Bi 213 PLANT HORMONES 2010

OBJECTIVES

The purpose of this experiment is to demonstrate the ability of phytohormones to regulate growth and development during the life cycle of plants. Specifically, you will learn how certain physiological processes can be controlled during senescence.

INTRODUCTION

Higher plants undergo a complex pattern of growth and development during their life cycle. For more than 100 years, plant physiologists have tried to understand the mechanisms by which this pattern of growth and development is regulated. During this time, various endogenously produced molecules have been identified as having the property of altering one or more aspects of growth and development. These molecules are called plant hormones, plant growth substances, or phytohormones. Most experiments with phytohormones have involved exogenous application of extracted hormones of synthetic analogues to plants or plant tissues. These experiments have demonstrated numerous dramatic effects of hormones on plant growth and development.

Relatively little is known about the mechanisms by which these molecules exert their effects. This problem of mechanism of action is compounded by the fact that each phytohormone usually produces multiple effects, so the initial site of action is often obscured. The observation that phytohormones are active at very low concentrations, 10^{-6} M or less, suggests that phytohormones act by binding tightly to specific “receptor" molecules. The receptor might be, for example, a protein, that is activated by the binding of a particular phytohormone.

Groups of Phytohormones

To date, five major groups of phytohormones have been established. These groups are listed here, together with a brief description of the physiological effects that they evoke.

A. Auxins

Indole-3-acetic acid (IAA) is a naturally occurring auxin. IAA is synthesized from trytophan in the above-ground meristematic tissues and then undergoes polar transport down the stem. Stimulation of cell elongation in stems is the best known effect of auxin, but it also plays a role in other processes such as apical dominance. The chemical 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (α -NAA) are two of the many synthetic auxins that are known.

B. Gibberellins

Gibberellic acid (GA_3) is only one of more than 60 naturally occurring gibberellins. These diterpenoid compounds are synthesized from geranylpyrophosphate in leaf primordia and roots. GA_3 is readily translocated throughout most parts of the plants. Gibberellins cause profound stem elongation and, in some plants, enhance other processes such as seed germination and fruit set.

C. Cytokinins

Zeatin was the first naturally occurring cytokinin to be identified in plants. Synthetic cytokinins such as kinetin and N6-benzyladenine (BA) were discovered previously. The major effect of cytokinins is a stimulation of cell division. Cytokinins also have other effects, however, including cell enlargement and regulation of senescence. Zeatin and most synthetic cytokinins are thought to be synthesized in roots and also in developing fruits.

D. Abscisic Acid

Abscisic acid (ABA) is another naturally occurring phytohormone. ABA is an isoprenoid compound and is synthesized in both roots and leaves. This hormone is translocated readily in the plant. ABA acts to inhibit, rather than stimulate, many physiological functions. Effects of ABA include closure of stomata, dormancy of axillary buds, abscission of certain fruits, and inhibition of seed germination.

E. Ethylene

Ethylene gas is produced by many plant tissues, although ripening fruits are an especially rich source of this phytohormone. The immediate biosynthetic precursor of ethylene on plants is 1-aminocyclopropane-1-carboxylic acid which itself is formed from S-adenosylmethionine. Ethylene stimulates fruit ripening, accelerates senescence, and alters other processes such as bud growth in pea seedlings. Ethylene moves readily through the plant by the process of diffusion.

HORMONAL CONTROL OF LEAF SENESCENCE

INTRODUCTION

Senescence of leaves is marked by a switch from anabolic (or steady state) to catabolic activities within the cells leading to general losses in amounts of protein, RNA, chlorophyll, etc. Senescing leaves are very evident in deciduous trees every autumn. The control of leaf senescence may involve the levels of various hormones and/or inhibitors. Cytokinins very markedly slow the rate of senescence and may play an in vivo role in maintaining vigorous, healthy leaves. On the other hand, ethylene appears to have the opposite effect, and may be the endogenous senescence accelerator in leaves.

To study the role of cytokinins in leaf senescence, most investigators have made use of the fact that isolated leaves or leaf discs rapidly senesce when floated on water. Adding cytokinins to the solution slows senescence visibly, and such an experimental set-up can be used to examine biochemical parameters of the senescence process as affected by cytokinins (or ethylene). This is what you will do when you measure the effects of kinetin and ethylene on chlorophyll loss from leaf tissue.

METHODS

The First Week of the Plant Hormone Lab

1. Bean plants will brought to the lab for use in this experiment, one bean plant per lab group.

2. Use a sharp #3 cork borer to cut 35-40 discs from the two primary leaves. To do this, remove the primary leaves from the plant and lay them topside down on several layers of paper toweling. Press the cork borer down firmly and evenly on the desired area of the leaf. Try to avoid major veins of vascular tissue.

3. As you cut the discs, place them in a 400-ml beaker containing about 200 ml of deionized water.

4. Obtain 5 petri dishes and number them 1-5, as indicated in Table I. Add 15 ml of deionized water to each of dishes 1 and 2. Add 15 ml of N6-benzyladenine (BA) solution at 1.3×10^{-4} M, 1.3×10^{-5} M, or 1.3×10^{-6} M to dishes 3, 4, 5, respectively.

5. Use a spatula or forceps to transfer 5 leaf discs from the beaker to a Kimwipe tissue. Gently blot the 5 discs dry with the tissue, and then measure and record in Table I the combined mass of the 5 discs. After weighing, float the 5 discs adaxial side up (i.e., topside up) on the solution in dish number 1.

6. In a similar manner, select, dry, and weigh groups of 5 discs for each of the other dishes, number 2-5.

7. Select another 5 leaf discs. After drying and weighing these, drop them into an empty screw-top test tube labeled "6. Initial." Close the tube, wrap it with aluminum foil, and store it in a freezer until you harvest the other leaf discs from the petri dishes.

8. Incubate the discs in the petri dishes at about 25°C. Incubate dish 1 in continuous light. Incubate dishes 2-5 in the dark.

9. Examine the discs after 2 days of incubation, and note any visible differences. Then continue the incubation as before.

The Second Week of the Plant Hormone Lab

10. Harvest the discs after 7 days of incubation. Label 5 test tubes with numbers 1-5, and transfer the 5 discs from each dish into the corresponding tube. Retrieve tube number 6 containing the 5 discs that were stored in the freezer.

11. Add 10 ml of 80% ethanol to the discs in each of the 6 tubes. Cap each tube with a marble.

12. Place the tubes in 75-78°C water bath for 35 minutes to extract the chlorophyll from the leaf discs. The water bath must be heated electrically. The alcohol is flammable, so do not use a Bunsen burner and do not allow an open flame in the area. The bath should be set up in a fume hood, if one is available.

13. After 35 minutes, remove the tubes from the bath and allow them to cool.

14. Carefully, use forceps to remove the leaf discs (now white) from the tubes and discard them. Check the volume of each extract with a 10-ml graduated cylinder and add 80% ethanol to restore the volumes to 10 ml.

*Note: for the steps below, all solutions measured with a spectrophometer MUST be performed using the appropriate cuvette (a special tube designed for spectrophometer use)

15. For each pigment extract, measure and record in Table I the absorbances at 645 nm and 663 nm. Calibrate the spectrophotometer to $A_{645} = 0.000$ and $A_{663} = 0.000$ on blanks composed of 80% ethanol.

16. For each extract, calculate the combined concentrations of chlorophyll a and b according to the formula:

$$(\text{chl a + b})\ (\mu\text{g/ml}) = 20\ A_{645} + 8\ A_{663}$$

17. For each extract, calculate the final mass of chlorophyll a and b per mg of initial fresh mass. To do this, use the combined mass of the 5 discs, as measured the previous week, and the fact that the alcohol extracts 10-ml volumes. Thus:

$$\frac{\text{(chl a+b) } \mu\text{g}}{\text{fresh mass mg}} = \frac{\text{(chl a+b) } (\mu\text{g/ml}) \times (10\text{ml})}{\text{mass of 5 discs (mg)}}$$

18. For extracts 1-5, calculate the final amount of chlorophyll per fresh mass as a percentage of the initial amount, i.e., divide (chl a + b) / fresh mass for each extract by the (chl a + b) / fresh mass of extract 6.

Name:

TABLE I: HORMONAL CONTROL OF SENESCENCE

Dish/Tube Number	Treatment	Combined Mass of 5 Discs (mg)	A_{645}	A_{663}	chl (a+b) (µg/ml)	$\frac{\text{chl (a+b)}}{\text{fresh mass}}$ (µg/mg-check units)	chl (a+b) Retained $\frac{\text{Final}}{\text{Initial}} \times 100\%$
1	Water, Light						
2	Water, Dark						
3	1.3×10^{-4}M BA, Dark						
4	1.3×10^{-5}M BA, Dark						
5	1.3×10^{-6}M BA, Dark						
6	Initial						---