Hormones and the Motor Response of Root Gravitropism

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Gravitropism is a growth movement which results from the response of roots and shoots to gravity. The root cap/tip (the terminal 1.0 to 1.5 mm of the root) is the site of perception of the gravitational force. Gravicurvature occurs in the elongation zone of roots, which is 2 to 6 mm behind the root cap/tip.

According to the Cholodny-Went hypothesis (Digby and Firn, 1980; Went and Thimann, 1937), the gravitropic response of roots is controlled by the lateral movement of a growth inhibitor across a root when the root is placed in a horizontal position in a gravitational field. Auxin is redistributed by lateral transport toward the lower side of the horizontally-oriented root. The accumulation of auxin in the lower portion of the root results in a supraoptimal auxin concentration. Since auxin is inhibitory to root growth, the supraoptimal concentration of auxin inhibits root growth in the elongation zone on the lower side of the root. The upper portion of the root contains optimal levels of auxin. The optimal levels of auxin stimulate growth of the upper portion of the root. This differential rate of elongation between upper and lower halves of the root results in curvature. Although this hypothesis is widely accepted, an alternative hypothesis has been proposed.

The alternative hypothesis for root gravitropism is called the Root Cap Inhibitor Model. In this model, abscisic acid (AbA) is substituted for IAA as the growth inhibitor. Pilet and Rivier (1981) found that AbA is present in root caps. They proposed that AbA accumulates in the lower hemisphere of the elongation zone of horizontally-oriented roots. Additionally, Pilet and Chanson (1981) observed that exogenously-applied AbA can inhibit root elongation in maize.

However, several other researchers have demonstrated that AbA promotes root elongation within the time period required for expression of gravicurvature. Mulkey *et al.* (1983) found that the initial effect of AbA is stimulation of root growth over a wide range of concentrations of AbA (Figure 1).

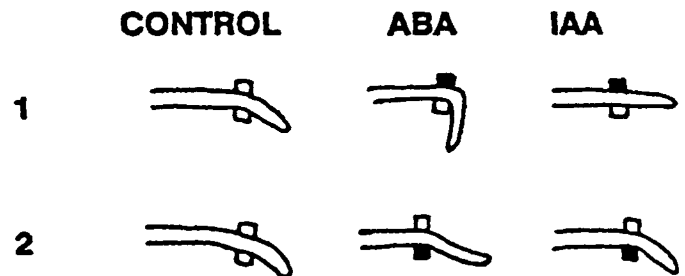

Figure 1. Comparative effects of unilateral application of AbA and IAA on gravitropism in roots. 1) Hormone applied to the top of the elongation zone (closed square = hormone; open square = agar only). 2) Hormone applied to the bottom of the elongation zone (closed square = hormone; open square = agar only). (After Mulkey *et al.*, 1983)

The inhibition of root growth is observed only in high concentrations of AbA (0.1 to 1 μM) and with prolonged exposure to AbA (more than 12 hours). Gravicurvature is complete within 2 hr. These data discount the involvement of AbA in the motor response of gravitropism of roots.

Furthermore, the effects of auxin on root elongation are consistent with its suggested role as a growth inhibitor in gravitropism. IAA strongly inhibits root growth at concentrations higher than 0.1 μM (Mulkey, *et al.*, 1982; Thimann, 1937). The evidence for AbA as an inhibitor of root growth is less consistent (Jackson and Barlow, 1981). AbA has been reported to inhibit root elongation (Pilet and Chanson, 1981), to have no effect on root elongation (Gaither, *et al.*, 1975) or to promote root elongation (Abou-Mandour and Hartung, 1980; Gaither, *et al.*, 1975; Yamaguchi and Street, 1977).

In this laboratory exercise, a simple agar block method is used to examine the effect of plant hormones on the elongation zone of a root during

gravitropism. This method is simple, but has many applications to verify the role of plant hormones in gravitropic curvature.

GOALS OF THE EXPERIMENT

1. Comparison of the effect of unilateral application of IAA and AbA on asymmetric growth of roots.
2. Examine the involvement of plant hormones (IAA and AbA) in the gravitropic response of roots.

TIME REQUIREMENT

0.25 hour (approximately) 3 days prior to experiment to soak grain
1.00 hour (approximately) 1.5-2 days prior to experiment to plant grain
1.00 hour prior to experiment to prepare agar block
2.00 hours experiment running time

MATERIALS AND EQUIPMENT

Abscisic acid (AbA)
Agar
Chamber for humidified box
Dark room
Disposable plastic petri dishes, 100x15 mm
Forceps
Grain (corn)
Hot plate
Indole 3-acetic acid (IAA)
Paper towel
Photographic paper (black and white)
Plastic trays and tub
Plexiglas
Razor blade
Screw, two machine screws (1.5" x 8/24 or 8/32)
Small block of wood
Thread
Time lapse video cassette recorder and camera
Window putty

METHOD

Seedling Preparation. Corn grains are soaked overnight in running tap water to prevent anaerobiosis. The grain germinates between wet paper towels on plastic trays in a vertical position. To obtain straight primary roots you should place the corn grains in rows on a tray covered with 2-3 layers of paper towel. Cover the grains with 3 or 4 layers of paper towels; place another tray over final layer of towels to hold the paper towels and grain in place. Position the trays vertically in a shallow tub containing 1-2 inches of water. Primary roots of approximately 1.5-2.0 cm should be used for the experiment. This should require 2-3 days of growth, depending upon the cultivar and temperature.

Incorporation of Indole-3-acetic acid and Abscisic acid. For agar plates containing IAA or AbA and plain, prepare 100 ml of 1% of non-nutrient agar solution. The solution is boiled to dissolve the agar and poured in 100 x 15 mm plastic petri dishes (10 ml of solution per plates). Plates are prepared to contain 0.01 mM IAA, 0.1 mM AbA, and no hormone. The poured plates are placed on a level surface to cool.

Preparation of Agar Block. Prepare a marking block as illustrated in Figure 2. The marking block is constructed of two machine screws which are glued to a small block of wood. The machine screws act as guides and spacers for thread, which is wrapped around the block/screws. Using this wood block, press the surface of agar plates horizontally, then vertically to make a grid of small squares on the surface of the agar plates. Using the razor blade, carefully cut the surface of agar plates along the scars to produce uniform agar blocks.

Application of Agar Block. Carefully pick up a agar block with forceps and place it on the elongation zone of root which is 4-6 mm from the root tip. The agar blocks, which may or may not contain hormones, are placed on the top or bottom surface of root depending on the experiment.

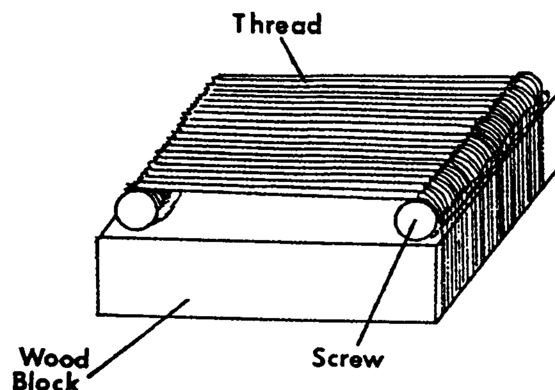

Figure 2. Design of wood block for making the scar on the surface of agar plates to prepare the agar blocks.

Preparation of Seedling Holder. A holder for the seedlings is made with pieces of Plexiglas as shown in Figure 3. The size of the bottom and top portion of holder is 50 x 70 mm, and the standitself is 30 x 150 mm. This holder will allow placement of 10 seedlings along each side. To maintain humidity around the holders, individual humidity chambers can be prepared by removing the mouth-end from 500 ml tissue culture flasks. A square of paper towel is moistened and placed against one of the inside walls of the flask. Inexpensive humidity chambers can be made by cutting the top from 2 liter plastic beverage bottles. The lower half of the bottles can be lined with moist paper towels and inverted over the seedling holder. For large classes, a small aquarium (2-5 gallon) can be lined with moist towels; a square of window glass can be used as a lid. The key to the success of this experiment is to maintain a very high humidity level within the chamber.

EXPERIMENTAL PROCEDURE

1. Prepare the humidified chamber with paper towels and distilled water.
2. Select 50 seedlings with primary roots of 1.5-2.0 cm in length.
3. Place the seedlings in a horizontal orientation on the Plexiglas holder with window putty (Figure 3). Ten seedlings should be placed along each side of the holder. In this experiment, 3 holders should be used.
4. Place the holders into humidified chambers after applying agar block to the elongation zone of roots as follows:

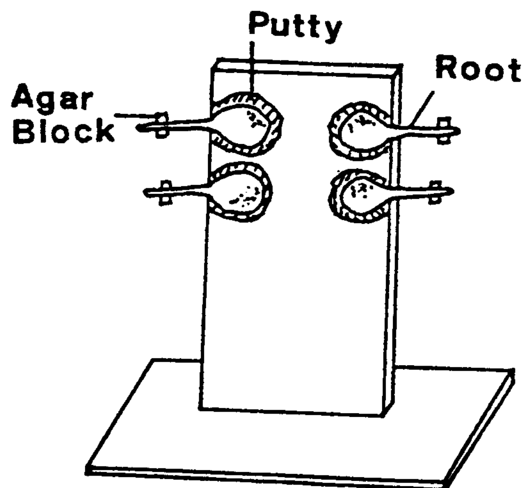

Figure 3. Diagram of seedling holder to observe the curvature of roots.

   a) plain agar blocks on both side (control).
   b) **top:** 0.1 mM AbA;
      bottom: plain agar block
   c) **top:** plain agar block;
      bottom: 0.1 mM AbA
   d) **top:** 0.01 mM IAA;
      bottom: plain agar block
   e) **top:** plain agar block;
      bottom: 0.01 mM IAA
5. Incubate the roots in the humidified chamber for 1 hour. Observe the curvature periodically.
6. If a time lapse video cassette recorder is available for use in observing the curvature, the roots may be allowed to respond for several hours or overnight.
7. If a time lapse video cassette recorder is not available, take the holder to the dark room after 1 hour. Place the photographic paper behind the holder, then illuminate the light for a very short time (approx. 1 sec) to get the shadow of roots. Develop the photographic paper. A shadowgraph will be produced with a black background and white root shapes. Measure the degree of curvature with a protractor.

OBSERVATION AND QUESTIONS

Measure the curvature of roots which are applied the agar blocks containing IAA or AbA. Compare the effect of IAA and AbA on the gravitropic curvature.

Is there any difference in the presentation time and rate of curvature between the treatments with the agar blocks containing hormones on top and bottom portion of roots?

What is the major difference between control and hormone treated roots?

SUGGESTIONS FOR ADDITIONAL EXPERIMENTS

1. Measure and compare the root elongation rate in the presence of 0.01 mM IAA or 0.1 mM AbA during short (up to 12 hours) and long time periods (up to 3 days).
2. Apply other plant hormones such as gibberellic acid, kinetin, or ethylene (in the form of Ethephon) to the roots.
3. Apply ethylene agonists, such as silver ions, AVG (1 μM) orcobalt ions to the gravi-responding roots. The interaction of auxin and ethylene in root elongation has been well documented (see Mulkey *et al*, 1982).

Literature Cited

Abou-Mandour, A., and W. Hartung. 1980. The effect of abscisic acid on growth and development of intact seedlings, root and callus cultures and stem and root segments of *Phaseolus coccineus*. *Z Pflanzenphysiol.* 100:25-33.

Digby, R. D., and J. Firn. 1980. The establishment of tropic curvatures in plants. *Ann. Rev. Plant Physiol.* 31:131-148.

de la Torre, D., J. L. Diez, J. F. Lopez-Saez, and G. Gimenez-Martin. 1972. Effect of abscisic acid on the cytological components of the root growth. *Cytologia.* 37:197-205.

Gaither, D. H., D. H. Lutz, and L. E. Forrence. 1975. Abscisic acid stimulates elongation of excised pea root tips. *Plant Physiol.* 55:948-949.

Jackson, M. B., and P. W. Barlow. 1981. Root geotropism and the role of growth regulators from the cap: a re-examination. *Plant Cell Environ.* 4:107-123.

Mulkey, T. J., K. M. Kuzmanoff, and M. L. Evans. 1982. Promotion of growth and shift in the auxin dose/response relationship in maize roots treated with the ethylene biosynthesis inhibitors aminoethoxyvinylglycine and cobalt. *Plant Sci. Lett.* 25:43-48.

————, M. L. Evans, and K. M. Kuzmanoff. 1983. The kinetics of abscisic acid action on root growth and gravitropism. *Planta.* 157:150-157.

Pilet, P. E., and A. Chanson. 1981. Effect of abscisic acid on maize root growth: a critical examination. *Plant Sci Lett.* 21:99-106.

————, and L. Rivier. 1981. Abscisic acid and distribution in horizontal maize root segments. *Planta.* 153:453-458.

Thimann, K. V. 1937. On the nature of inhibitions caused by auxin. *Am. J. Bot.* 24:407-412.

Went, F. W., and K. V. Thimann. 1937. *Phytohormones.* Macmillan: New York.

Yamaguchi, T., and H. E Street. 1977. Stimulation of the growth of excised cultured roots of soya