Bot. Bull. Academia Sinica 25: 191-196 (1984)

SENESCENCE OF RICE LEAVES

XII. Effects of 1, 3-Diaminopropane, Spermidine and Spermine

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(Received April 14, 1984; Accepted June 17, 1984)

Abstract

The effect of polyamines, spermidine and spermine, and 1, 3-diaminopropane on senescence of detached leaves of rice was investigated. 1, 3-Diaminopropane, like spermidine and spermine, significantly retarded senescence of detached rice leaves under dark condition. Under light condition, 1, 3-diaminopropane, spermidine and spermine retarded protein loss but promoted chlorophyll degradation. 1, 3-Diaminopropane was more effective in retarding senescence than spermidine or spermine. β-Hydroxyethylhydrazine, an inhibitor of polyamine oxidase, reversed the observed effect of polyamines under light condition but not under dark condition. It is apparent that, under light condition, the effect of polyamines on senescence is possibly mediated through their conversion to 1, 3-diaminopropane. Ca^{2+} competitively reduced the polyamine or 1, 3-diaminopropane effect, suggesting that an initial attachment to a membrane site shared with Ca^{2+} is required for the antisenescencent effect of polyamines or 1, 3-diaminopropane.

Key words: *Oryza sativa*; leaf senescence; diaminopropane; β-hydroxyethylhydrazine; spermidine; spermine.

Introduction

Polyamines, such as spermidine (Sd) and spermine (Sm) are synthesized almost in all biological systems, including higher plants (Smith, 1977). Polyamines have been reported to retard leaf senescence (Altman, 1982; Cohen *et al.*, 1979; Kaur-Sawhney and Galston, 1979). This conclusion was mainly based on the fact that chlorophyll degradation of detached leaves was retarded by exogenously applied polyamines. Recently, we also found that polyamines strongly retarded senescence of detached leaves (Cheng and Kao, 1983). However, the retardation

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effect is mainly localized in those areas around the cut edges of detached leaves (Cheng and Kao, 1983).

1,3-Diaminopropane (DAP), an oxidation product of the naturally occurring polyamines, occurs in many cereal plants (Smith, 1980). The oxidation is catalyzed by polyamine oxidase, a cell wall-localized enzyme in oat leaves (Kaur-Sawhney *et al.*, 1981). Endogenous DAP level has been reported to decrease in attached oat leaves with increasing age of seedlings and in excised leaves with increasing time of dark incubation, suggesting that DAP, like polyamines, may be involved in the control of senescence (Kaur-Sawhney *et al.*, 1982). Shih *et al.* (1982) first demonstrated that exogenous DAP indeed retarted leaf senescence. Since then, no similar work has been reported.

The present work was undertaken to study the effect of detached rice leaves. Our data demonstrated that DAP is more effective than polyamines in retarding leaf senescence.

Materials and Methods

Plant Materials and Incubation Condition

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as described elsewhere (Kao, 1980). Leaf samples (3 cm from tip) were collected from the third leaves of seedlings at 10 days after planting. Since polyamines are not readily transported in leaf cells (Cheng and Kao, 1983). Leaf segments (3 cm) were further sliced into 1-cm pieces, and thirty 1-cm segments were incubated in test solution in a 50-ml flask. The flasks were then incubated at 27°C in darkness or in the light (14 Wm^{-2}).

Determinations of Chlorophyll and Protein

Chlorophyll and protein were extracted and determined as described before (Kao, 1980) and expressed as A_{665} and A_{700} per thirty 1-cm segmets, respectively.

Results and Discussion

The senescence of excised rice leaves was followed by measuring the decrease of chlorophyll or protein. Fig. 1 shows the effect of DAP, Sd and Sm on chlorophyll and protein content in leaf segments treated with dark or light. DAP, like Sm and Sd, strongly retarded the decrease of both chlorophyll and protein content under dark condition. Under light condition, DAP and polyamines retarded the decrease of protein content but promoted chlorophyll degradation. The promotion of chlorophyll degradation by DAP and polyamines indicates that, in addition to the normal mechanism, an irreversible photooxidation process may be involved. Increased chlorophyll degradation under light condition has also

been demonstrated in rice leaf segments treated with metal chelators (Kao, 1984a) and straight-chain alcohols (Kao, 1984b). It is also clear from Fig. 1 that DAP was more effective in retarding senescence than polyamines under both light and dark conditions.

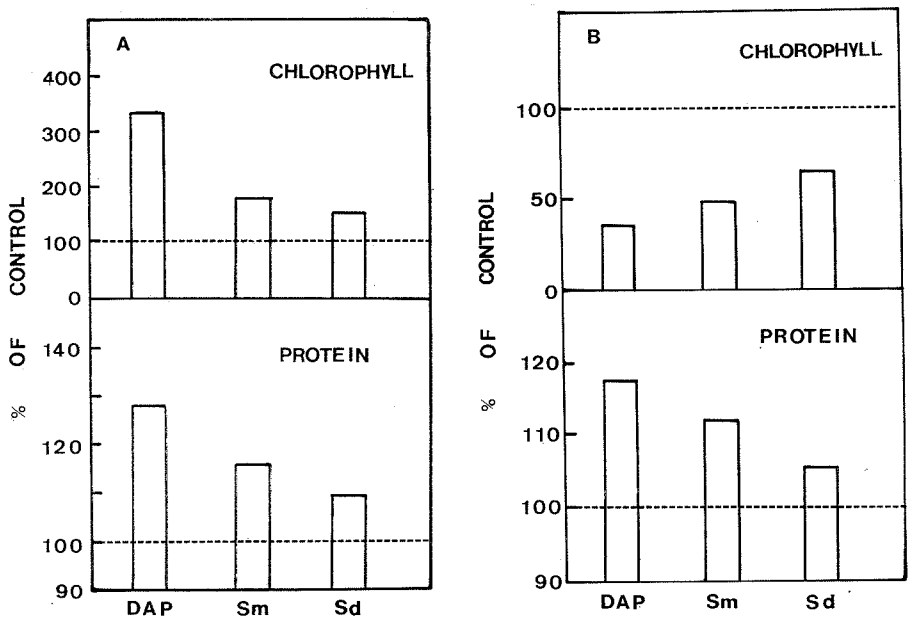

Fig. 1. Effect of DAP, Sm and Sd on the chlorophyll and protein content in excised rice leaves treated with dark (A) or light (B). Chlorophyll and protein were determined after 3 and 4 days in light and darkness, respectively.

Since DAP is a metabolite of polyamine oxidation, DAP may be accumulated when leaf segments are incubated on polyamine-containing medium. Shih *et al.* (1982) provided evidence that DAP was indeed accumulated when excised oat leaves were incubated on Sd-containing medium. It seems that the effect of polyamines on senescence may be mediated through their conversion to DAP. This hypothesis can be easily tested by using β-hydroxyethylhydrazine (HEH), an inhibitor of polyamine oxidase (Kaur-Sawhney *et al.*, 1981; Shih *et al.*, 1982). If the hypothesis is correct, then the addition of HEH into polyamine-containing medium should reverse the observed effect of polyamines. It is true that HEH reversed the effect of polyamines under light condition (Table 1). However, HEH did not show such effec under dark condition (Table 1). Furthermore, leaves treated with HEH under light condition had lower chlorophyll level as compared with the control. This may be due to the decrease of endogenous DAP level by HEH via its inhibition of polyamines oxidase. Thus, polyamine oxidase is apparently involved in senescence process of leaves under light condition, but not under dark condition.

Table 1. *Interaction of HEH on the effect of polyamines in regulating chlorophyll content in rice leaf segments under light or dark treatment*

Chlorophyll was determined after 3 and 4 days in light and darkness, respectively.

Treatment	Chlorophyll (A_{655})	
	Light	Dark
H_2O	1.17±0.04	0.49±0.02
HEH, 0.1 mM	0.89±0.01	0.65±0.04
Sd, 10 mM	0.55±0.02	0.77±0.02
Sm, 10 mM	0.47±0.04	0.66±0.01
HEH, 0.1 mM+Sd, 10 mM	0.69±0.01	0.86±0.02
HEH, 0.1 mM+Sm, 10 mM	0.60±0.02	0.74±0.02

Table 2. *Prevention by calcium of DAP and Polyamine effect on chlorophyll content in leaf segments treated with light or darkness*

The concentration of calcium, DAP and polyamines is 10 mM, respectively. Chlorophyll was determined after 3 and 4 days in light and darkness, respectively.

Treatment	Chlorophyll (A_{665})	
	Light	Dark
Water control	1.15±0.02	0.40±0.01
Ca^{2+}	1.13±0.04	0.38±0.02
DAP	0.35±0.02	0.81±0.01
Sd	0.59±0.03	0.75±0.02
Sm	0.51±0.01	0.67±0.03
DAP+Ca^{2+}	0.80±0.05	0.67±0.01
Sd+Ca^{2+}	0.70±0.01	0.59±0.01
Sm+Ca^{2+}	0.68±0.02	0.48±0.01
Ca^{2+} (1 day)+DAP*	1.10±0.05	0.38±0.02
Ca^{2+} (1 day)+Sd*	1.17±0.01	0.42±0.02
Ca^{2+} (1day)+Sm*	1.14±0.03	0.39±0.01
DAP (1 day)+Ca**	0.42±0.01	0.68±0.02
Sd (1 day)+Ca**	0.68±0.02	0.58±0.03
Sm (1 day)+Ca**	0.57±0.01	0.54±0.01

* Leaf segments were treated with calcium chloride for 1 day and then followed with DAP or polyamines.

** Leaf segments were treated with DAP or polyamines for 1 day and then followed with calcium chloride.

Since DAP and polyamines are positively charged at cellular pH and polyamines are known to bind strongly to negative sites on membranes, ribosomes and nucleic acids (Abraham and Pihl, 1981; Bachrach, 1973), they could possibly affect leaf senescence by virtue of this cation property. If this is true, treatments with another cation such as calcium should reverse the effect of DAP and polyamines. The data in Table 2 showed that the effect of DAP and polyamines on senescence in darkened excised leaves was reduced in the presence of calcium chloride. Likewise, in the light, where DAP or polyamines promoted chlorophyll degradation, the presence of Ca^{2+} partially inhibited the DAP or polyamine effect. Calcium, however, was not effective under both light and dark conditions if the leaves were first treated with DAP as polyamines for 1 day and then transferred to calcium chloride solution. Similarly, DAP, Sd or Sm was not effective if the leaves were first treated with calcium chloride and then with DAP, Sd or Sm (Table 2), Calcium sulfate had the same effect as calcium chloride in reversing the effect of DAP or polyamines (data not shown). The results suggest that the effect of DAP or polyamines on senescence occurs via an initial binding of DAP or polyamines to membrane site which shares with calcium ion. The results of Kaur-Sawhney *et al.* (1979) and Shih *et al.* (1982) also supported the hypothesis that DAP and polyamines affect senescence by virtue of cation property.

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水稻葉片老化之研究

(十二) 1, 3-Diaminopropane, Spermidine 與 Spermine 之效應

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本研究主要探討 Polyamines 如 Spermidine 與 Spermine 以及 1, 3-Diaminopropane 對水稻切離葉片老化之影響。1, 3-Diaminopropane 如同 Spermidine 與 Spermine 能顯著的延緩切離葉片在暗中老化。在光線下，它們能延緩蛋白質之分解，但是促進葉綠素之分解。1, 3-Diaminopropane 延緩老化之效果大於 Polyamines。利用 Polyamine oxidase 抑制劑研究之結果顯示光線下 Polyamine 延緩老化之效應可能是由於其代謝爲 1, 3-Diaminopropane 所致。Ca^{2+} 改變 Polyamine 與 1, 3-Diaminopropane 之效應，說明了 Polyamine 與 1, 3-Diaminopropane 必須先與膜結合後始能表現延緩老化之效應。