

Short Communication

Influence of Cobalt on Soybean Hypocotyl Growth and Its Ethylene Evolution

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ABSTRACT

Development of dark-grown "Clark" soybean (Glycine max [L.] Merr.) seedlings is abnormal at 25 C but normal at 20 and 30 C. At 25 C, hypocotyls swell and fail to elongate normally; lateral root formation and seedling ethylene evolution are enhanced.

Co²⁺ promoted hypocotyl elongation of etiolated "Clark" soybean seedlings by 28% when grown at 25 C. The same growth-promoting concentration reduced hypocotyl thickness and primary root elongation by 28 and 43%, respectively. Co²⁺ inhibited ethylene production both of intact seedlings and of apical 1-cm hypocotyl segments with attached epicotyls and cotyledons by 65 and 69%, respectively. These results suggest that Co²⁺ exerts its effects on the hypocotyl growth by inhibiting ethylene production, and also confirm our previous conclusion that abnormal ethylene production at 25 C is responsible for the inhibition of hypocotyl elongation and for its swelling.

Hypocotyl elongation of certain soybean cultivars, e.g. "Clark," is inhibited by about 50% when grown at 25 C for 9 days in darkness. At 20 and 30 C the hypocotyl grows normally as do other cultivars, e.g. "Mandarin," at all three temperatures (1, 2, 8, 9). We (7, 8) have presented evidence that the high rate of ethylene production by "Clark" seedlings at 25 C causes the inhibition. It has been found (3, 6) that Co²⁺ salts enhance elongation of cucumber hypocotyl segments and prevent their IAA-induced lateral swelling by inhibiting the IAA-induced ethylene production. Basal ethylene production of pea epicotyl segments has also shown to be inhibited by Co²⁺ (5). I investigated whether Co²⁺ would have similar morphological and physiological effects on the hypocotyl of intact "Clark" seedlings. This report presents a case of Co²⁺ manipulating the hypocotyl growth of an intact plant by inhibiting its temperature-induced ethylene production.

MATERIALS AND METHODS

Seeds of (Glycine max [L.] Merr.) cv. "Clark" (U.S. Regional Soybean Lab., Urbana, Ill.) were surface-sterilized in 10% (v/v) Whitex for 10 min, rinsed with distilled H₂O, and soaked in various concentrations of aqueous Co(NO₃)₂ for about 1 hr. Seeds were aligned between two sheets of germinating paper (6 × 35 cm) with the embryos upward. Sheets were rolled into a cylinder and were stood in 60 ml of Co²⁺ solution in a 700-ml glass jar. Plants were grown at 25 C in darkness. Three days after planting ungerminated seeds, seedlings with broken cotyledons, seed coats, and inverted seedlings were discarded. All the manipulations were conducted under a dim green light, consisting of a 25-w incandescent lamp filtered through two layers of green Cinemoid and one layer of blue cellophane.

In measuring endogenous ethylene production, five 3-day-old seedlings wrapped in germinating paper were transferred to a 100-ml graduated cylinder containing 10 ml of either distilled H₂O or 0.5 mM Co²⁺ solution. Each treatment consisted of four graduated cylinders. At time intervals indicated, each cylinder was sealed for 4 hr and then 1-ml samples were withdrawn with a Hamilton gas-tight syringe for analysis. Apical 1-cm hypocotyl segments with attached cotyledons and epicotyl were prepared from the 6-day-old seedlings as previously described (7). Ten such segments were transferred to a 39-ml flask 5 to 40 min after cutting and sealed. After closure for 3 hr, 1-ml samples were withdrawn for ethylene measurement. Each flask contained 0.5 ml of either distilled H₂O or Co²⁺ solution (0.5 mM). Each treatment consisted of three flasks. Ethylene was measured in a Varian 2400 gas chromatograph equipped with a hydrogen flame ionization detector and a stainless steel column (213 x 0.32 cm) packed with activated alumina and operated at 50 C.

RESULTS

Effects on Hypocotyl Growth. Co²⁺ promoted hypocotyl elongation of intact seedlings over a concentration range of 0.1 to 1 mM (Fig. 1), with a maximum at 0.5 mM (Fig. 2). Concentration of 0.5 mM also prevented hypocotyl swelling by decreasing its fresh weight per unit length from 45.1 to 32.6 mg/cm. A concentration of 10 mM inhibited elongation as compared with water controls.

Effects on Root Growth. Visual observation of roots of seedlings grown in 0.5 mM Co²⁺ solution showed a general inhibition of root growth (Fig. 2). After 9 days of growth the average primary root lengths of seedlings grown in water and in Co²⁺ solution were 15.1 and 8.6 cm, respectively.

Effect on Ethylene Production. Co²⁺ at a concentration of 0.5 mM inhibited ethylene production of the intact seedlings (Fig. 3). The inhibition was first evident at 4 days. The same concentration of Co²⁺ decreased the ethylene production by excised parts consisting of apical 1-cm hypocotyl segments with attached cotyledons and epicotyl from 1.50 in water controls to 0.60 nl/hr·g fresh weight.

DISCUSSION

Evidence has been presented (7, 8) that inhibition of elongation and swelling of "Clark" soybean hypocotyl are caused by a high rate of ethylene production when seedlings are grown at 25 C. The present results show that Co²⁺ promotes elongation and decreases thickness of hypocotyl of these seedlings. Similar responses to
Co²⁺ have been reported for excised pea epicotyl and cucumber hypocotyl (3, 6, 10, 11), but not for intact seedlings. Inasmuch as Co²⁺ inhibits both ethylene production of the intact seedlings and of the apical 1-cm hypocotyl segments with cotyledons and epicotyl attached, I concluded that Co²⁺ exerts its effects on the soybean hypocotyl growth, at least in part, by inhibiting ethylene formation. This mode of action for Co²⁺ has also been suggested by other investigators (3–5).

LITERATURE CITED
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