Auxin Movement During Root Gravitorpism

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There have been many attempts to elucidate the effect of auxin on root growth and gravitropic response since Thimann (1937) observed the dose-response relationships of auxin on root and stem elongation.

Indole 3-acetic acid (IAA) is the active form of auxin in most plant species. Auxin is an essential factor in the control of root and stem growth and development. Auxin can either stimulate or inhibit cell elongation in roots, depending on concentration. Thimann (1937) suggested that the auxin effects on root elongation are similar to the effects observed on stem or coleoptile tissue. Since he was unable to observe significant promotion of root elongation by auxin, he suggested that root cells are more sensitive to auxin than stem or coleoptile cells.

While auxin has several physiological functions in plant organs, many researchers have focused on the role of auxin in gravitropism in plants. Gravitropism is a plant growth movement induced by either a gravitational stimulus or by mass acceleration (e.g., by centrifugation). Roots exhibit positive gravitropic movement particularly in primary roots. During past years, the Cholodny-Went hypothesis (Digby and Firn, 1980; Went and Thimann, 1937) has been widely accepted to explain the mechanism of gravitropism in plants, although there is an alternative hypothesis that has been proposed. According to these researchers (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 (Figure 1). 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, supraoptimal concentrations of auxin inhibit growth of the elongation zone on the lower side of the root. The upper portion of the root contains optimal levels of auxin that stimulate growth of this upper portion. This differential rate of elongation between upper and lower halves of the root results in curvature.

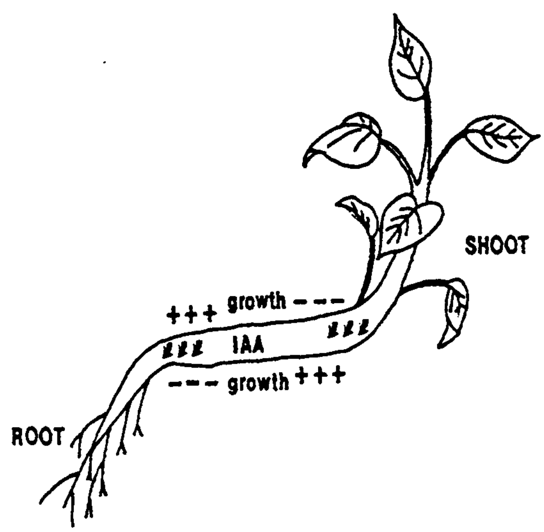

Figure 1. Diagrammatic representation of gravitropism in plants. Changes in the rate of elongation along the upper and lower surfaces of the shoot or root are indicated by +++ (promotion) or --- (inhibition). The direction of the movement of IAA in each organ is represented by the arrows.

Many researchers have reported a variety of experiments consistent with the concept that the site of graviperception for the root is the root cap (Björkman and Leopold, 1987; Moore and Evans, 1986; Poovaiah *et al.*, 1987; Wendt *et al.*, 1987). However, gravitropic response does not occur in the root cap, but in the elongation zone which is 2-6 mm behind the root cap. Based on a variety of experiments, authors have suggested that the growth inhibitor may be either auxin (Jackson and Barlow, 1981; Mulkey and Evans, 1981, 1982; Mulkey *et al.*, 1983; Steen and Hild, 1980) or abscisic acid (AbA; Pilet and Chanson, 1981; Pilet and Rivier, 1981). Some authors discount AbA as a growth inhibitor in gravistimulated roots (Lee *et al.*, 1990, Mulkey *et al.*, 1983). Thus, it is widely accepted that auxin plays the central role in the maintenance of the asymmetric elongation rates observed during gravicurvature of roots.

In this laboratory exercise, auxin movement in the elongation zone of root is observed during gravitropism using a simple donor-receiver agar block system.

Laboratory Exercise

GOALS OF THE EXPERIMENT

1. Examine the effect of gravity on IAA movement in the elongation zone of root.
2. Examine the time-dependent IAA distribution in the elongation zone of gravistimulated roots.
3. Examine the IAA movement related to the IAA transport inhibitors such as TIBA (2,3,5-triiodo benzoic acid) and NPA (naphtylphthalamic acid).

TIME REQUIREMENT

- 0.25 hours (approximately) 3 days prior to experiment to soak grain
- 1.00 hours prior to experiment to prepare agar blocks
- 1.00 hour (approximately) 1.5-2.0 days prior to experiment to plant grain
- 1.50 hours prior to experimentforo pretreatment as needed
- 2.00 hours experiment running time
- 2.00 hours (approximately) after the experiment to setup and measure radioactivity)

MATERIALS AND EQUIPMENT

- Agar
- Disposable gloves
- Disposable plastic petri dishes, 100x15 and 150x15 mm
- Filter paper (Whatman No.1)
- Forceps
- Grain, corn
- Hot plates
- Oxygen Tank
- Paper Towel
- Plastic trays and tub
- Radioactive IAA (30,000 cpm per block and concentration of IAA is approximately 10^{-9} to 10^{-10} M)
- Razor blade
- Scintillation counter
- Screws, two machine screws (1.5" x 8/24 or 8/32)
- Small block of wood
- Thread
- Tissue culture flask (200 ml) with syringe needle (20G 1 1/2)
- Window putty (Mortite)

METHODS

Seedling Preparation. Corn grains are soaked overnight in running tap water to prevent anaerobiosis. Grains germinate 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.

Application of Radioactive IAA. For donor (containing radioactive IAA) and receiver (plain) agar plates, 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 plate). One of the plates contains radioactive IAA (30,000 cpm; 10^{-9} to 10^{-10} M IAA) with a drop of food dye to distinguish radioactive and plain agar plates. The poured plates are placed on a level surface and allowed to solidify.

Preparation of Agar Blocks. Prepare a marking block as illustrated in Figure 2. The 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 block horizontally, then vertically to make small squares. Using the razor blade, carefully cut the agar along the scars of the thread to obtain uniform squares of agar.

Pretreatment of Root. Roots are pretreated with the desired chemical or hormone solution for proper time periods, depending on experiments (usually for 1 hour). Roots are attached vertically to the wall of a tissue culture flask using window putty.

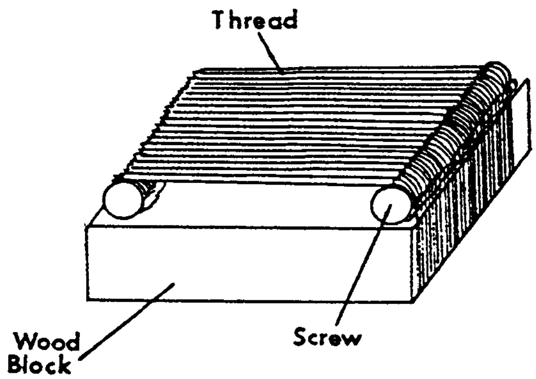

Figure 2. Design of wood block for making the scar on the surface of agar plates to prepare the agar block.

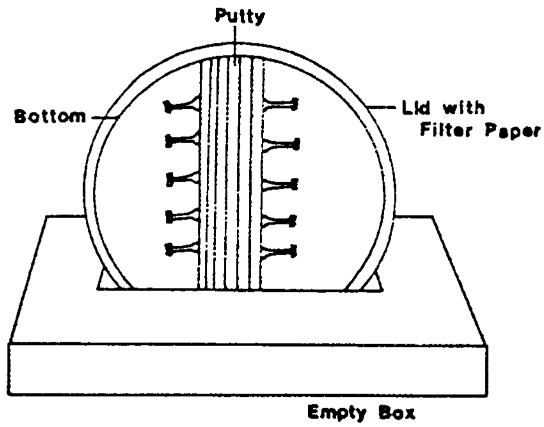

Figure 3. Diagram of putty holder in the bottom of petri dish (humidified chamber) and a stand for petri dishes to keep them in vertical position using a empty box.

The flask is filled with a solution of the test compound. The solution is oxygenated using a syringe which is inserted and glued through the wall of the flask. After the pretreatment, roots are transferred to the humidified disposable petri dishes.

EXPERIMENTAL PROCEDURE

1. Prepare the humidified disposable petri dishes, as many as need, by adding wetted filter paper circles to the top half of each dish.
2. Make a holder for seedlings with window putty in the center of bottom of petri dish (150x15 mm). The putty strip is vertical across the bottom of petri dishes (Figure 3).
3. Prepare a stand for petri dishes to keep them in the vertical position. A small empty box with a slit in the top is ideal.
4. Select 10 corn seedlings with primary roots 1.5 cm in length for each petri dish. Roots are pretreated (if needed) with testing chemical solution while in a vertical position.
5. Attach the seedlings to the putty in the humidified chamber so that the roots are oriented in a horizontal position; 5 seedlings should be attached to each side (Figure 3).
6. Apply donor agar block containing radioactive IAA on the top surface of the elongation zone (2-4 mm from the tip) of the roots on one side of the putty strip and on the bottom surface of the roots on the other side of the putty strip (Figure 3).
7. Apply the receiver block on the opposite surface of the roots directly opposite of the donor block (Figure 3).
8. Incubate the roots for proper time period (45 or 90 min), then collect the donor and receiver agar blocks from both surfaces of root.
9. Measure the radioactivity of the donor and receiver agar block by scintillation counting.
10. Calculate the ratio of radioactivity in the agar blocks (amount which moved into the bottom receiver/amount which moved into the top receiver) to determine the IAA movement.

OBSERVATIONS AND QUESTIONS

What is the significance of the ratio of radioactivity in receiver agar block if ratio is greater than 1 or less than 1?

Which direction did IAA move? Could the amount of IAA which moved be considered significant? Does this method measure the total amount of IAA which moved? Why or why not?

What degree of curvature was obtained by the roots during the time selected for auxin transport? Does the degree of curvature and the ratio of auxin in the receiver blocks correlate?

SUGGESTIONS FOR ADDITIONAL EXPERIMENTS

1. Observe the IAA movement in different time periods.

2. Determine the effect of an auxin transport inhibitor (NPA or TIBA) on IAA movement.

3. Pretreat the roots with ethylene biosynthesis inhibitors such as cobalt ion (0.1 mM) and/or 1 μM AVG (aminoethoxyvinylglycine), then observe the IAA movement in the elongation zone of root during gravitropism.

4. Pretreat the roots with ethylene-producing agents such as Ethephon (1 mM) or 1 μM ACC (1-aminocyclopropane-1-carboxylic acid) which is a precursor of ethylene, then observe the IAA movement in the elongation zone of root during gravitropism.

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