Hormonal Regulation Of Growth in Plants

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WHY IS IT IMPORTANT to study hormonal regulation in plants? Since the pioneering work of Francis Darwin, in the 1890s, on the participation of hormones in the light-induced curvature responses in grass seedlings, hormones have been found to regulate a host of important developmental processes in plants. These include growth-curvature responses (geotropism and phototropism), bud dormancy and bud break, seed dormancy and germination, root initiation, flowering, and fruit maturation. Applications of such hormonal regulation have been directly seen today in the use—and misuse—of herbicides (like 2,4-D and 2,4,5-T); the control of flowering time in pineapple; the increase of fruit size in grapes, figs, and tomatoes; the development of seedless tomatoes and figs (parthenocarpic fruit development); the control of sex expression in squashes; the control of ripening of fruit in cold storage; the induction of rooting in stem cuttings that are difficult to root; and, most recently, the development of single-cell cultures of higher plants to achieve somatic cell hybridization.

Many investigations of the effect of gibberellic acid (GA_3) on growth in plants have been suggested (Morholt et al. 1966; Galbraith 1968). We have chosen oat (*Avena*) stem segments and rice (*Oryza*) coleoptile sections to illustrate hormonal regulation in plants because of their remarkably large growth responses to gibberellin-type hormones; the fact that the basic growth phenomenon here involves cell elongation; the rapidity of the response; and the ease of preparing segments, especially of oat, and of making measurements. This system, in contrast to others, shows one of the most striking growth responses known in isolated plant tissues (fig. 1).

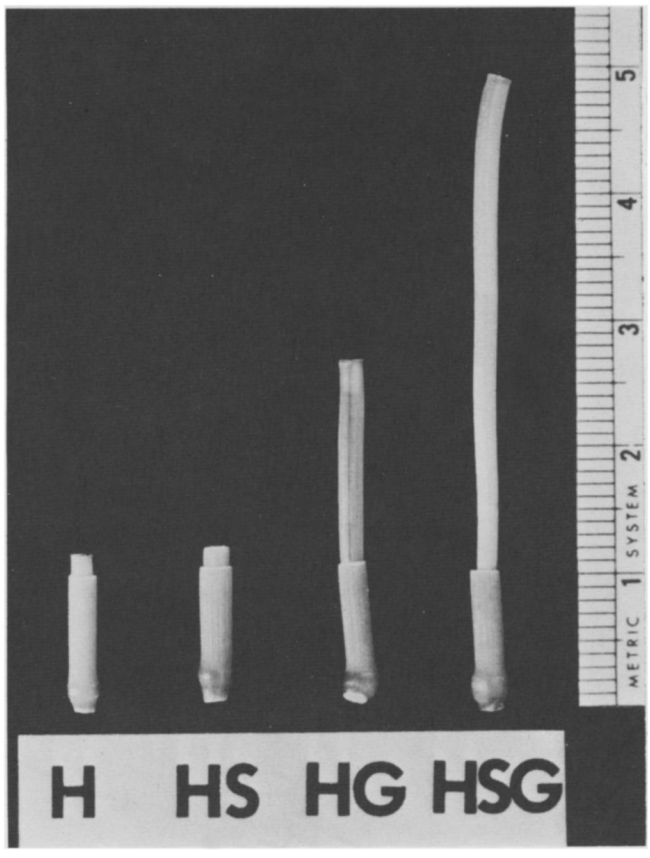

Fig. 1. Growth responses of oat (*Avena*) stem segments to gibberellin and sucrose after a 66-hour incubation period. *H*, Hoagland's solution (control); *HS*, Hoagland's solution + 0.1 M sucrose; *HG*, Hoagland's solution + 30 μM gibberellic acid; *HSG*, Hoagland's solution + 0.1 M sucrose + 30 μM gibberellic acid.

Materials and Equipment

The materials and equipment needed by each student or group of students are the following:

- 40–45-day-old oat plants
- Razor blade cutting device (see fig. 2)
- Styrene foam coffee cup
- Cheesecloth
- Distilled water
- Clorox, 10% (0.5% sodium hypochlorite)
- Petri dishes (10 cm diameter)
- Gibberellic acid, 30 μM, freshly prepared
- Sucrose, 0.1 μM, freshly prepared
- Filter papers (9 cm diameter)
- Pipettes (2 ml, 5 ml, 10 ml)
- Incubators controlled at 27–28 °C
- Paper towels

Peter B. Kaufman (pictured) is professor of botany, University of Michigan, Ann Arbor, Mich. 48104. Sarvjit L. Soni (photo not available), formerly a postdoctoral fellow in the University of Michigan botany department, lives at 10 Donway East, Apt. E–10, Don Mills, Ont. M3C 1X7, Canada. **Kaufman** is a 1949 graduate, in plant science, of Cornell University and has his Ph.D. from the University of California at Davis. He has taught at Michigan since 1956. He is the author of many papers, chiefly on plant growth regulators, and is a coauthor of *Laboratory Experiments in Plant Physiology* (1974: Macmillan Co.). **Soni** is a 1960 graduate, in botany, of Punjab University, India, and he received his M.Sc. and Ph.D. degrees from that institution; he also has a B.Ed. from the University of Toronto. He, too, has published extensively on plant growth mechanisms, and he is a specialist in the elucidation of plant ultrastructures by electron microscopy.

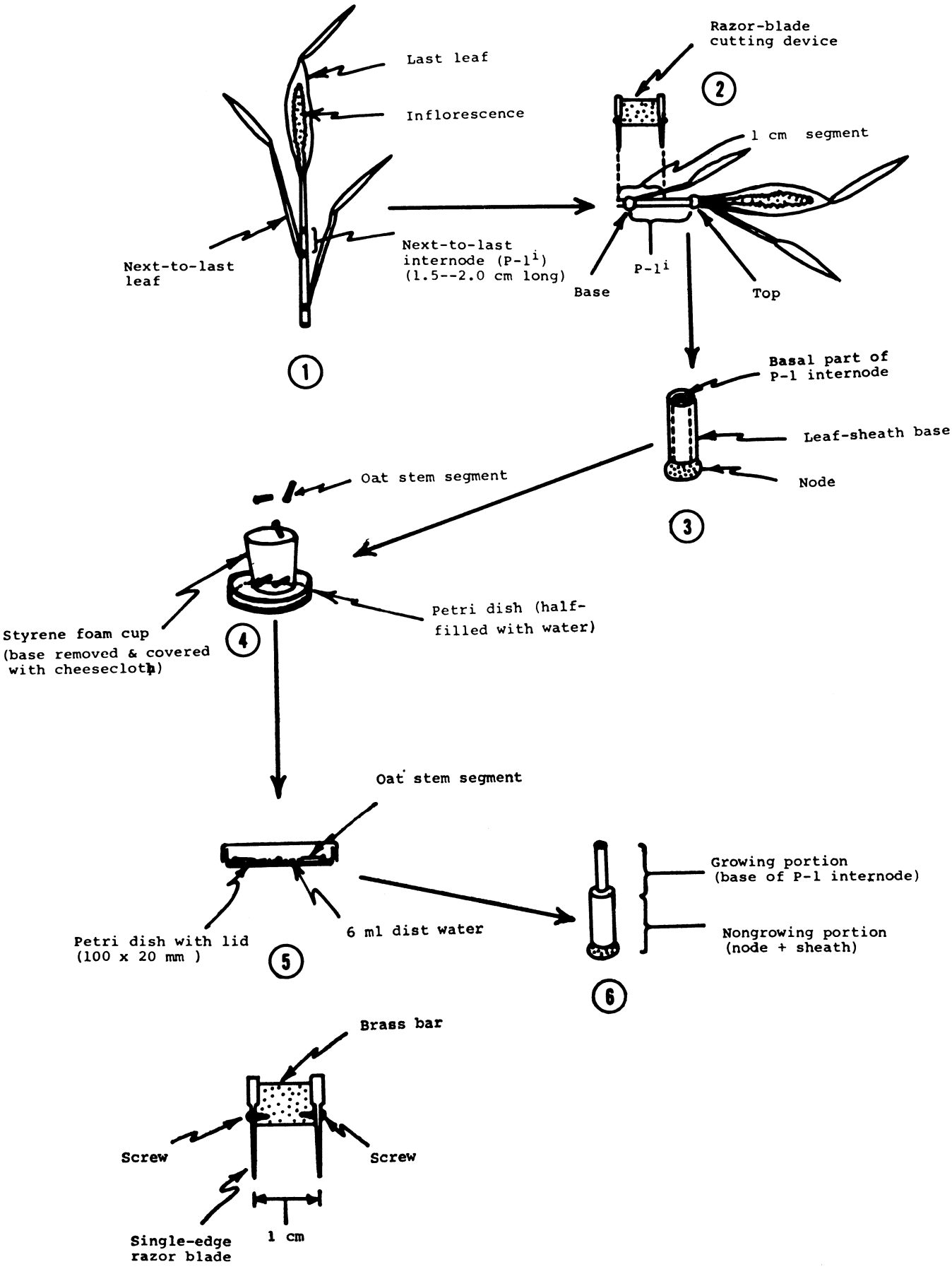

Fig. 2 (opposite). Procedures. *1*, oat shoot (40-45 days old). *2*, method of cutting a 1-cm stem segment from the oat shoot. *3*, 1-cm oat stem segment. *4*, placing of stem segments in styrene foam cup, to surface-sterilize them in 10% Clorox, followed by vigorous washing in distilled water. *5*, culture set-up for stem segments in a Petri dish. *6*, typical linear growth of internode portion in an oat stem segment, illustrating the growing part to be measured.

Procedures

Oat Stem Segments

The students are provided with pails of water containing the oat shoots having next-to-last, or p-1, internodes (fig. 2, *1*) 1–2 cm long. (These shoots may be kept in a refrigerator or cold-room for periods up to 10 days prior to use in the lab for this experiment without any decrease in segment growth response.) The shoots are cut from 40–45-day-old plants. *Keep all shoots immersed in water halfway above their bases,* even during the time the stem segments are being cut.

Prepare 1-cm stem segments from the oat shoots. These segments should be excised from the bases of p-1 internodes as shown in fig. 2, *2*. The teacher should demonstrate this, so that the students can follow carefully. Hold the shoot up to the light, to identify the node through the leaf sheath (nongrowing supporting "jacket" surrounding the internode) and the basal portion of p-1 internode (contains intercalary meristem, which generates new cells for the internode).

Place the segments, immediately, in distilled water (fig. 2, *4*) as the segments are cut. For this, use the styrene foam coffee cup: cut off the base, cover the base with cheesecloth, and place the cup in a Petri dish of distilled water.

After cutting all the segments, transfer them in the cup to a dish of the 10% Clorox for exactly 2 minutes —longer times injure the tissue—to surface-sterilize the stem segments and thus to prevent microbial contamination. To stop the Clorox treatment, wash the segments vigorously in distilled water for 2–3 minutes, to remove residual Clorox.

Experimental Treatments

Experiment	*Number of segments*
H_2O control	20
GA_3 (30 μM)	20
Sucrose (0.1 M)	20
GA_3 (30 μM) + sucrose (0.1 M)	20

Culturing Segments for Growth Experiments

To each Petri dish (fig. 2, *5*) add a 9-cm disk of filter paper and 6 ml of treatment solution per dish. *Do not confuse volumes*: these are critical, because too much solution prevents segment growth, due to the anaerobic conditions, and too little solution causes the segments to dry out. Mark the dishes for the different treatments.

Now transfer segments (previously surface-sterilized and thoroughly washed) with clean forceps—20 segments per dish. Lay all segments horizontally in the dish and more or less equidistant from each other on the filter paper. Transfer segments quickly, and start with water or sugar first, as called for. *Do not mix solutions by using the same forceps without washing forceps between treatments.* GA_3 is active in promoting growth at concentrations as low as 10^{-8} M in these segments.

Incubate the segments in the dark at 27–28 °C.

Growth Measurements

Measure net changes in the length of each segment with a millimeter ruler (a plastic ruler split in half longitudinally works best) to the nearest 0.5 mm, as shown in fig. 2, *6*.

Measure the segments at 24, 48, and 72 hours. (If the experiment is started on Monday, the growth response is completed by Thursday.)

Plot the data (growth rate vs. incubation time) on graph paper.

Additional Treatments

To illustrate hormonal interaction, additional treatments may include the following: 10^{-5} M kinetin; 10^{-5} M kinetin + 30 μM GA_3; 10^{-5} M indole-3-acetic acid (IAA); and 10^{-5} M IAA + 30 μM GA_3.

To evaluate the protein synthesis requirement, the drug cycloheximide (10 μg/ml) + GA_3 (30 μM) treatment may be added, *but be very careful in pipetting this toxic drug.* It is therefore best to use either a syringe or a rubber bulb on the pipette when adding cycloheximide to the Petri dish. After completing the experiment, all cycloheximide-containing solutions in Petri dishes should be decanted carefully into a plastic jar. *No cycloheximide, under any circumstances, is to be dumped in any lab sink.* While measuring lengths of segments, do not touch the segments with cycloheximide; use forceps to handle these segments, and wash the ruler in distilled water thoroughly after measuring.

Rice Coleoptile Sections

In addition to oat stem segments, rice coleoptile sections can be used. The method is almost the same, except that coleoptile sections require different preparation.

Rice seeds are surface-sterilized with 10% Clorox for 2 minutes, then thoroughly washed in 8–10 changes of distilled water. This treatment prevents any microbial contamination in the incubation period. 50 seeds are placed on a disk of filter paper in a 5-cm Petri dish containing 9 ml of distilled water and are incubated at 30 °C.

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come to mind; only a few will be raised here. We hope they stimulate your thinking.

How can you explain the slight differences, if any, in body temperature? How does the human body use these differences?

What is the optimum temperature for the development and maturation of an ovum? How is this regulated? What is the optimum temperature for the development and maturation of spermatozoa? How is this regulated?

The above constitutes the presentation to the class. I will conclude with remarks addressed to the present reader.

Exercises of this nature have several advantages. They provide the students with an opportunity to perform "hands on" science. The students handle and manipulate the instruments and materials, and they experience what occurs in actual laboratory situations. The students collect data and evaluate them. Within limits, this exercise allows the student to construct his own experiments for further study; this may be the most valuable aspect of the exercise.

The equipment used in this exercise is reliable, inexpensive, and reusable. Transmitters made in the lab (Mackay 1968) should be coated with epoxy, then paraffin, then silicone, to keep body fluids away from the components. Commercially made transmitters (I have used those of the Mini-Mitter Co., P.O. Box 88210-G, Indianapolis, Ind. 46208) usually come embedded in epoxy and inserted in a plastic capsule, ready for paraffin–silicone coating and use.

The batteries used in the transmitter are small hearing-aid batteries. In transmitters such as those described above, battery life is several months in continuous use. When not in use, transmitters may be stored in a refrigerator or a freezer; this extends the life of the battery considerably.

References

MacKay, R. S. 1968. *Bio-medical telemetry: sensing and transmitting information from animals to man.* John Wiley & Sons, New York.

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For Trail Riders

If you can't persuade trail-bikers to become hikers, you might at least get them to read *Trail Riders' Guide to the Environment,* by Shaun Bennett, a consultant to the American Motorcycle Association. The booklet, which the National Wildlife Federation says is "responsibly written," can be ordered from the Motorcycle Industry Council, Inc., 1001 Connecticut Ave., N.W., Washington, D.C. 20036.

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After about 96 hours, 5-mm sections from the coleoptiles (1–1.5 cm long) are isolated 3 mm below the tips in yellowish-green light (a fluorescent lamp covered with one sheet, each, of green and amber Plexiglas "G" or other suitable plastic sheet). All sections are collected in distilled water before an experiment is started. 20 coleoptile sections are floated in a 5-cm Petri dish containing 15 ml of the test solution and are incubated at 23 °C in the dark. Length of the coleoptile sections is measured to the nearest 1 mm with a ruler.

Summary

These investigations illustrate induction of growth by gibberellic acid. The students gain a keener insight into the nature of the growth process and how it is regulated by a hormone and by sugar. They develop skill in experimenting with the plant tissue in vivo—observing and measuring the growth of the segments or sections and interpreting the data.

This investigation can be extended to the study of the hormonal regulation of growth by hormone interaction, the requirement of key metabolites for hormone-induced growth, and the regulation of growth by protein synthesis.

REFERENCES

Galbraith, D. I. 1968. Lab manual for *Biological science: principles and patterns of life.* Holt, Rinehart & Winston of Canada, Ltd., Toronto.

Morholt, E., et al. 1966. *A sourcebook for the biological sciences.* Harcourt, Brace & World, Inc., New York.

The Educated Man

That man, I think, has had a liberal education who has been so trained in youth that his body is the ready servant of his will, and does with ease and pleasure all the work that, as a mechanism, it is capable of; whose intellect is a clear, cold, logic engine, with all its parts of equal strength, and in smooth working order; ready like a steam engine, to be turned to any kind of work, and spin the gossamers as well as forge the anchors of the mind; whose mind is stored with knowledge of the great and fundamental truths of Nature, and of the laws of her operations; and who, no stunted ascetic, is full of life and fire, but whose passions are trained to come to heel by a vigorous will, the servant of a tender conscience; who has learned to love all beauty, whether of Nature or of art, to hate all vileness, and to respect others as himself.

Thomas Henry Huxley (1825–95),
"A Liberal Education and Where to Find It"