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RESPONSE OF WHITE HALF-RUNNER BEAN HYPOCOTYL TO CERTAIN AMINO ACIDS AND TO ALFALFA CHLOROPLASTS

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ABSTRACT—Excised white half-runner bean hypocotyls were used to determine the cytokinin effects of certain β-hydroxyamino acids and of alfalfa. The D-isomer of β-hydroxyvaline was equal to the L-isomer in stimulating hypocotyl growth. This shows that the effect is not due to the aldolase enzyme, which is widely distributed in nature, because aldolase is specific for L-isomers. The stimulation by both isomers could be due to similarity of structure to the cytokinin, zeatin, or it could be due to metabolism of β-hydroxyvaline to glycine and acetone. Alfalfa chloroplasts stimulated elongation of etiolated white half-runner hypocotyls.

The D- and L-isomers of β-hydroxyvaline and β-methoxyvaline, amino acids which cannot be made by aldocondensation, were first completely resolved by Edwards (1963, 1964) and Edwards and Minthorn (1968). These isomers are not available commercially. Field and garden studies conducted over the past thirty years with Tween 20 liposomal chloroplast extracts from alfalfa meal have yielded interesting results (Edwards, unpublished). Ries et al. (1977) reported that alfalfa mulch greatly increased the yield of tomatoes. Ries et al. (1977) isolated triacontanol from alfalfa and studied triacontanol as a plant growth factor. Bhalla (1981) found triacontanol to be unreliable as a growth factor. This work seems to indicate that as yet unidentified growth factors may be present in alfalfa. In this study, white half-runner bean hypocotyl was used to demonstrate the growth effects of alfalfa chloroplasts and of certain amino acids.

Auxins and cytokinins are growth hormones. Zeatin, the important cytokinin for plants, is an isopentenyl adenine. The opines are special amino acids synthesized by plant cells when a copy of transfer DNA is transferred and integrated into random sites of a plant's chromosomes (Lehninger et al., 1993). In this study, isopentyl and other amino acids were used to demonstrate the relationship between amino acid structure and growth of white half-runner bean hypocotyl.

MATERIALS AND METHODS

Commercial alfalfa meal, sold by Archer Daniels Midland Company, Decatur, Illinois containing 17% protein, was used to extract chloroplasts. Ten grams of alfalfa meal with chloroplasts were transferred to a pre-chilled mortar containing 10 ml of sucrose-Tris-HCl solution (0.25 M sucrose in 0.01 M Tris), pH 7.9, at 1°C and ground until there was no further change in appearance, according to the method of Robyt and White (1987). The chloroplasts settle to the bottom of the mortar leaving the debris suspended. The mortars were emptied into 15 ml centrifuge tubes that were centrifuged at 1000 g for 60 sec. The supernatant was discarded. If needed, 2 ml of the sucrose-TRIS solution was added and the tubes again vortexed. The chloroplasts pellets were vortexed for 3–5 sec. in Tween 20 (0.01%).

Commercial white half-runner beans were grown in sterile potting soil in the absence of light until the sprouts were tall enough to harvest 3.0 cm of hypocotyl according to the methods of Schlagnhaufer et al. (1984). The hypocotyl segments were measured with a vernier caliper, and placed on strips of sterile gauze (2.5 × 6 cm) soaked with deionized water in sterile petri dishes. The control gauze strips had only deionized water. To compare growth-stimulating effects, three drops of different substrates were added to the strips. The substrates tested included: enantiomers of β-hydroxyvaline (4 mg/ml), β-methoxyvaline (4 mg/ml), chloroplasts extracted in Tween 20 (0.01%), chloroplasts/Tween 20 plus the auxin indole acetic acid (0.05 M), and chloroplasts/Tween 20 plus the plant growth regulator kinetin (10 drops; 0.001 M). The excised hypocotyls were allowed to grow in darkness for 30 h at 25°C, and again measured with a vernier caliper. The hypocotyl lengths after 30 h are summarized in Table 1.

In the second experiment growth comparisons were made with β-hydroxyvaline and β-methoxyvaline, chosen because of previous work with these hydroxyamino acids; serine, chosen because of its unique one-carbon metabolism in which serine is converted to glycine and a one-carbon unit used to synthesize amino acids, purines and pyrimidines; and zeatin, chosen because it is an important plant growth hormone. Hypocotyls were allowed to grow in darkness for 30 h at 25°C and, then, measured with a vernier caliper.

Etiolated white half-runner bean hypocotyls (3.0 cm), measured with a vernier caliper, were excised from seedlings that were just slightly more than 3.0 cm. The hypocotyl segments were placed on 2.5 × 6 cm strips of sterile gauze, soaked in deionized water, in sterile petri dishes. The controls had gauze strips with deionized water. The other petri dishes had three drops of substrate added. The hypocotyl lengths after 30 h, and the substrate concentrations are summarized in Table 2.

RESULTS AND DISCUSSION

Data collected in this study are summarized in Tables 1 and 2. Analysis of variance was used in compiling the statistics. Chloroplasts in Tween 20 produced significantly more growth in

TABLE 1. Effect of amino acids, isolated chloroplasts, and growth factors on hypocotyl growth.

Substrates	Hypocotyl length (cm) after 30 h[1]
Control	3.08 ± 0.020[c]*
D-β-Hydroxyvaline (4 mg/ml)	3.36 ± 0.040[b]
L-β-Methoxyvaline (4 mg/ml)	3.16 ± 0.024[c]
L-β-Hydroxyvaline (4 mg/ml)	3.32 ± 0.020[b]
Chloroplasts/Tween 20	4.16 ± 0.040[a]
Indoleacetic acid (0.05M + chloroplasts/Tween 20	3.12 ± 0.020[c]
Kinetin (10 drops of 0.001 M) + chloroplasts/Tween 20	2.76 ± 0.051[d]

[1] Values are mean ± *SEM*.

* Means followed by the same letter are not significantly different ($P > 0.05$).

white half-runner bean hypocotyl than the other substrates (Table 1). It is interesting to note that the kinetin/chloroplasts/Tween mixture was inhibitory to hypocotyl growth. It could be inferred that kinetin, a plant growth regulator, reverses the chloroplast enhancement of white half-runner bean hypocotyl elongation. D-β-Hydroxyvaline was second in producing hypocotyl elongation; indoleacetic acid/chloroplasts/Tween showed the same growth as the controls.

The results of the second experiment are summarized in Table 2. D-β-methoxyvaline produced the greatest growth; L-serine was next while DL-serine, L-β-hydroxyvaline, and D-β-hydroxyvaline were not significantly different when the substrate concentration was 2 mg/ml. Isopentyl amino acids appear to stimulate elongation in white half-runner bean hypocotyl. The results summarized in Tables 1 and 2 indicate that with white half-runner bean hypocotyl, the substrates do not appear to be stereospecific for D- and L-enantiomers, which indicates the growth stimulation is not due to the aldolase enzyme.

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TABLE 2. Effect of β-hydroxy and β-methoxyvaline, serine and zeatin on white half-runner bean hypocotyl growth.

Substrates	Hypocotyl length (cm) after 30 h[1]
L-β-Hydroxyvaline (2 mg/ml)	3.30 ± 0.045[c]*
DL-Serine (2 mg/ml)	3.30 ± 0.052[c]
L-Serine (2 mg/ml)	3.48 ± 0.017[b]
L-β-Methoxyvaline (2 mg/ml)	3.97 ± 0.033[a]
Zeatin (2 drops of 2.5 mg/ml)	3.18 ± 0.017[de]
L-β-Hydroxyvaline (2 mg/ml)	3.22 ± 0.031[cd]
Control	3.10 ± 0.000[e]

[1] Values are mean ± *SEM*.

* Means followed by the same letter are not significantly different ($P > 0.05$).

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