Localized Effect of Polyamines on Chlorophyll Loss¹

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The effect of polyamines, spermidine and spermine, on senescence of detached leaves of rice, wheat and soybean was investigated. The decline of chlorophyll in rice leaf segments was promoted by polyamines during the first three days and in soybean leaf discs during the first day of incubation. However, the decline in chlorophyll was later retarded by polyamines. Polyamines were more effective in retarding senescence of detached wheat leaves at a higher concentration (10 mM) or in narrower segments. The retardation of chlorophyll loss by polyamines was mainly localized in those areas around the cut edges of detached leaves or near large veins of soybean primary leaves. This suggests that polyamines are not readily transported in leaf cells.

Key words: Chlorophyll loss — Localized effect — Mobilization effect — Polyamines — Spermidine — Spermine.

Polyamines have been found in all higher plants studied (Smith 1981). They are known to play an important regulatory role in plant growth and differentiation (Galston and Kaur-Sawhney 1980). Recently, 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 Chl degradation of detached leaves was retarded by exogenously applied polyamines.

The present study was undertaken to determine the effect of polyamines on Chl loss of rice, wheat and soybean leaves. Our data demonstrated that the polyamine effect on Chl loss is quite localized.

Materials and Methods

Plant materials—The plants studied consisted of rice (*Oryza sativa* cv. Taichung Native 1), wheat (*Triticum aestivum* cv. Taichung 30) and soybean (*Glycine max* Merr. cv. Tainung 4). Rice and soybean seedlings were grown as previously described (Hsia and Kao 1978, Kao 1980). Wheat seeds were sown in vermiculite and grown in a greenhouse with 20°C days and 15°C nights. Unless otherwise stated, leaf segments consisted of the apical 3 cm of the third leaves of 9-day-old rice seedlings and the first leaves of 8-day-old wheat seedlings. Leaf discs (8 mm in diameter) were taken from primary leaves of 14-day-old soybean seedlings.

A group of ten leaf discs or segments was floated on 10 ml of test solution in a Petri dish. Incubation was carried out in darkness at 27°C for rice and wheat and 30°C for soybean.

Chl determination—Chl was extracted and determined as described before (Kao 1980). Chl was expressed as A_{665} per ten segments or discs.

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Abbreviations: Chl, Chlorophyll; Sd, Spermidine; Sm, Spermine.

Fig. 1 The effect of polyamines on Chl level of rice leaf segments. Three centimeters segments (a) and 1 cm segments (b) were treated with 10 mM Sd or Sm in the dark. Chlorophyll level was expressed in A₆₆₅ per 10 segments (a) and per 30 segments (b).

Results

The effect of polyamines on the Chl loss of rice leaf segments—Fig. 1a shows that the decrease of Chl in rice leaf segments was accelerated by Sd or Sm (10 mM) during the first three days. Sd and Sm retarded the decline in Chl only during the last day of incubation. These results are contrary to those of Altman (1982), Cohen et al. (1979) and Kaur-Sawhney and Galston (1979), who reported polyamines retarded the decrease of Chl in excised radish and oat leaves during the whole period of incubation. When rice leaf segments (3 cm) were further sliced into 1 cm

Fig. 2 The effect of polyamine concentrations on Chl level of wheat leaf segments. Leaf segments were treated with Sd (a) and Sm (b) in the dark.

Fig. 3 The effect of polyamines on the Chl level in soybean leaf discs. Leaf discs were treated with 10 mM Sd or Sm in the dark.

fragments, and thirty 1 cm segments were incubated in the same concentration of Sd or Sm solution, it was found that Sd and Sm retarded the loss of Chl during the last 2 days of incubation, although Sm showed some promotion of Chl loss during the first two days (Fig. 1b).

The effect of polyamines on the Chl loss of wheat leaf segments—The decrease of Chl in detached segments of wheat leaves was promoted slightly by 1 mM but retarded by 10 mM Sd (Fig. 2a). Sm was also found to be more effective at 10 mM than at 1 mM (Fig. 2b). Sm, as demonstrated with rice segments, was more effective in retarding the decrease of Chl level in 1 cm wheat segments than in 3 cm wheat segments (data not shown).

The effect of polyamines on the Chl loss of soybean leaves—For soybean leaf discs, polyamines slightly promoted Chl loss during the first 24 h, but significantly retarded it during later incubation (Fig. 3). When leaf discs of various sizes were treated with 10 mM Sd and Sm in the dark, it was found that the retardation effect was quite localized (Fig. 4). Only the peripheral area of discs remained green or became even more green. On the other hand, the central areas became more

Fig. 4 The effect of polyamines on the Chl loss of soybean leaf discs. Leaf discs were treated with 10 mM Sd or Sm for 48 h in the dark. The diameters of discs from left are 18, 15, 11, 8 mm, respectively.

Fig. 5 The effect of polyamines on the Chl loss of soybean primary leaves. Leaf petioles were submerged basally in 10 mM Sd or Sm solution for 48 h in the dark.

yellow as compared with the control. Larger discs usually showed larger areas of acceleration of Chl loss than smaller ones. This effect is very rapid and becomes evident after 24 h of dark incubation (data not shown). For rice and wheat leaf segments, the retardation effect of polyamines on Chl loss was also localized along the cut edges (data not shown).

When a mature primary leaf of 14-day-old soybean seedlings was excised and the petiole was submerged in a 10 mM solution of polyamine, retention of Chl occurred mainly around the large veins, whereas the rest of the leaf showed an acceleration of Chl loss (Fig. 5).

Discussion

Polyamines have been reported to retard leaf senescence and Chl loss (Altman 1982, Cohen et al. 1979, Kaur-Sawhney and Galston 1979, Kaur-Sawhney et al. 1982, Smith 1982). The present work, in general, supports these findings. However, our results demonstrate that the retardation of Chl loss by polyamines was localized to those areas along the cut edges of detached leaves or along the large veins of soybean leaves. Kaur-Sawhney and Galston (1979) also reported that Chl loss was localized in the peeled area of leaf blade. The localized effect seems to suggest that polyamines are poorly transported in leaf cells. This is not surprising if the polycationic nature of polyamines is considered. Localization effect of polyamines would explain the results that polyamines are more effective at higher concentration or in segments with smaller width.

From visual observations, it was found that those areas away from the cut edges of detached leaves or large veins of soybean primary leaves usually showed an acceleration of Chl loss when compared with the control. This seems to suggest that those areas receiving polyamines cause mobilization of substances, which are presumably essential to retard Chl loss, from those areas away from the cut edges of detached leaves or large veins of soybean leaves. In other words, polyamines appear to create a new source-sink relationship. This would explain why the loss in Chl level was enhanced by polyamines during the early phase of incubation but retarded during the later phase. The mobilization effect of polyamines seems to be quite similar to that of cytokinins (Leopold and Kawase 1964, Mothes and Engelbrecht 1961).

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