4 and 5) suggests that the synthesis of specific protein(s) responsible for the retardation or the promotion of senescence process is possibly regulated at the level of polyadenylation of mRNA. Jupp (1980) also reported that incorporation of radioactively labelled adenine into poly-(A)RNA was detectable in leaf tissue during senescence. Though 2′-deoxyadenosine acted similarly as cordycepin on the senescence of rice leaves (Tables 4 and 5), we do not know whether 2′-deoxyadenosine also inhibits the polyadenylation of mRNA.

Literature Cited

Arnon, D. I. and R. K. Chain. 1975. Regulation of ferredoxin-catalyzed photosynthetic phosphorylation. Proc. Natl. Acad. Sci. USA **72**: 4961-4965.

Avron, M. and N. Shavit. 1965. Inhibitors and uncouplers of photophosphorylation. Biochim. Biophys. Acta **109**: 317-331.

Bowman, J. B., S. E. Mainzer, K. E. Allen, and C. W. Slayman. 1978. Effects of inhibitors on plasma membrane and mitochondrial adenosine triphosphatases of *Neurospora crassa*. Biochim. Biophys. Acta **512**: 13-28.

Briskin, D. P. and R. J. Poole. 1983. The plasma membrane ATPase of higher plant cells. What's New in Plant Physiol. **14**: 1-4.

Chou, S. B. and C. H. Kao. 1986. Senescence of rice leaves. XIII. Role of protein synthesis in regulation of senescence in detached leaves. Mem. Coll. Agric. Natl. Taiwan Univ. **26**: 13-17.

Darnell, J. E., L. Philipson, R. Wall, and M. Adesnik. 1969.

The selective interruption of nucleolar RNA synthesis in HeLa cells by cordycepin. J. Cell Biol. **41**: 510-520.

De Greef, J. A., W. L. Butler, T. F. Roth, and H. Fredericq. 1971. Control of senescence in *Marchantia* by phytochrome. Plant Physiol. **48**: 407-412.

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

Haber, A. H., D. J. Thompson, P. L. Walne, and L. L. Triplett. 1960. Non-photosynthetic retardation of chloroplast senescence by light. Plant Physiol. **44**: 1619-1628.

Harold, F. M. 1972. Conservation and tansportation of energy by bacterial membrans. Bacteriol. Rev. **36**: 172-230.

Hinds, G. and C. P. Whittingham. 1963. Reduction of ferrocyanide by chloroplasts in presence of nitrogenous bases. Biochim. Biophys. Acta **75**: 194-202.

Hopkinson, J. M. 1966. Studies on the expansion of the leaf surface. VI. Senescence and the usefulness of old leaves. J. Exp. Bot. **17**: 762-770.

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

Hurng, W. P., L. Y. Su, and C. H. Kao. 1986. Senescence of rice leaves. XVI. Regulation by light. Bot. Bull. Academia Sinica **27**: 163-174.

Jagendorf, A. T. and M. Avron. 1959. Inhibitors of photosynthetic phosphorylation in relation of electron and oxygen transport pathways. Arch. Biochem. Biophys. **80**: 246-237.

Jupp, D. J. 1918. Nucleic acids and proteins during development of leaves of *Festuca pratensis*. Ph. D. Thesis, Univ. Durham.

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

Koo, 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.

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.

Lloyd, E. J. 1980. The effects of leaf age and senescence on the distribution of carbon in *Lolium temulentum*. J. Exp. Bot. **31**: 1067-1079.

Malik, N. S. A. and K. V. Thimann. 1980. Metabolism of oat leaves during senescence. VI. Changes in ATP level. Plant Physiol. **65**: 855-859.

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

Siev, M., R. Weinberg, and S. Penman. 1969. The selective incorporation of nucleolar RNA synthesis in HeLa cells. J. Cell Biol. **41**: 510-520.

Sugiura, M. 1963. Effect of red and far-red light on protein and phosphate metabolism in tobacco leaf discs. Bot. Mag. Tokyo **76**: 174-180.

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.

水稻葉片老化之研究
(十七) 抑制劑之效應

洪偉屏[1] 高景輝[2]
臺灣菸葉研究所[1]
臺灣大學農藝學系[2]

本試驗係利用與光合作用及能量有關之抑制劑及腺嘌呤衍生物，探討光線延緩水稻切離葉片老化之可能機制。水稻切離葉片於黑暗下老化之可能原因亦一併探討。非循環性光磷酸化反應抑制劑 DCMU 不能降低光線之效應，但循環性光磷酸化反應之抑制劑 methylamine 與氯化銨，則可降低光線效應，顯示光線之效應係由於其可經由循環性光磷酸化反應產生 ATP。此項推論可由光照下 CCCP 加速而 ATP 延緩切離葉片老化之結果得到支持。試驗結果亦顯示黑暗下切離葉片之所以老化可能由於 ATP 之合成能力降低。釩酸鹽之結果顯示 ATP 之能否被利用亦與老化有關。

腺嘌呤衍生物 adenine、adenosine、2′-deoxyadenosine 及 3′-deoxyadenosine 於黑暗下處理切離葉片均有延緩老化之效果。光照下，adenine、adenosine 亦可延緩老化之效果，但 2′-deoxyadenosine 與 3′-deoxyadenosine 却有促進葉片老化之效果。過去之試驗結果，顯示光照下可能有某種蛋白質之合成而延緩葉片老化，而黑暗下則可能有某種蛋白質之合成而使得葉片老化。3′-deoxyadenosine 之結果似乎顯示此類蛋白質合成之控制層次可能爲 mRNA 之 poly-adenylation。