

SENESCENCE OF RICE LEAVES

VIII. The Regulation of Proline Accumulation During Senescence

CHANG YING WANG and CHING HUEI KAO

*Department of Agronomy, National Taiwan University,
Taipei, Taiwan 107, Republic of China*

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Abstract

Several chemically unrelated compounds were used to study the regulation of proline accumulation during senescence of detached rice leaves. Cytokinins, naturally occurring and synthetic, which are known to retard senescence in the dark, prevented the accumulation of proline. Other agents such as cycloheximide and α , α' -dipyridyl which have been shown to retard senescence also inhibited the accumulation of proline. Under light condition, very smaller amount of proline was accumulated as compared with the dark control. Abscisic acid (ABA) enhanced the accumulation of proline in the light, which may be due to a concomitant effect of the promotion of senescence by ABA. Though benzyladenine (BA) had no effect on proline accumulation under light condition, BA inhibited ABA-induced accumulation of proline. All these results seem to support the suggestion that proline accumulation may be a manifestation of changes in metabolism associated with excision and consequent senescence.

Introduction

Applied abscisic acid (ABA) has been shown to induce proline accumulation in excised leaves of barley and *Lolium temulentum* (Aspinall *et al.*, 1973) and *Pennisetum tyhoides* (Huber, 1974; Eder and Huber, 1977). The response is not universal, however, since tobacco and tomato plants do not accumulate proline in response to applied ABA (Aspinall and Paleg, 1981; Wample and Bewley, 1975). When kinetin or gibberellic acid is supplied to excised, stressed barley or radish leaves the accumulation of proline is inhibited (Aspinall and Paleg, 1981). Cytokinins have also been shown to reduce proline accumulation induced by ABA (Eder and Huber, 1977), and to reduce the proline content of excised cucumber cotyledons (Udayakumar *et al.*, 1976).

The previous work in this laboratory showed that proline content increased

greatly in rice leaf segments when incubated in the dark (Wang *et al.*, 1982). It seems that rice leaf segments provide good system to study the regulation of proline accumulation.

The present communication describes the regulation of proline accumulation in rice leaf segments by several chemically unrelated compounds.

Materials and Methods

Rice (*Oryza sativa* cv. Taichung 1) seedlings were cultured as described previously (Kao, 1980). The apical 3 cm of the third leaves of 9-day-old seedlings was used for experiments. A group of 10 segments was floated on 10 ml of test solution in a 50 ml flask. Incubation was carried out in darkness or light at 27°C. Each treatment contained four replications.

Chlorophyll and α -amino nitrogen were extracted and determined as described before (Kao, 1980). Chlorophyll and α -amino nitrogen were expressed as A_{665} and A_{570} per ten segments, respectively.

Proline was extracted and determined according to the method described previously (Wang *et al.*, 1982). Briefly, leaf segments were homogenized with 3% sulfosalicylic acid and centrifuged. The supernatant was treated with acetic acid and acid-ninhydrin, boiled for 1 hour and extracted with toluene. Then its absorbance at 520 nm was read. Proline content was expressed as μ mole per ten segments.

Results

The senescence of excised rice leaves was followed by measuring the decrease of chlorophyll and the increase of amino nitrogen. Benzyladenine (BA) significantly retarded rice leaf senescence at the concentration from 10^{-7} to 10^{-4} M. It is interesting to find that BA in the same range of concentrations as those which retarded leaf senescence inhibited proline accumulation in the dark. Fig. 1 shows the chlorophyll, amino nitrogen and proline levels after 4 days in the dark.

It has been reported that both naturally occurring and synthetic cytokinins are effective in retarding senescence of detached soybean and rice leaves (Kao, 1978; Yu and Kao, 1981). The inhibition of proline accumulation and the retardation of senescence by various cytokinins are, in general, parallel (Figs. 2 and 3). Compared with those cytokinins with straight chains at 6-N position of adenine such as isopentenyladenine, *n*-hexyladenine and zeatin, cytokinins with ring structure such as BA and kinetin are more effective in preventing proline accumulation and senescence in detached rice leaves.

Cycloheximide has been shown to prevent senescence of rice leaves in the dark (Kao, 1978). Fig. 4 shows that cycloheximide also inhibited proline accumulation in the dark.

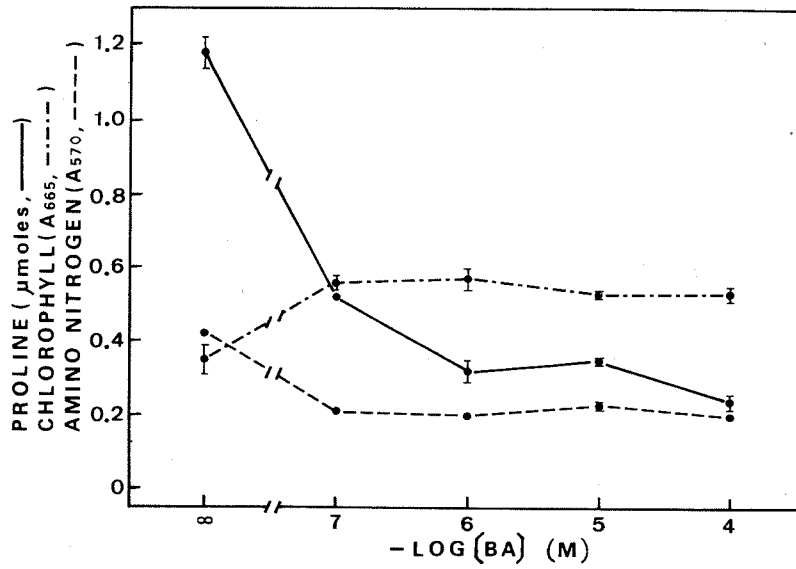


Fig. 1. Effects of BA concentrations on chlorophyll, amino nitrogen and proline levels in detached rice leaves incubated in the dark for 4 days.

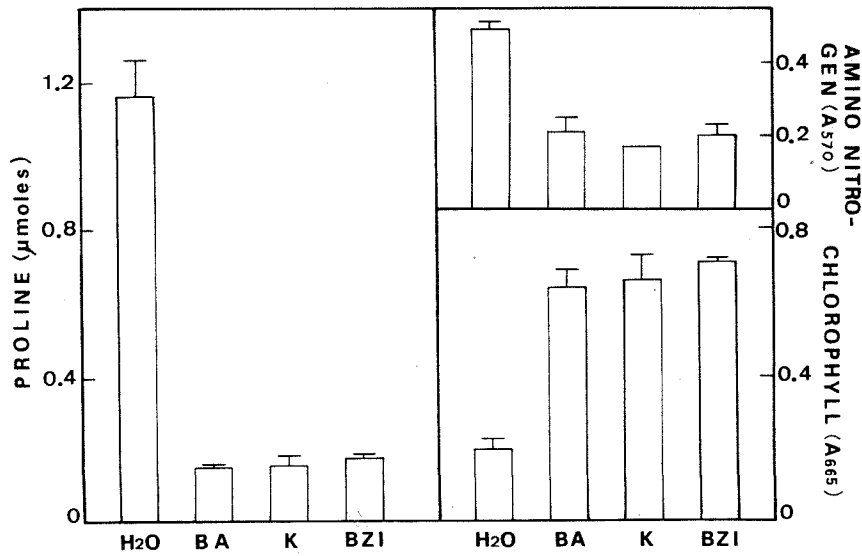


Fig. 2. Effects of benzyladenine (BA), kinetin (K) and benzimidazole (BZI) on chlorophyll, amino nitrogen and proline levels in detached rice leaves incubated in the dark for 4 days. The concentration of BA, K and BZI was 10^{-5} M.

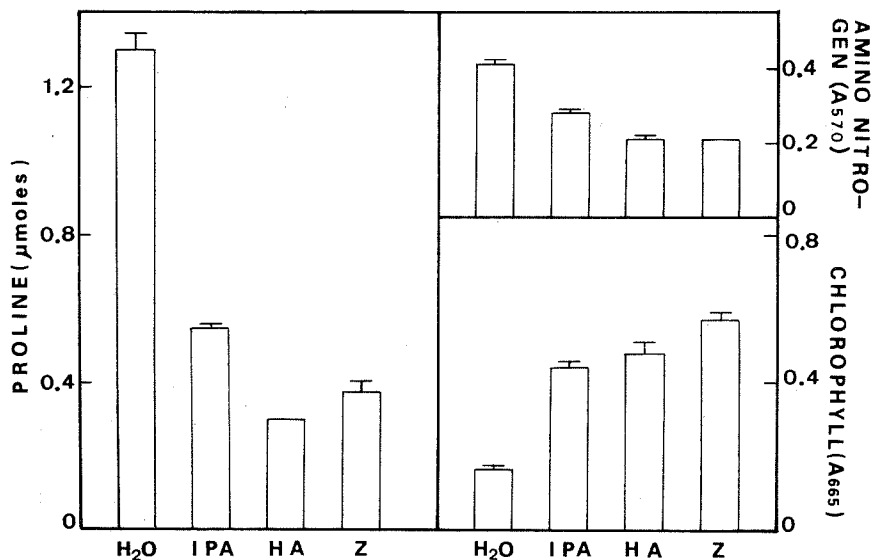


Fig. 3. Effects of isopentenyladenine (IPA), *n*-hexyladenine (HA) and zeatin (Z) on chlorophyll, amino nitrogen and proline levels in detached rice leaves incubated in the dark for 4 days. The concentration of IPA, HA and Z was 10^{-5} M.

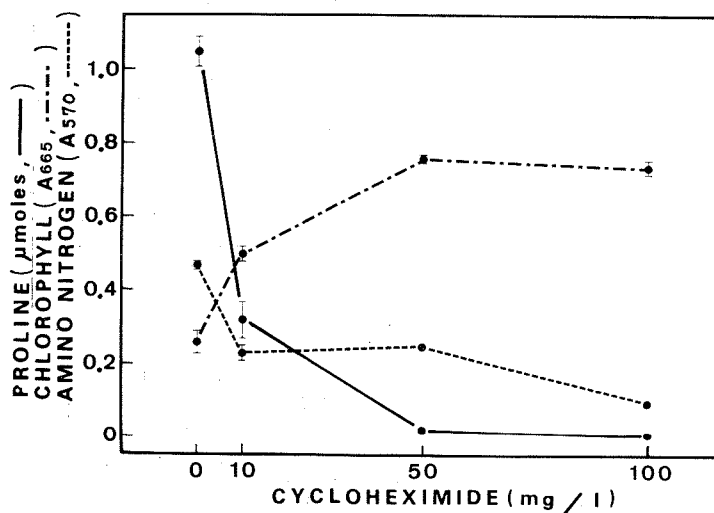


Fig. 4. Effects of cycloheximide concentrations on chlorophyll, amino nitrogen and proline levels in detached rice leaves incubated in the dark for 4 days.

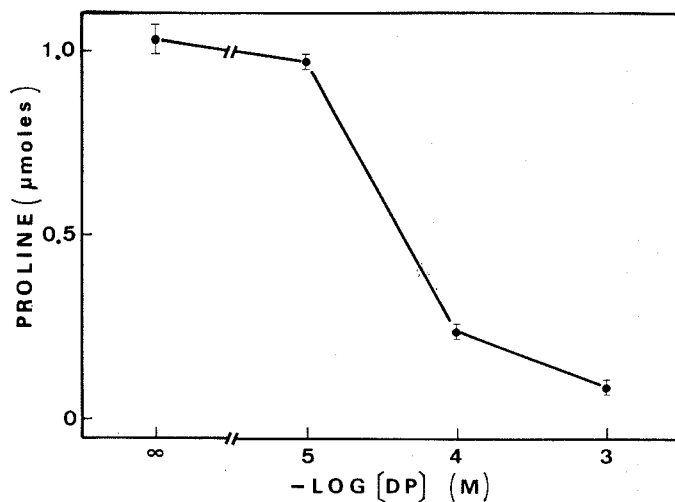


Fig. 5. Effects of α, α' -dipyridyl (DP), concentration on proline level in detached leaves incubated in the dark for 4 days.

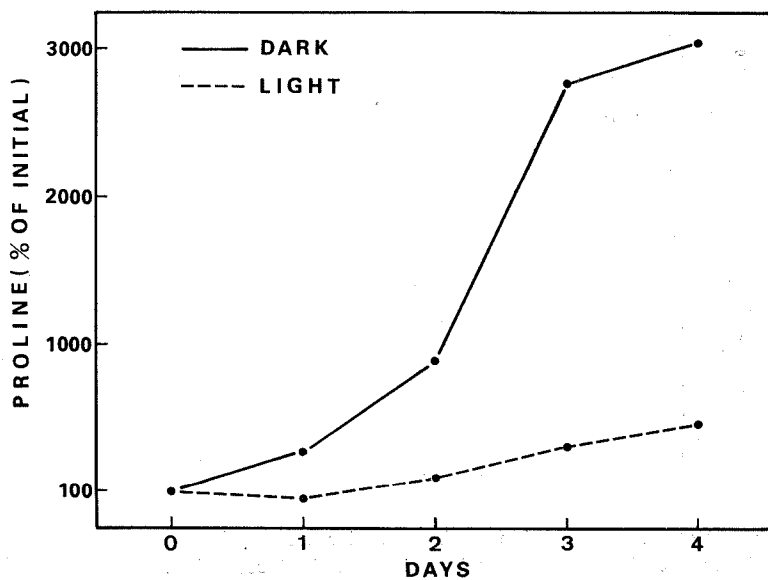


Fig. 6. Effect of light (14 Wm^{-2}) on proline level in detached rice leaves.

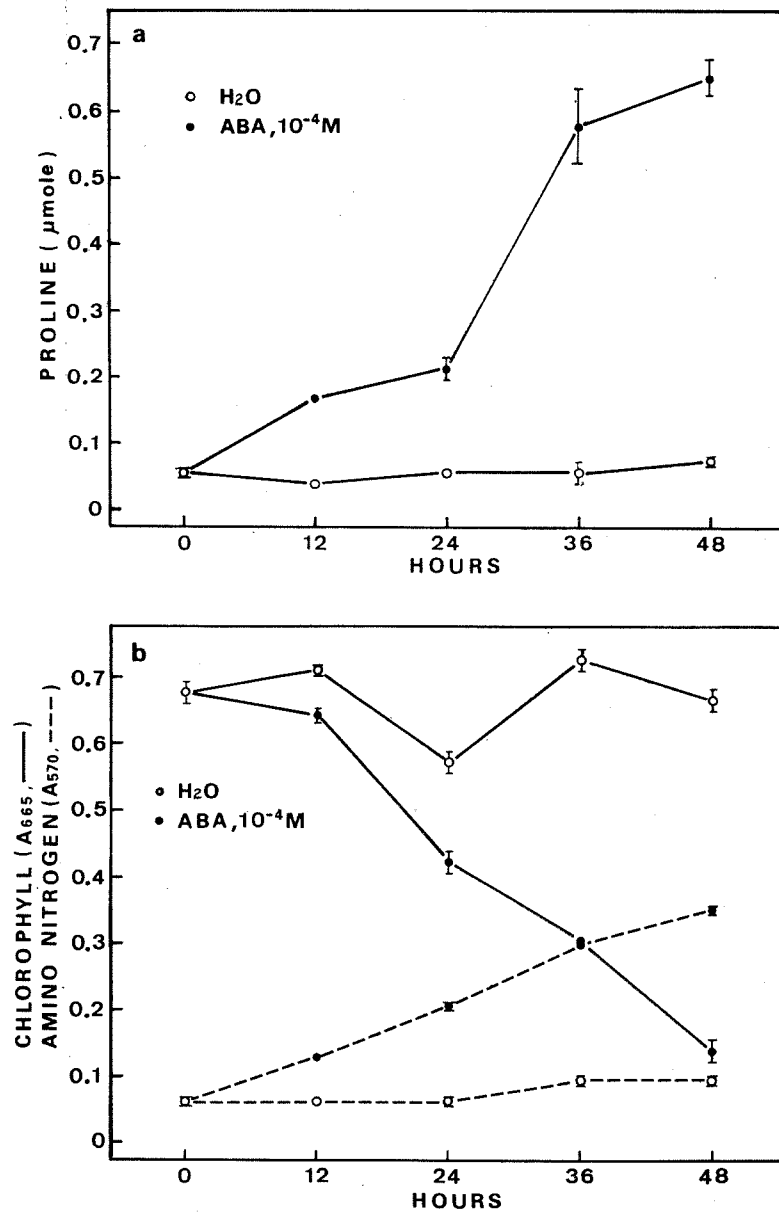


Fig. 7. Effect of ABA on proline level (a), and chlorophyll and amino nitrogen levels (b) in detached rice leaves incubated in light condition.

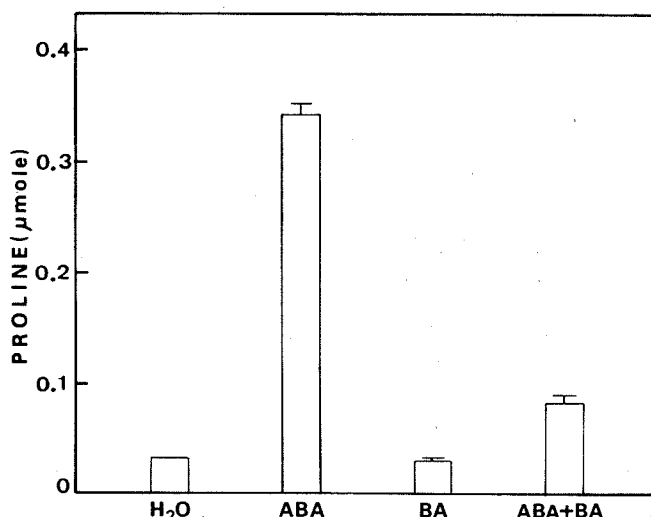


Fig. 8. Effect of BA (10^{-5} M) and ABA (10^{-4} M) on proline level in detached rice leaves incubated in light condition for 48 h.

Thimann and Satler (1979b) has pointed out that α, α' -dipyridyl retarded the senescence of detached oat leaves in the dark. Our unpublished data also showed that α, α' -dipyridyl has senescence retardation effect in detached rice leaves when incubated in the dark. Fig. 5 presents evidence that α, α' -dipyridyl also prevents the accumulation of proline in the dark.

Under light condition, very smaller amount of proline was accumulated (Fig. 6). When leaf segments were treated with ABA under light condition, it was found that ABA effectively promoted both proline accumulation and leaf senescence (Fig. 7a and 7b). Aspinall *et al.* (1973) have demonstrated marked accumulation of free proline in the excised leaves of barley and *Lolium temulentum* in response to ABA treatment. Our results support, in general, these findings and contribute additional data. Though BA had no effect on proline accumulation under light condition, BA inhibited ABA-induced accumulation of proline (Fig. 8).

Discussion

Our previous work demonstrated that proline accumulated greatly during senescence of detached rice leaves (Wang *et al.*, 1982). It was also proved that the accumulation of proline during senescence, at least in part, resulted from an increase rate of synthesis, possibly due to a disruption of the normal feedback inhibition of proline synthesis (Wang *et al.*, 1982). The present work indicates that treatments that retarded senescence inhibited proline accumulation, while agent that promoted senescence promoted proline accumulation. These results

further support our previous suggestion that proline accumulation which occurs in rice leaf segments after incubation on water may be a manifestation of changes in metabolism associated with excision and consequent senescence.

ABA is known to increase rapidly in excised wheat leaves during water stress (Aspinall, 1980; Hsiao, 1973; Wright and Hiron, 1969). The consequences of the accumulation of ABA during water stress are stomatal closure and proline accumulation (Aspinall, 1980). Recently, Radin and Ackerson (1982) suggested that ABA controlled stomatal closure during slower water stress. ABA has also been reported to increase during senescence (Even-Chen and Itai, 1975; Gepstein and Thimann, 1980). Our previous work demonstrated that proline content increased greatly in detached rice leaves during senescence. Thimann and Satler (1979a, b) proposed recently that stomatal closure is responsible for leaf senescence. The metabolic consequences of water stress have frequently been compared with those of senescence. Brady *et al.* (1974) demonstrated that early events in the senescence of turgid and water stressed leaves are similar. Our results seem to suggest that stomata may also be closed during senescence of detached rice leaves.

It has been proposed that the accumulation of proline during water stress may be mediated through stress effects on one or more effector compounds (Aspinall and Paleg, 1981). The kinetics of proline accumulation also allow the possibility of an intermediate stage between induction of water stress and the biochemical events leading to proline accumulation. Plant hormones may be the candidates for such an intermediary role. Our result indicated that proline accumulation was modified by cytokinins and ABA. Furthermore, BA inhibited ABA-induced proline accumulation. It seems that water stress induces the changes in endogenous cytokinin (Itai *et al.*, 1973) and ABA concentrations which in turn regulate the accumulation of proline in leaf tissue.

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水稻葉片老化之研究

(八) 老化過程中脯氨酸累積之控制

王長瑩 高景輝

國立臺灣大學農藝學系

本研究主要是利用幾種化學構造不同之化合物來研究水稻切離葉片老化過程中脯氨酸累積之控制。不論是人工合成之 cytokinins 或者是自然產生之 cytokinins 都可延緩葉片老化，同時也可抑制脯氨酸之累積。其他化合物如 cycloheximide、 α, α' -dipyridyl 凡能延緩葉片老化者都可抑制脯氨酸之累積。在光線下，脯氨酸累積的非常少。而 ABA 處理可顯著的增加脯氨酸的累積，此可能是由於 ABA 促進葉片老化的結果。雖然在光線下，BA 對於脯氨酸的累積沒有影響，但是 BA 可降低 ABA 所促進之脯氨酸累積。這些結果似乎支持以前所提出的假設：葉片切離後之老化過程中會有明顯之代謝變化。