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BIOASSAY AND CORN COLEOPTILES

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Introduction

In the 1930s, Dr. F. W. Went and R. Bonner conducted investigations into the factors regulating cell elongation in plants and their subsequent effect on plant growth. These studies eventually led to the isolation of a plant growth hormone called auxin. Auxin was finally identified chemically as indole-3-acetic acid or IAA. Throughout these investigations the presence, absence or dilution of auxin was accessed by observing the growth of coleoptiles of the oat plant (*Avena*) which are extremely sensitive to small variations in concentration of IAA. Coleoptiles are sheathlike organs surrounding the leaves of the growing shoots of plants in the grass family (Fig. 1). This technique of using a biological structure to detect the presence, absence or concentration of a chemical compound is call a *bioassay*.

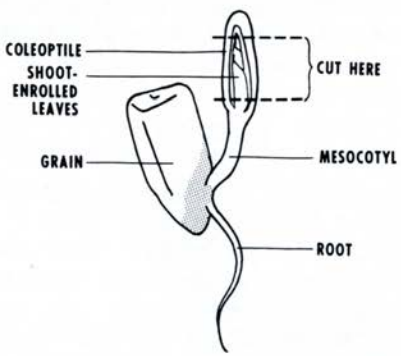

Fig. 1. Longitudinal section of a kernel of corn.

Once IAA was isolated and identified it was possible, by manipulating concentrations, to study the manner in which these compounds regulated plant growth. Eventually new compounds were synthesized that were similar in chemical structure to IAA but had never occurred naturally in plants. These chemical mimics or analogues became useful in the development of modern herbicides and other plant growth regulating substances.

In studying these new compounds the bioassay techniques developed by Went are still utilized not only in assessing their concentrations but also in

comparing their effects with those of natural plant hormones. Oat coleoptiles, because of their sensitivity and the mass of data collected on their responses to growth-inducing compounds, remain the primary bioassay organs of plant physiologists. However, oat coleoptiles, because of their small size, are difficult for beginning students to work with in the classroom. Another member of the grass family, corn (*Zea*) is much easier to experiment with because of its larger size and like oats, its coleoptiles are sensitive to varying dilutions of IAA. The remainder of this paper outlines a method of reproducing Bonner's coleoptile section tests using the "rag doll" method of rearing corn coleoptiles and by identifying the basic techniques in bioassaying the role of plant growth regulators in cell elongation. Unlike Went's curvature tests, Bonner's section method is simpler in applicability and is more useful over a wider variety of plant hormone concentrations.

Materials

The following materials are required in conducting this investigation.

200-kernels of Golden Bantam sweet corn.
2-double-edged razor blades.
2-¼ - inch round headed stove bolts, 2 inches long.
20-¼ - inch washers.
5-Petri dishes.
2-pipettes graduated to 0.01 ml and 0.1 ml.
1 lb. of table sugar.
1 gm of IAA.
20-paper towels folded to 10.5 x 24.5 cm.
1-large-mouthed, gallon mayonnaise jar.
100 ml of isopropyl or ethyl alcohol.
1000 ml of distilled water.
1 roll of aluminum foil.
1-plastic ruler graduated in mm.
1-glass marking pencil.
1-forceps.

Procedure

1. Wash about 200 kernels of corn to remove any fungicide. Three washings should be adequate.

2. Soak the kernels for 24 hours at room temperature to initiate embryonic growth.

3. Place nine pre-soaked kernels, about an inch apart, along the center line of the length of a prefolded paper towel, orienting the tips of the kernels toward the long edge of the towel.

4. Beginning at the short edge of the towel, roll the towel, with the grains, into a package. The wet paper will keep the package intact without

fasteners. Be sure to mark which direction the tips of the kernels are pointing in the rolled towel.

5. Pour a ¼ - inch layer of water in the bottom of a wide-mouthed, gallon jar.

6. Place the towel package, with the kernel tips oriented downward, into the gallon jar to facilitate germination. Orientation is important since the root of the embryo emerges downward from the tip of the kernel while the shoot grows upward. If this procedure is not followed, you will be rewarded with an unwanted demonstration of geotropism.

7. Repeat the packaging process with the remaining kernels until the supply of kernels is exhausted.

8. Cover the jar with aluminum foil and place the jar in the dark at room temperature. The corn will germinate and grow and the coleoptiles will be ready for experimentation in four or five days.

9. Prepare a 0.1m IAA stock solution by dissolving 0.175 gm of IAA in 3 ml of isopropyl or ethyl alcohol and pour into a graduated, 100 ml volumetric beaker and fill the beaker to the 100 ml mark with distilled water. If possible refrigerate the solution or store in a dark place.

10. Have students compute the amount of IAA stock solution required to be added to 20 ml of distilled water to make different dilutions for experimental treatment solutions. Recommended dilutions are 0.0 M IAA (control); 0.001 M IAA; 0.00005 M IAA; and 0.000005 M IAA. It is to be noted that all solutions are mixed using 20 ml of distilled water as a standard dilutant since the final volume produced must fit into a Petri dish. The following equation is used in computing the dilutions:

$$\frac{V_1}{V_2} = \frac{C_2}{C_1}$$

In the preceding equation, C_1 represents the concentration of the stock solution; C_2 represents the desired diluted concentration; V_2 represents the volume of distilled water (20 ml) to be added to the stock solution; and V_1 represents the volume of the stock solution necessary to obtain the desired diluted concentration (C_2).

Sample Problem

How many ml of 0.1 M IAA stock solution must be added to 20 ml of distilled water to make a dilution of 0.001 M IAA?

$$\frac{X}{20\ ml} = \frac{0.001\ M}{0.1\ M}$$

$$(0.1)\ (X) = (20)\ (0.001)$$

$$X = .2\ ml\ of\ IAA\ stock\ solution$$

11. Prepare a 10% stock solution of sucrose.

12. Have students compute how much of the 10% sucrose solution must be added to 20 ml of water to form a 1% sucrose solution using the same dilution method as previously outlined.

13. Prepare a coleoptile cutter as follows: Insert two stove bolts through the slots of a double-edge razor blade, until the blade is flush with the head of the stove bolt. Next place enough washers on each stove bolt to space the second razor blade from 6 to 10 mm from the first blade. Place the second blade over the ends of the two bolts and push it flush against the washers. Add one washer to each bolt and screw a nut on the end of each stove bolt until it is secure.

14. On the day of the experiment students can pipette the pre-calculated volumes of sucrose and IAA stock solutions into four Petri dishes and add 20 ml of water to each dish. Label each dish to identify the following treatments:

Solution A (control) ..... 1% sucrose in 0.0 M IAA
Solution B ..... 1% sucrose in 0.001 M IAA
Solution C ..... 1% sucrose in 0.00005 M IAA
Solution D ..... 1% sucrose in 0.000005 M IAA

15. After the test solutions have been prepared in the Petri dishes, take the corn shoots from the germination jar. (At the end of four or five days, the yellow leaves should make up about 20 mm of the shoot length.) Working in a dim light, remove the packages one at a time. Select the straightest shoots and using the cutter, rapidly cut a segment from each shoot about 2 mm **from** the tip of the shoot (Fig. 1). (The tip is excluded because it is a source of auxin.) Store all segments in a Petri dish filled with distilled water. Ten to fifteen segments are needed for each treatment. When a sufficient number of segments have been cut, use a forceps to randomly transfer an equal quota of segments to each

treatment solution. Some sections may float in the water and an effort should be made to keep segments from contacting one another.

16. Cover the labeled Petri dishes and put them in a dark place at room temperature for 24 hours.

17. From the remaining material have students cut at least 12 sements to determine the average length of a typical segment to the nearest 0.5 mm. Record this value.

18. After 24 hours examine the contents of the Petri dishes. You will note that some segments are curved producing a problem for accurate measurement. Whatever procedure you devise to minimize measurement errors induced by curvature, keep the procedure constant. Record the measurement for each segment length in a separate column for each treatment. Compute the mean segment length for each treatment and record this value.

Discussion

By comparing the mean segment length of coleoptiles in each treatment, students will learn that the maximum cell elongation occurs when the IAA concentration is approximately 0.00005 M. Higher concentrations inhibit cell elongation and lower concentrations provide insufficient auxin for optimum cell development.

The variations in segment length observed in each treatment provide the basis for teaching the need of statistical analysis. At this time the student "T" test might be introduced to test the significance of the differences between any pair of treatments. A discussion of the cause of curvature could also be pursued.

Once the student has learned this technique, the bioassay can be varied to include exploration of other growth regulating substances involving cell elongation, such as fungicides and herbicides. These latter substances should be added to test solutions containing sucrose with or without IAA, in parts per million. Such investigations will show that some concentrations of herbicides completely block the action of IAA, in other cases herbicides may stimulate cell elongation beyond that amount attributable to IAA alone.

For both student and teacher, the techniques presented here provide an inexpensive, efficient, simple method of studying chemical factors that effect plant growth and explores the potential of the bioassay as an important research tool.

References

Andus, L. J. 1969. *Plant Growth Substances, 2nd ed.* L. Hill, London.

Bentley, J. A. 1950. An examination of a method of auxin assay using the growth of isolated sections of *Avena* coleoptiles in test solutions. *J. Expt. Bot.* 1: 201-13.

Bentley, J. A. 1962. Analysis of plant hormones. In: D. Glick (Ed.) *Methods of Biochemical Anal* 9: 75-125.

Bentley, J. A. and S. Housley. 1954. Bio-assay of plant growth hormone. *Physiol. Plantarum* 7: 405-420.

Bonner, J. 1933. The action of the plant growth hormone. *J. Gen. Physiol.* 17: 63.

Crosby, D. G., R. V. Berthold, and R. Spencer, Jr. 1961. Naturally occurring growth substances. II. An improved straight growth test and its application. *Plant Physiol.* 36: 48-51.

Devlin, R. M. 1966. *Plant Physiology*. Reinhold Publ. Corp., New York, pp. 420-421.

Leopold, A. C. 1964. *Plant Growth and Development*. McGraw-Hill Book Co., Inc., New York.

Mitchell, J. W. and Livingston, G. A. 1968. *Methods of studying plant hormones and growth-regulating substances*. Agric. Handbook No. 336, U.S.D.A. pp. 23-25.

Nitsch, J. P. and C. Nitsch. 1956. Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight-growth test for auxin. *Plant Physiol.* 31: 94-111.

Schneider, C. L. 1938. The interdependence of auxin and sugar for growth. *Amer. J. Botany* 25: 258-270.

Sirois, J. C. 1966. Studies on growth regulators. I. Improved *Avena* coleoptile elongation test for auxin. *Plant Physiol.* 41: 1308-1312.

Van Overbeek, J. 1959. Auxins. *The Botanical Rev.* 25 (2): 269-350.

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

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Plant Growth Hormones

The farmer of the future may one day spray chemicals that would simultaneously increase growth of specific crops while killing competing weeds, according to Dr. Robert Bandurski of Michigan State University.

"But most of the auxin molecules normally found in plants are in a bound form," Dr. Bandurski explained. "They are attached to some other molecule - an X factor."

Until recently, the X factor was unknown because it was split off by harsh chemical purification techniques.

Using new purification and analysis techniques, scientists in Dr. Bandurski's laboratory have uncovered the missing X factor.

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