



Calcium in the regulation of corn leaf senescence by light

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Abstract. The participation of Ca^{2+} in light-retarded senescence of detached corn leaves was examined. Application of Ca^{2+} chelators (EGTA and BAPTA) promoted light-retarded senescence. The effect of EGTA could be reversed by adding Ca^{2+} , though Ca^{2+} itself had no effect on senescence in the light. Ca^{2+} channel blockers (verapamil and lanthanum), a Ca^{2+} ionophore A23187, and calmodulin antagonists (chlorpromazine, trifluoperazine, W-7 and compound 48/80) also promoted light-retarded senescence. Results seem to suggest that light may retard senescence by maintaining a normal transmembrane flux of Ca^{2+} . Since agents known to be agonists of the phosphatidylinositol second messenger system (serotonin and deoxycholate) can partially substitute for light, the phosphatidylinositol pathway is likely to be an important second messenger involved in coupling light action to senescence retardation.

Key words: Calcium; Corn; Leaf senescence; Light; Phosphatidylinositol.

Introduction

Leaf senescence is known to be retarded by exogenously applied Ca^{2+} (Ferguson *et al.*, 1983; Poovaiah and Leopold, 1973). On the other hand, elevated cytosolic Ca^{2+} has been reported to promote senescence (Leshem *et al.*, 1984). These results indicate that calcium plays an important role in regulating leaf senescence. Light has been found to retard senescence in leaves of several species (Thomas and Stoddart, 1980). Several lines of evidence indicate that cytosolic Ca^{2+} is involved in the stimulus-response coupling in many light-mediated phenomena (Hepler and Wayne, 1985; Poovaiah and Reddy, 1987). Thus, a study of the role of Ca^{2+} in light-regulated leaf senescence might yield useful information. The work described here con-

cerns the question of Ca^{2+} involvement in the photocontrol of leaf senescence.

Materials and Methods

Corn seeds (*Zea mays* L. cv. Tainung 351) were grown as described previously (Huang and Kao, 1992). Apical 2.5-cm segments excised from the primary leaves of 7-day-old corn seedlings were used. Two leaf segments were placed vertically in test tubes with the cut ends submerged in 2 ml of test solution and incubated at 27°C for 4 days, either under light ($16.7 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) provided by Gro-lux fluorescent lamps or in darkness. All test solutions were adjusted to pH 5.5.

Total chlorophyll was extracted and determined following the method of Arnon (1949) and is expressed as mg/gFW.

All chemicals used in this study were purchased from Sigma Company, U. S. A. EGTA and BAPTA solutions were prepared by dissolving the chemicals in 10 drops of 1 N NaOH and then adding the appropriate amount of distilled water. Verapamil and A23187 were dissolved in 10 drops of ethanol (95%). After adding the

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²**Abbreviations:** BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-N, N, N', N'-tetraacetic acid; EGTA, ethyleneglycol-bis-(β -aminoethyl ether)-N, N, N', N'-tetraacetic acid; PI, phosphatidylinositol.

appropriate amount of distilled water the solutions were heated to 80°C to remove the ethanol. W-7 was dissolved in 10 drops of 1N HCl, and the appropriate amount of distilled water was then added. Other chemicals were dissolved in distilled water.

Results and Discussion

The senescence of detached corn leaves was followed by measuring the decrease of chlorophyll. Data in Table 1 show that light significantly retarded senescence of detached corn leaves. The effectiveness of Ca^{2+} in retarding chlorophyll degradation under light conditions was tested as shown in Fig. 1. The chlorophyll level was not significantly affected by adding Ca^{2+} in the range 0.1-5 mM. It appears that light-retarded senescence of detached corn leaves does not require Ca^{2+} in the incubation medium.

EGTA is a specific Ca^{2+} chelator used in many systems (Daye *et al.*, 1984; Karege *et al.*, 1982; Lehtonen, 1984). The chelator BAPTA has been reported to be much more selective for Ca^{2+} than EGTA (Tsien, 1980). Data of Figures 1 and 2 show that both EGTA and BAPTA were effective in promoting senescence of detached corn leaves. The effect of EGTA could be reversed by the addition of Ca^{2+} (Fig. 1).

To further characterize the role of Ca^{2+} in light-retarded senescence of detached corn leaves, experiments were carried out with putative calcium channel blockers. Verapamil, a phenyl-alkylamine derivative (Janis *et al.*, 1985) and lanthanum chloride (LaCl_3) (Fineran and Gilbertson, 1978) were applied to detached corn leaves in the light. Both verapamil and La^{3+} were found to be effective in causing the decrease of chlorophyll levels (Fig. 3).

When 10 μM A23187, a Ca^{2+} ionophore (Pressman, 1976), was added to detached corn leaves under light conditions of 4 days, senescence was significantly promoted (Table 2).

In a recent report, we presented evidence that failure to maintain normal transmembrane flux of Ca^{2+} is a key cause of dark-induced senescence of detached corn leaves (Huang and Kao, 1992). If this suggestion is correct, then the retardation of corn leaf senescence by light is most likely mediated through the maintenance of normal transmembrane flux of Ca^{2+} . Once the normal transmembrane influx of Ca^{2+} under light condition is disturbed or blocked, senescence promotion in

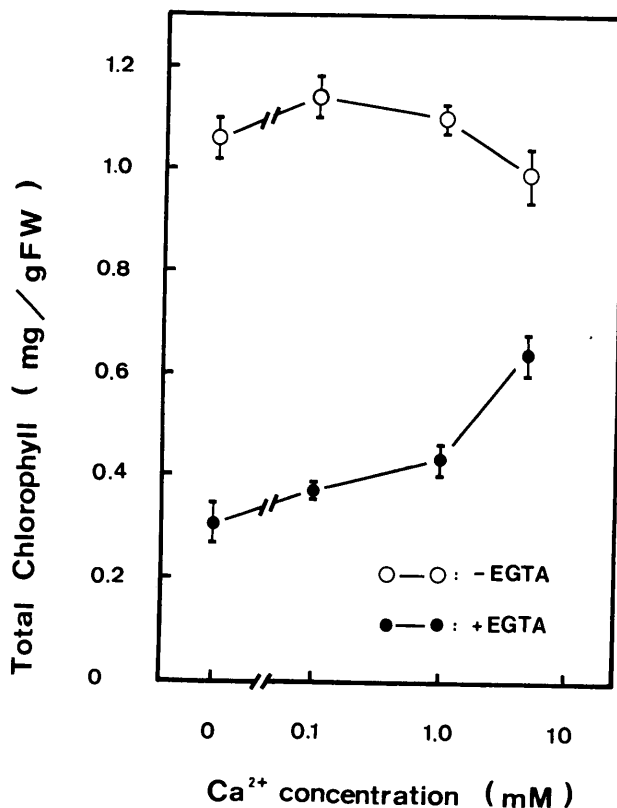


Fig. 1. Effects of CaCl_2 in the presence and absence of EGTA (5 mM) on chlorophyll levels in detached corn leaves. Chlorophyll was determined after 4 days in the light. Bars represent standard errors ($n=4$).

Table 1. Effect of light on chlorophyll levels in detached corn leaves

Values are means with standard errors ($n=4$).

Treatment	Total chlorophyll, mg/gFW
Initial	2.22 ± 0.03
4 days in darkness	0.72 ± 0.04
4 days in light	1.24 ± 0.05

Table 2. Effect of A23187 on chlorophyll levels in detached corn leaves. Total chlorophyll level was determined after 4 days in the light

Values are means with standard errors ($n=4$).

Treatment	Total chlorophyll, mg/gFW
Control	1.56 ± 0.03
A23187	1.17 ± 0.03

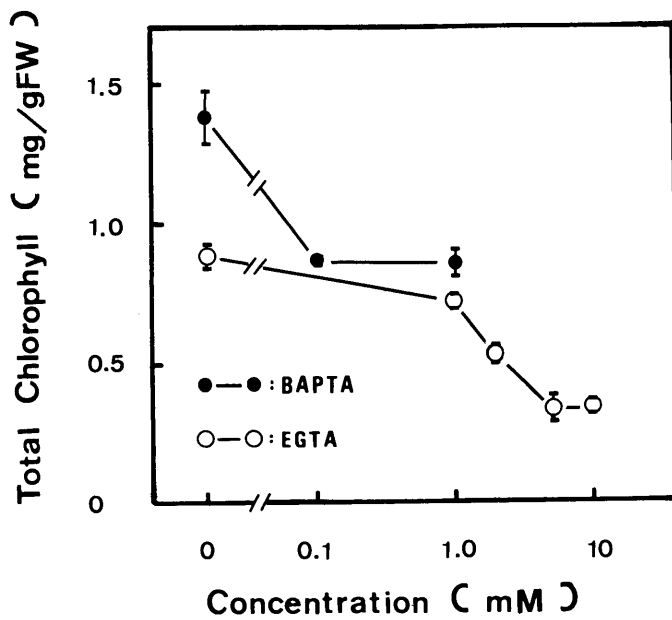


Fig. 2. Effects of EGTA and BAPTA on chlorophyll levels in detached corn leaves. Chlorophyll was determined after 4 days in the light. Bars represent standard errors (n=4).

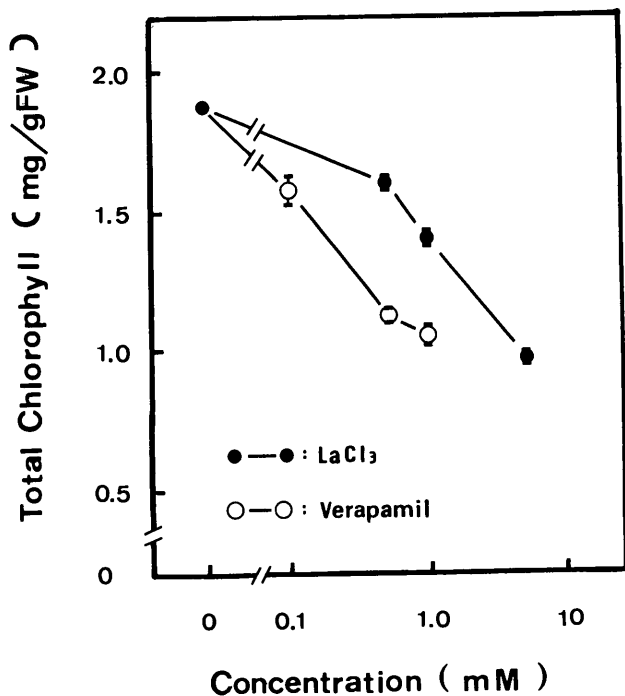


Fig. 3. Effects of verapamil and LaCl₃ on chlorophyll levels in detached corn leaves. Chlorophyll was determined after 4 days in the light. Bars represent standard errors (n=4).

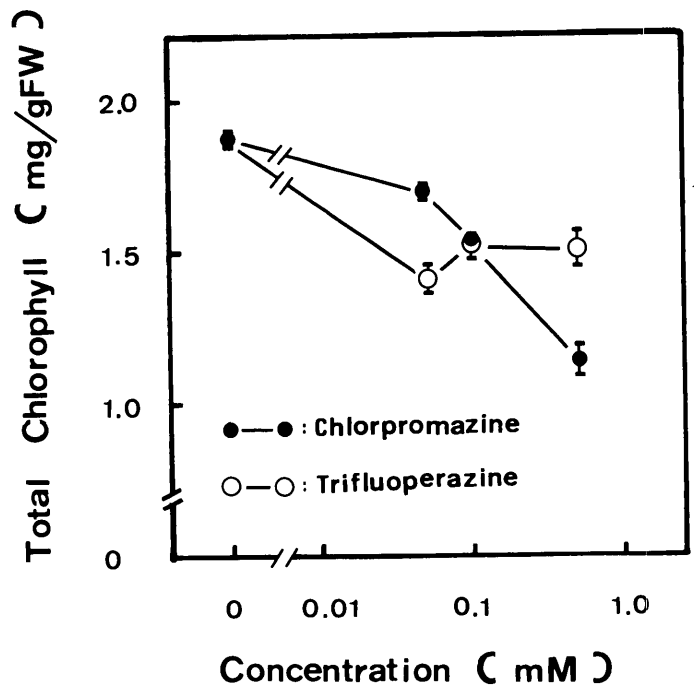


Fig. 4. Effects of chlorpromazine and trifluoperazine on chlorophyll levels in detached corn leaves. Chlorophyll was determined after 4 days in the light. Bars represent standard errors.

corn leaves would be expected. This is indeed the case as judged from the results described above.

Calmodulin is now known to function in almost all plant and animal cells as a transducer of small Ca²⁺ concentration changes into major physiological responses (Poovaiah and Reedy, 1987). A major enzyme under calmodulin control is a plasma membrane Ca²⁺-ATPase, which pumps Ca²⁺ out into the wall of plant cells (Dieter, 1984). Light has been shown to stimulate Ca²⁺ efflux from oat cells (Hale and Roux, 1980). It seems that light may retard senescence of corn leaves through the stimulation of Ca²⁺ efflux via calmodulin-modulated Ca²⁺-ATPase. The fact that calmodulin antagonists (chlorpromazine, trifluoperazine, W-7 and compound 48/80, Hidaka and Tanaka, 1982) promoted senescence of corn leaves in the light (Fig. 4 and Table 3) tends to support this suggestion.

Cleavage and phosphorylation of membrane phosphatidylinositol (PI) is known to result in a transient rise in cytosolic Ca²⁺ levels (Kikkawa and Nishizuka, 1986; Poovaiah and Reddy, 1987). PI turnover has been shown to be the second messenger in stimulus-response coupling in numerous phenomena (Berridge, 1987). The

components of the PI pathway of signal transduction have been demonstrated in plants (Boss and Massel, 1985; Drobak and Ferguson, 1985; Heim and Wagner, 1986; Morse *et al.*, 1987; Rincon and Boss, 1987). Thus, we tested agents, such as serotonin (5 - hydroxy - tryptamine) and deoxycholate, which are known to serve as agonists for the PI second messenger system for their effects on senescence of detached corn leaves. Detached corn leaves were treated with serotonin or deoxycholate in the dark. Both serotonin and deoxycholate were effective in retarding senescence in the dark (Table 4).

Since PI agonists can mimic the effect of light in retarding senescence, it appears that the PI pathway is

Table 3. Effect of W-7 and compound 48/80 on chlorophyll levels in detached corn leaves. Chlorophyll was determined after 4 days in the light

Values are means with standard errors (n=4).

Treatment	Total chlorophyll, mg/gFW
W-7 (mM)	
0	0.96±0.08
0.1	0.92±0.04
0.2	0.73±0.06
0.5	0.56±0.05
1.0	0.57±0.04
Compound 48/80 (µg/ml)	
0	0.96±0.08
50	1.11±0.16
100	1.00±0.12
200	0.72±0.01

Table 4. Effect of agonists of the phosphoinositide pathway on chlorophyll levels in detached corn leaves. Total chlorophyll was determined after 4 days in darkness

Values are means with standard errors (n=4).

Treatment	Total chlorophyll, mg/gFW
Serotonin (mM)	
0	0.74±0.05
0.5	0.83±0.06
1.0	1.06±0.01
5.0	1.60±0.17
Deoxycholate (mM)	
0	0.72±0.04
0.1	1.31±0.02
0.5	1.51±0.03
1.0	1.61±0.05

likely to be an important second messenger involved in coupling light action to senescence retardation. However, the role of the PI second-messenger system in light-retarded senescence will not be firmly established until the release of soluble inositol phosphates is shown to occur in light-retarded detached corn leaves.

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鈣與光線延緩玉米葉片老化之關係

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本研究主要在探討光線所延緩玉米切離葉片的老化是否需要鈣的參與。處理鈣離子的螯合物(EGTA 與 BAPTA)，可加速切離葉片在光線下的老化。EGTA 的效果可經由添加鈣離子而消失，但鈣離子本身不影響切離葉片在光線下的老化。鈣離子的通路抑制劑(verapamil 與 lanthanum)，鈣離子的 ionophore A23187 以及 calmodulin 的抑制劑(chlorpromazine, trifluoperazine, W-7 與 compound 48/80)亦可加速切離葉片在黑暗中的老化。我們的結果似乎顯示光線是經由維持正常的鈣離子運移而延緩老化。由於 phosphatidylinositol 系統的輔助劑(agonists)如 serotonin 與 deoxycholate 可取代光線的效果，因此 phosphatidylinositol 途徑很可能是光線延緩葉片老化的二次訊息。