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Inhibition of Maize Primary Root Elongation by Spermidine: Effect on Cell Shape and Mitotic Index

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Abstract. Spermidine applied for 18 h to intact maize seedlings through their roots reduces root growth 70%, and the effect is reversible. Histological observations of longitudinal sections of 0.4-cm root apical segments from 2-day-old maize seedlings grown for 18 h in 0.5 mM $CaSO_4$ solution with or without 1 mM spermidine contribute to the explanation of spermidine-dependent slow root growth. In the meristematic zone a strong reduction of the mitotic index and in the elongation zone an inhibition of cell elongation occur simultaneously. Cell shape analysis along the growth axis of the maize root apex expressed in terms of form factor (FCircle) values substantiates the dual effect of spermidine on mitotic activity and cell elongation.

Polyamines (PA) are organic polycations distributed widely in all living cells. Their involvement in various cell functions such as cell division, growth and differentiation, fruit ripening, senescence inhibition, and embryo formation has been recognized, but their basic mechanism of action has not yet been established (Smith 1985, Altmann 1989). Although high levels of PA are generally related to cell-division and other plant growth and development processes, recently some inhibitory effects of exogenously applied PA have been described. Among them are the reversible inhibition of maize root elongation after PA pretreatment (Gatta et al. 1992, de Agazio et al. 1992), the growth enhancement of soybean roots as a consequence of PA biosynthesis inhibition (Garmanik and Frydman 1991), and the inhibition in isolated embryonic axes of chickpea seeds of the activity of RNA polymerase after treatment with spermine (Spm) (Bueno et al. 1993). Most of these effects have been observed in tissues rich in amine oxidases, suggesting a possible involvement of the oxidation products of PA (Gatta et al. 1992; de Agazio et al. 1992).

In a previous paper we reported that spermidine (Spd) pretreatment induced 50% inhibition of root extension in intact maize seedlings after 24 h. This phenomenon was accompanied by an early differentiation of xylem tissues and a strong autofluorescence of vascular parenchyma, xylem, and rhizodermis (de Agazio et al. 1992). These findings suggest a reduction of cell expansion by Spd without excluding a possible inhibiting effect on cell division. The aim of this work was to study the morphological and cytological events induced in maize root apical segments by exogenous Spd in order to understand the mechanism of root growth inhibition in maize.

Materials and Methods

Plant Material

Maize seeds (*Zea mays* L., hybrid line Plenus V516), supplied by the Dekalb Centre (Chiarano, Italy), were rinsed continuously with tap water for 8 h and then germinated in the dark at 27 °C in a controlled growth chamber over three layers of filter paper moistered with 0.5 mM $CaSO_4$ solution. Two-day-old seedlings were selected on the basis of their root length (2.5 cm) and transferred to a fresh growth solution in the presence or absence of 1 mM Spd for 18 h, unless otherwise stated. The growth rate of intact primary root was followed for up to 114 h.

Root Segment Elongation

Eight root segments (0.8 cm long) deprived of 2 mm of apex (subapical segments) cut from controls and Spd-pretreated seedlings were put in line, and 8 h after transferring them to fresh

Abbreviations: PA, polyamine(s); Spm, spermine; Spd, spermidine.

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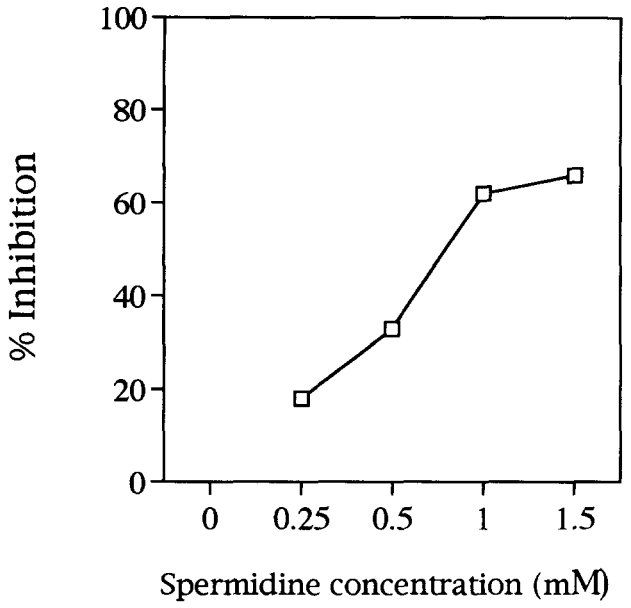

Fig. 1. Inhibition of root growth at different concentrations of exogenous spermidine. Two-day-old maize seedlings were treated with or without spermidine for 18 h.

growth solution, their total length was measured, as reported by Sacchi and Cocucci (1992).

Histological Analysis

Four-mm-long maize root apices were fixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 5% sucrose, pH 7.2, then rinsed for 15 min in the same buffer, postfixed for 1 h in 1% OsO_4 in 0.1 M cacodylate buffer with 5% sucrose, pH 7.2, and finally Epon embedded. Semithin sections 2 mm thick were cut with an LKB Ultratome III and stained with alkaline methylene blue and toluidine blue. The sections were observed under a Zeiss light microscope and analyzed using a Kontron electron Zeiss Vidas 25 image analyzer program. Mitotic figures were counted with respect to nucleolated cells visible in the sections.

Form Factor

Cortical cell shape was analyzed by circularity shape factor calculated at 0.25, 1.50, 2.75, and 4.0 mm from the apex according to the user's manual of Vidas III equation

$$\text{FCircle} = \frac{4 \times \pi \times \text{area}}{\text{perim.}^2}.$$

The values of this FCircle range from close to 0 for elongated or irregular objects to 1 for circular objects.

Data reported refer to a single typical experiment with three replicates per treatment. At least three

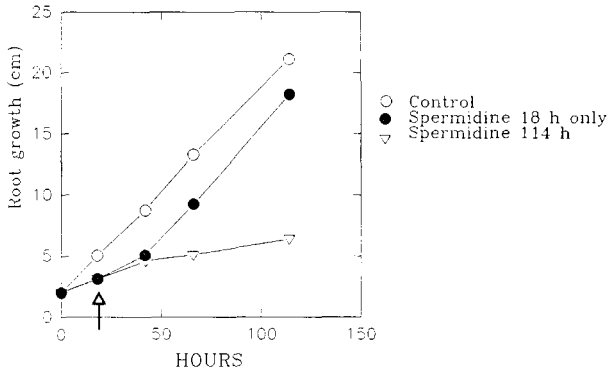

Fig. 2. Effect of exogenous spermidine on root growth. Two-day-maize seedlings were treated with or without 1 mM spermidine for 18 or 114 h. The *arrow* indicates the time of spermidine removal.

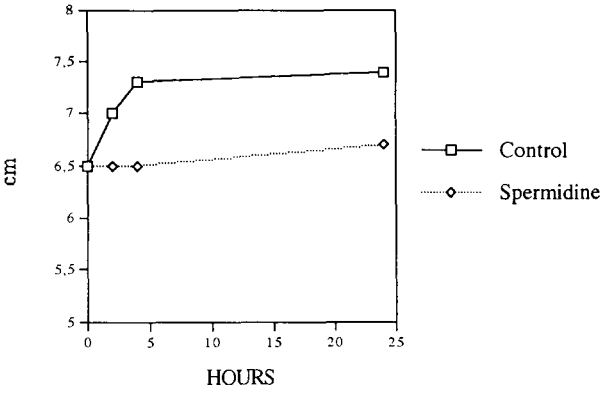

Fig. 3. Effect of spermidine pretreatment on root segment elongation. Two-day-old maize seedlings were treated with or without spermidine for 18 h. Eight subapical segments (0.8 cm long) of control and spermidine-treated roots were cut and put in line in Petri dishes. Cell elongation was measured by the difference between the total length of the segments immediately after and 8 h after transfer.

series of independent experiments gave similar results.

Results and Discussion

The inhibitory effect of Spd on the root growth rate of 2-day-old maize seedlings is dose dependent as reported in Figure 1. An inhibition close to the maximum was observed at 1 mM Spd. Therefore, this concentration of Spd was used in the experiments. No growth inhibition was observed when roots were grown in the presence of diamines, putrescine, or diaminopropane (data not shown). The inhibition caused by Spd was fully reversible as shown in Figure 2. The growth rate was inhibited 70% after 18 h

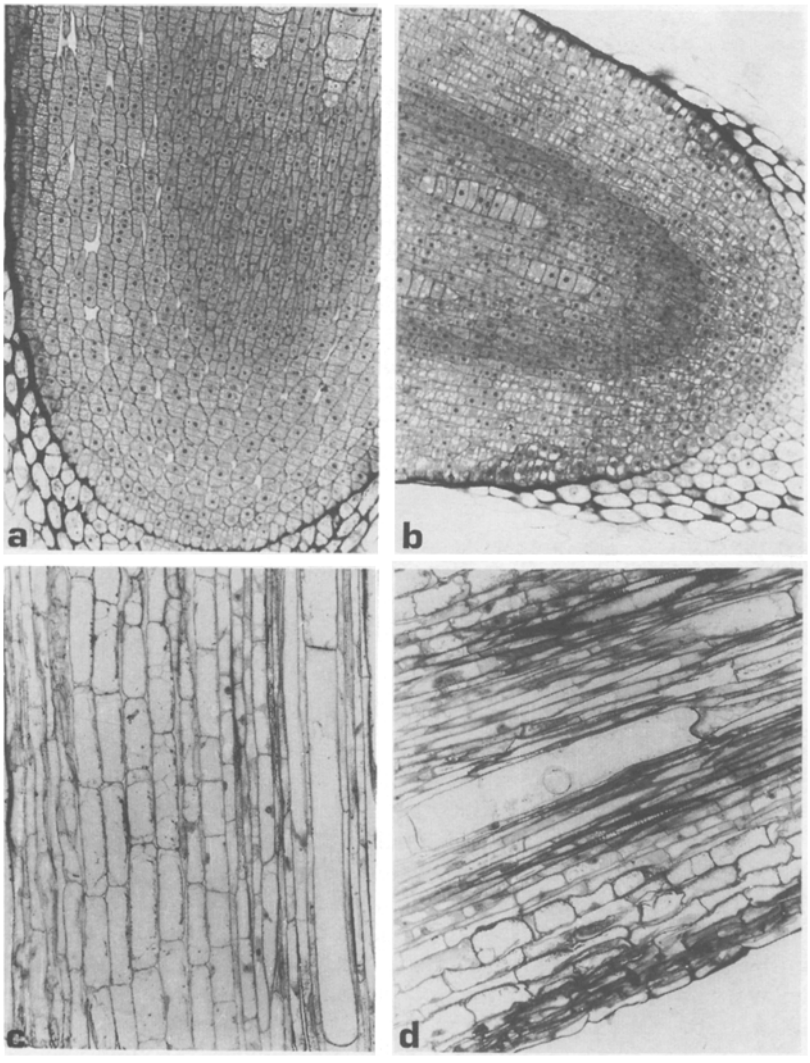

Fig. 4. Light micrograph of longitudinal sections from control (**a** and **c**) and Spd-pretreated (**b** and **d**) root tips taken at a distance of 0–0.5 mm (**a** and **b**) and 3.5–4.0 mm (**c** and **d**) from the apex. Magnification, ×220.

but was completely restored after 48–60 h when Spd treatment was removed from the external medium. On the contrary, a daily supply of Spd induced a continuous growth inhibition. Root subapical segments from 2-day-old maize seedlings treated with 1 mM Spd for 18 h and transferred in Petri dishes showed a complete inhibition of cell expansion (Figure 3). This result suggests that cell elongation is inhibited in the subapical segment derived from Spd-treated roots, probably because of increasing lignification and rapid differentiation (de Agazio et al. 1992).

Apical root structures were studied to obtain more information on the mechanisms that induced slow root growth when Spd was added to the growth medium. The mitotic index measured in longitudinal sections of apical segments appears strongly inhibited by Spd pretreatment. In particular, in control roots the mitotic index was 19% between 0.5 and 1.0 mm and 10% between 0 and 0.5 mm. In Spd-pretreated roots mitosis was visible only between 0 and 0.5 mm (mitotic index from 2 to 5%), whereas at a distance from the apex greater than 0.5 mm mitosis was completely absent. Figure 4 shows longitudinal sections at 0–0.5 mm and 3.5–4.0 mm from the apex of control and 18 h Spd-pretreated roots. In the 0–0.5 region of control roots (Fig. 4**a**), cells with a thin horizontal cell wall between them, derived from the phragmoplast after the last mitotic division, were visible. In Spd-treated roots (Fig. 4**b**) the shortest cells were absent, and walls were not visible, confirming a strong reduction in mitotic division. The analysis of cell length distribution carried out in control roots shows that at a distance of 0–0.5 mm from the apex, more than 70% of cells had a length between 4 and 4.5 mm; in Spd-treated roots almost all of the cells had a length greater than 5 mm (Fig. 5). The situa-

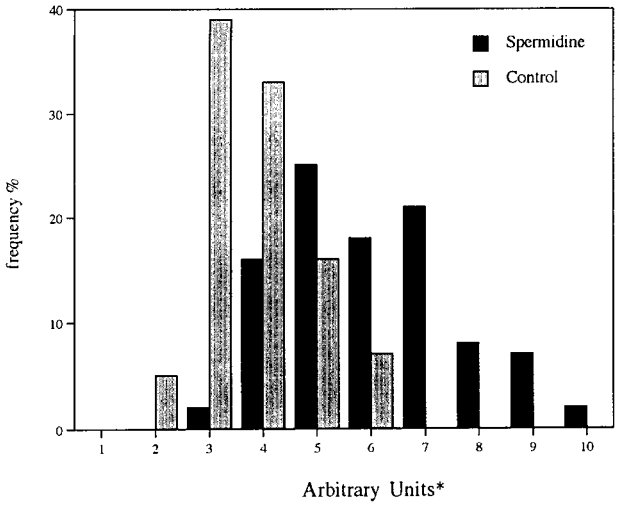

Fig. 5. Distribution of cell length at a distance between 0 and 0.5 mm from the apex in longitudinal sections of control and 18-h 1 mM spermidine-treated roots. One arbitrary unit corresponds to 1.35 mm.

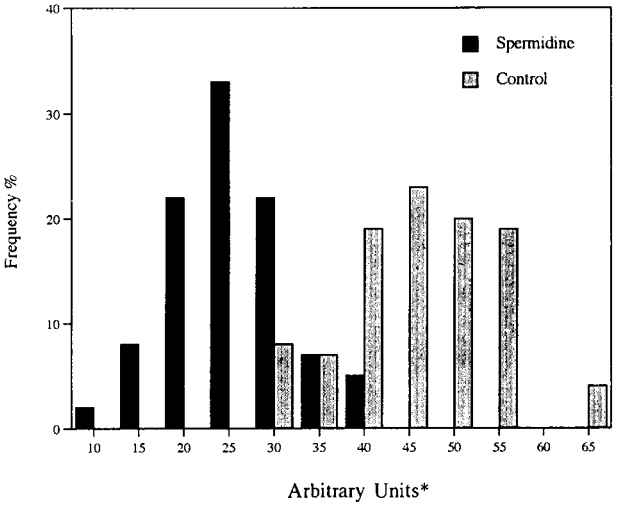

Fig. 6. Distribution of cell length at a distance between 3.5 and 4.5 mm from the apex in longitudinal sections of control and 18-h 1 mM spermidine-treated roots. One arbitrary unit corresponds to 1.35 mm.

tion was reversed in sections taken at a distance between 3.5 and 4 mm from the apex; in control roots, 75% of cells had a length between 45 and 75 mm, whereas in Spd-treated roots 90% of the cell length was less than 40 mm (Fig. 6).

Figure 4 shows other morphological differences between the controls and Spd-pretreated roots. Control maize root cells appear undifferentiated in the 0- to 0.5-mm region; their cytoplasm homogeneously fills the cell volume, and vacuoles were absent or small and infrequent (Fig. 4**a**). In Spd-pretreated roots, cells showed wide vacuoles, and walls appeared thicker than those of controls (Fig. 4**b**). In addition, the free spaces between cell columns (aerenchyma) in the cortical region were evident in control roots, but they were completely absent in Spd-pretreated roots. This is in agreement with the effects observed after treatment with putrescine (inhibition of aerenchima formation) and PA biosynthesis inhibitors (enhancement of aerenchima formation) described by Jackson and Hall (1993) in maize roots. Also, in the 3.5- to 4-mm region from the apex, Spd-treated roots (Fig. 4**d**) exhibited cell walls thicker than control roots, and lignified tracheas, which were not found in control roots (Fig. 4**c**).

Longitudinal sections in the 0- to 4-mm region showed distinctive changes in cell shape caused by Spd treatment. These changes can be expressed in terms of form factor (FCircle) values (Baluska et al. 1990). The FCircle can range from near 0 for a very elongated object to 1 for a circular object. In Figure 7 a comparison was made between the FCircle of cortical cells of control and Spd-pretreated roots. In control root apices, cell division decreased FCircle values at distances up to 1.50 mm from the apex. At distances greater than 1.50 mm, cell elongation increased FCircle values up to 2.75 mm, indicating an isodiametric shape of cells. The decrease of the FCircle value found at a 4.0-mm distance from the apex was due to a further increase of the longitudinal side. In Spd-pretreated roots, at a distance of 1.50 mm from the apex, cells have a near circular shape due to the absence of mitosis. Cell elongation decreased FCircle values at distances up to 2.75 mm from the apex; at distances greater than 2.75 mm, the cell increase was small in the transversal side and absent in the longitudinal side, causing an increase of FCircle value. A diagrammatic representation of cell shapes along the growth axis of the maize root apex in control and Spd-pretreated roots is shown in Figure 8.

In conclusion, the inhibition of root growth observed during Spd treatment of maize seedlings is a complex phenomenon due to reduction of both the mitotic index and cell elongation, accompanied by stiffening and lignification of the cell wall (de Agazio et al. 1992). Although much evidence supports the involvement of PA in various growth and developmental processes in higher plants (Smith 1985), it is not completely clear whether PA per se, compounds made from PA, or their catabolites are responsible for some of the observed effects. In several organisms, degradation products of Spd, like

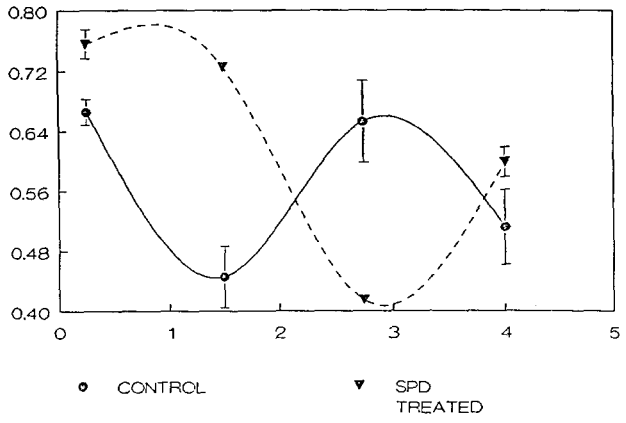

Fig. 7. Form factor (FCircle) of cortical cells at a distance of 0.25, 1.50, 2.75, and 4.0 mm from apex in longitudinal sections of control and 18-h spermidine-treated root. FCircle was calculated as reported in the Materials and Methods section.

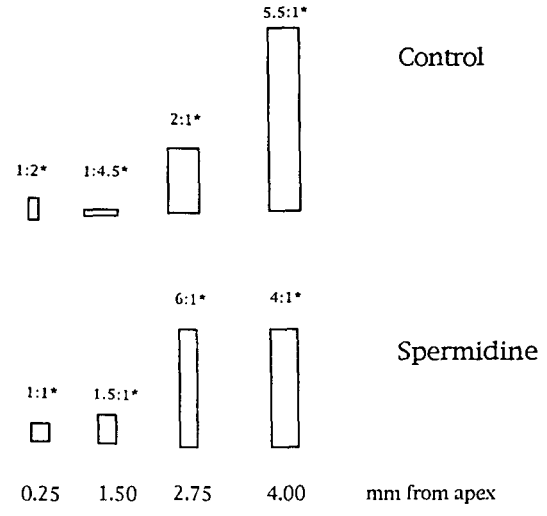

Fig. 8. Cell shape along the growth axis at a distance of 0.25, 1.50, 2.75, and 4.0 mm from the apex in longitudinal sections of control and 18-h spermidine-treated roots. *Asterisks* indicate Vertical/transversal cell axes.

H_2O_2 and aminoaldehydes, are known to elicit biological effects somehow similar to those reported in this paper (Bacharach et al. 1965, Gaugas and Deweg 1978, Cona et al. 1991, Showalter 1993). Therefore, the possibility exists that the observed effects of Spd in the inhibition of maize root growth are mediated by its degradation products.

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