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Endogenous polyamine levels and dark-induced senescence of detached corn leaves

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Abstract. The role of endogenous polyamines in the regulation of dark-induced senescence of detached corn leaves was investigated. Putrescine, spermidine, and diaminopropane were all present throughout senescence, but spermine was undetectable. Preceding the commencement of dark-induced senescence, there was a marked increase in putrescine levels. A slight increase in levels of spermidine and diaminopropane occurred only at an advanced stage of senescence. D-arginine and α-methylornithine caused a reduction in the levels of putrescine and a retardation of senescence. Benzyladenine, which retarded senescence, significantly decreased the levels of putrescine. The results suggest that an increase in endogenous putrescine level is likely to be the factor responsible for dark-induced senescence of detached corn leaves.

Keywords: Diaminopropane; Leaf senescence; Putrescine; Spermidine; Spermine; *Zea mays*.

Introduction

Polyamines have been implicated in several aspects of plant development (Evans and Malmberg, 1989). Diaminopropane, an oxidation product of naturally occurring polyamines, and exogenous polyamines are effective in retarding the senescence of excised leaves and protoplasts (Altman, 1982; Cheng and Kao, 1983; Cheng et al., 1984; Galston et al., 1978; Shih et al., 1982). Since polyamine levels have been observed to decrease in senescing leaves, it has been suggested that they play a role in the control of leaf senescence (Kaur-Sawhney et al., 1982; Srivastava et al., 1981). However, Birecka et al. (1984) reported that no significant decline of polyamines could be observed in oat (*Avena sativa* cv. Astro or Gary) and *Nicotiana alata* leaves, which exhibited typical dark-induced senescence syndrome. The present investigation was conducted to determine the role of endogenous polyamines in the control of senescence of detached corn leaves.

Materials and Methods

Plant Material and Incubation Conditions

Seedlings of corn (*Zea mays* cv. Tainong 1) were grown in vermiculite in a greenhouse under natural light at 30 °C day/25 °C night for 7 days, by which time the primary leaves were fully expanded. Two-and-a-half-centimeter apical segments excised from primary leaves were used. Leaf segments were placed vertically in test tubes with the cut end submerged in 2 ml of distilled water or test solution (5 mM D-arginine, 5 mM α-methylornithine, or 10 μM benzyladenine), and incubated at 27 °C in darkness.

Determination of Chlorophyll and Protein

Chlorophyll was extracted with 96% ethanol and determined by the method of Wintermans and De Mots (1965). For protein, leaf segments were homogenized in 25 mM sodium phosphate buffer (pH 7.5). The extract was centrifuged at 17,000 x g for 20 min, and the supernatant liquid was used for determination of protein by the method of Lowry et al. (1951). Chlorophyll and protein levels were expressed as mg/g fresh weight.

Determinations of Polyamines

Leaf segments were homogenized in 5% perchloric acid. Polyamines were determined using high performance liquid chromatography after benzoylation as described previously (Chen and Kao, 1991). The levels of polyamines were expressed as nmole/g fresh weight.

All experiments were repeated twice. Similar results and identical trends were obtained on each occasion. The data reported here are all from a single experiment.

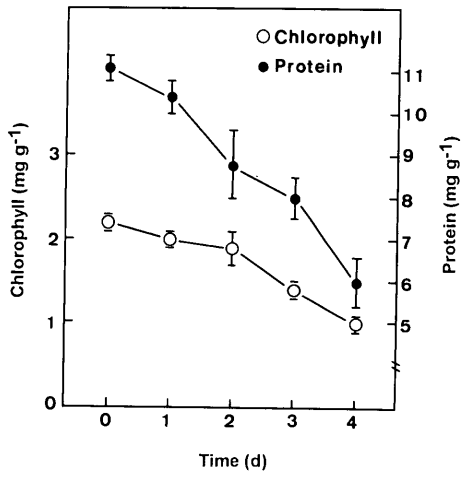

Fig. 1. Changes in levels of chlorophyll and protein in detached corn leaves incubated in darkness. Means ± SE, 4 replicates.

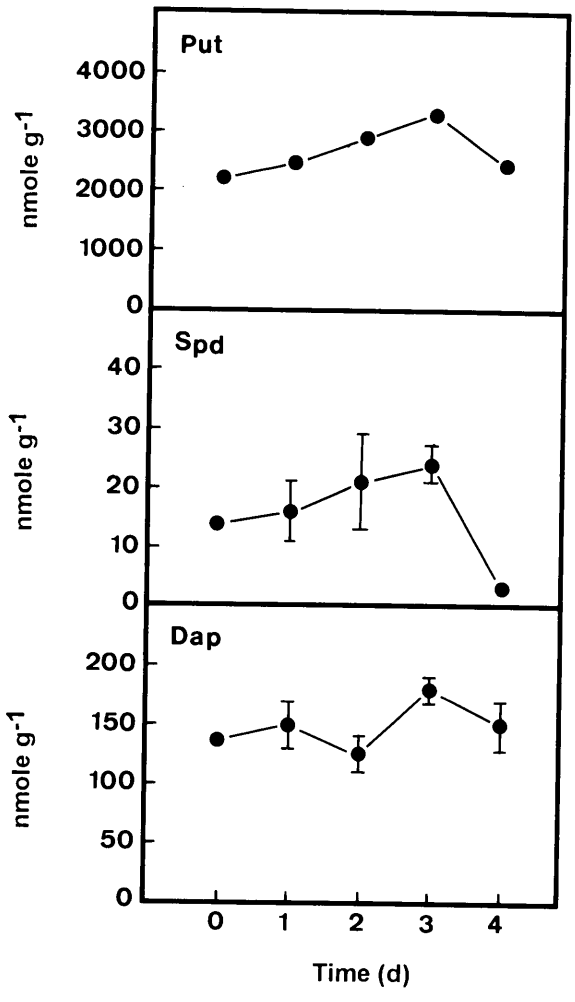

Fig. 2. Changes in levels of putrescine (Put), spermidine (Spd) and diaminopropane in detached corn leaves incubated in darkness. Means ± SE, 3 replicates. Only those SE larger than symbol size are shown.

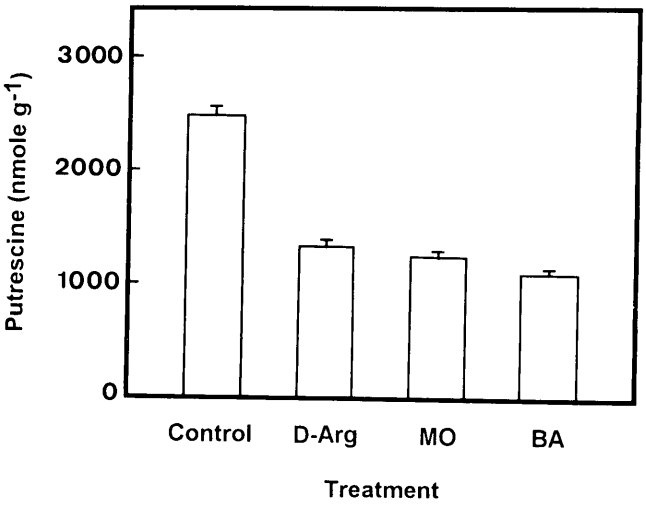

Fig. 3. Influence of D-arginine (D-Arg), α-methylornithine (MO) and benzyladenine (BA) on levels of putrescine in detached corn leaves incubated in darkness. Detached corn leaves were treated with 5 mM D-arginine, 5 mM α-methylornithine, or 10 μM BA. Putrescine levels were determined at 4 days after dark incubation. Means ± SE, 3 replicates.

Results and Discussion

The senescence of detached corn leaves was monitored by measuring the decrease in levels of chlorophyll and protein. Figure 1 shows that a decrease in levels of chlorophyll and protein was evident at 2 days after leaf detachment.

The present investigation showed that putrescine, spermidine, and diaminopropane (an oxidation product of spermidine and spermine) were present in detached corn leaves throughout senescence. Spermine, however, was undetectable in detached corn leaves during dark-induced senescence. Putrescine is the predominant polyamine present in detached corn leaves (Fig. 2). The level of putrescine in detached corn leaves increased immediately after leaf detachment, reached a maximum at day 3 and then decreased to basal level (Fig. 2). Clearly, the increase in putrescine preceded the commencement of senescence of detached corn leaves. The levels of spermidine and diaminopropane remained unchanged during the first 2 days of dark incubation (Fig. 2). A slight increase in levels of spermidine and diaminopropane occurred only at an advanced stage of senescence (3 days after incubation). Thus, spermidine and diaminopropane do not seem to play any role in the control of senescence of detached corn leaves.

Two different patterns of putrescine level have been reported during dark-induced senescence of detached leaves. In some cases, the putrescine level decreased during senescence (Chen and Kao, 1991; Kaur-Sawhney et al., 1982; Srivastava et al., 1981). In a second pattern, the putrescine level remained unchanged during senes-

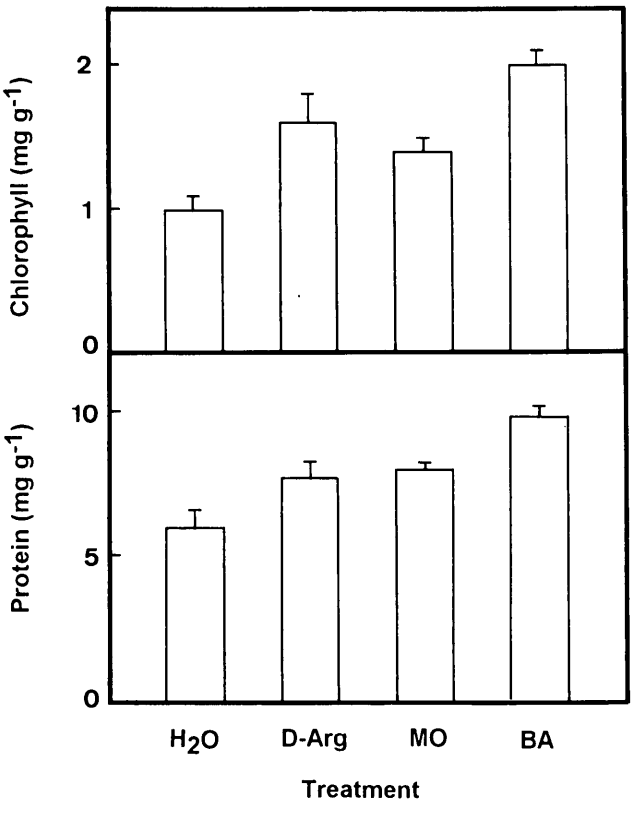

Fig. 4. Influence of D-arginine (D-Arg), α-methylornithine (MO) and benzyladenine (BA) on levels of chlorophyll and protein in detached corn leaves incubated in darkness. Detached corn leaves were treated with 5 mM D-arginine, 5 mM α-methylornithine, or 10 μM BA. Chlorophyll and protein levels were determined at 4 days after dark incubation. Means ± SE, 4 replicates.

cence (Birecka et al., 1984). We have observed here a third pattern: detached corn leaves exhibited an increase in putrescine during senescence.

It has been reported that putrescine accumulates in plants in response to various types of environmental stress (Flores, 1990). The results of the present investigation suggest that dark conditions can, like certain other stresses such as potassium and magnesium deficiency, excess ammonium ion, low pH, salinity, and osmotic stress, induce an increase in putrescine level.

Several experiments have shown that putrescine has a deleterious effect when fed to plant tissues (Coleman and Richards, 1956; DiTomaso et al., 1989; Roberts et al., 1986; Shevyakova, 1981). In fact, we observed that upon feeding putrescine to cut corn leaves a toxic symptom was produced. Birecka et al. (1984) suggested that exogenous polyamines could have a nonspecific action on senescence, an action apparently not exercised by endogenous polyamines. If the increase in levels of endogenous putrescine in detached corn leaves under dark conditions is the factor responsible for the senescence, then senescence is expected to be retarded by inhibitors of putrescine biosynthesis. As indicated in Figs. 3 and 4, D-arginine and α-methylornithine, which decreased the level of putrescine, indeed retarded the senescence of detached corn leaves in darkness (Figs. 3 and 4). It should be noted that levels of endogenous spermidine and diaminopropane were not affected by either inhibitor (data not shown).

The results reported here seem to indicate that an increase in putrescine levels is likely to be the factor responsible for the senescence of detached corn leaves in darkness. This conclusion is further supported by the observations that benzyladenine, a synthetic cytokinin, which retarded dark-induced senescence of detached corn leaves, also decreased the level of putrescine (Figs. 3 and 4).

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內生多元胺含量與暗中誘導玉米切離葉片老化關係之研究

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本研究主要是探討內生多元胺是否調控暗中誘導玉米 (台農一號) 切離葉片之老化。在老化過程中，玉米葉片含有 putrescine、spermidine 與 diaminopropane，但不含有 spermine。putrescine 是玉米葉片中主要的多元胺，其含量隨著老化的進行而增加，同時 putrescine 含量的增加早於葉綠素含量與蛋白質含量的降低。spermine 與 diaminopropane 含量僅在已老化的玉米切離葉片中，表現少許的增加。putrescine 合成的抑制劑 D-arginine 與 α-methylornithine 處理玉米切離葉片，可降低 putrescine 含量，同時延緩葉片的老化。benzyladenine 可延緩玉米切離葉片之老化，且顯著的降低 putrescine 含量。由結果顯示，內生 putrescine 含量的增加可能與黑暗誘導玉米切離葉片老化有關。

關鍵詞：Diaminopropane；葉片老化；Putrescine；Spermidine；Spermine；玉米。