Investigating Auxin Induced Formation of Adventitious Roots in Basil Cuttings

Objective: To determine how the presence of hormones can stimulate the development of new root growth in stem cuttings during vegetative propagation of basil plants.

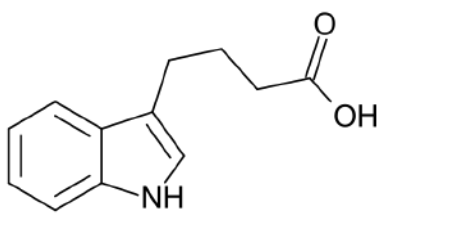
Introduction

Stem cutting is a common horticulture practice used to clone a wide variety of herbaceous and woody plants. In this technique, a plant stem is cut at an internode region of the stem and the cutting is placed in a container of water so that the cut end will be fully submerged. The plant tissues wounded by the cutting process will form a callus, a layer of soft tissue composed of unorganized parenchymal cells. New roots that begin growing out of the stem at and near the callus are referred to as adventitious roots (AR) because they do not originate from the embryonic root (radicle) of the plant.

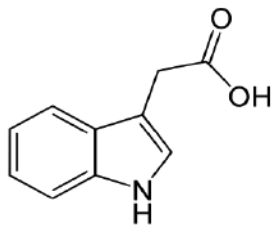
Figure 1: Plant stem anatomy and stem cuttings showing adventitious root growth.

The formation of ARs is induced by a category of hormones called plant growth regulators (PGRs) which include auxins, cytokinins (CKs) and gibberellins (GAs). A high ratio of auxin to cytokinin in the callus tissue favors the formation of roots, while a high cytokinin to auxin ratio favors shoot formation.

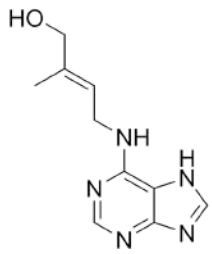
Commercial horticulture rooting products typically contain auxins such as indole-3-butyric acid (IBA) to increase auxin levels to encourage adventitious root growth. The gel from aloe vera leaves is commonly used by gardeners as a natural enhancer of rooting because it includes the auxin indole-3-acetic acid (IAA). This experiment will analyze the comparative effectiveness of a commercial rooting gel containing IBA with aloe vera gel containing IAA by observing the rate of callus formation and appearance of ARs as well as the number and growth rate of ARs in control and treatment groups of basil cuttings.



Indole-3-butyric acid (IBA - auxin)
https://commons.wikimedia.org/wiki/File:Indole-3-butyric_acid_structure.svg



Indole-3-acetic acid (IAA - auxin)
https://commons.wikimedia.org/wiki/File:Indol-3-ylacetic_acid.svg



Zeatin (cytokinin)
https://commons.wikimedia.org/wiki/File:Zeatin.png

Figure 2: Molecular structures of plant growth regulator hormones.

Before beginning this investigation, make sure you have done the following:

- **Read the lab handout and have completed the pre-lab Google form posted to BrightSpace.**
- **Follow the procedure carefully**. If you have questions about it, do not hesitate to ask for help.
- **Remember to make observations each day of your experiment**—create a rotation with your lab group so that someone makes observations of your plants on days your class is not meeting. Taking high quality images of your plants each day of the experimental window is highly encouraged so your group can make comparisons of the callus formation and rooting of the three groups.

Materials

- Dechlorinated tap water
- 50 mL test tubes (6)
- Test tube rack
- Dissection scissors
- Commercial root stimulator gel
- Aloe vera cutting
- Basil plant
- Grow lights/sunlit window
- Laminated observation sheet

Safety Considerations

- Goggles are required when working with glassware and chemical solutions.

Procedure

1. Label six test tubes A-F
2. Fill test tubes A-F with approximately 50 mL dechlorinated tap water.
3. Use scissors to cut six stems from a basil plant. The cut should be made in the middle of the second long internode. Remove the leaves from the node closest to the cut by cutting the petiole as close to the stem as possible without damaging the stem.
4. Place one stem each into test tubes A and B.
5. Dip two stems into the commercial rooting gel product and place one each in tubes C and D.
6. Dip two stems into the gel (latex) of a freshly cut aloe vera plant leaf and place one each into tubes E and F.
7. Move the six test tubes into a test tube rack and make sure the cut end of each basil cutting is fully submerged in the water. Add additional dechlorinated water as needed.
8. Place the test tube rack under grow lights or in a sunlit window.
9. Enter the date that Day 1 of this experiment was completed: _____.
10. Make observations of the cuttings once every 24 hours for a period of two weeks. Add dechlorinated tap water as needed to ensure the cut end of the stem is always submerged.
11. Record the day on which callus formation could first be observed, appearance of the first adventitious roots, and number and length of roots as growth occurs.
12. On the final day of observations, use dissection scissors to cut off the three longest ARs from each plant and determine the average length.
13. The basil cuttings can be planted in potting soil upon the completion of data collection.
14. Remaining liquids in the test tubes should be disposed of in the classroom sink and test tubes should be cleaned with a test tube brush and left on the drying rack.

DATA COLLECTION:

You and your lab group will make observations of your cuttings for the next two weeks (10 school days).

Table 1: Daily Observations of Basil Rooting				
Date	**Sample (water/root stim/aloe)**	**Callus present?**	**Number of Adventitious Roots**	**Written Observations**

CLASS DATA SET:

After recording the class data set presented in class you will use the Google Sheet available in BrightSpace to perform statistical analysis of your data. Once you have completed Table 2, create a graph to summarize the data.

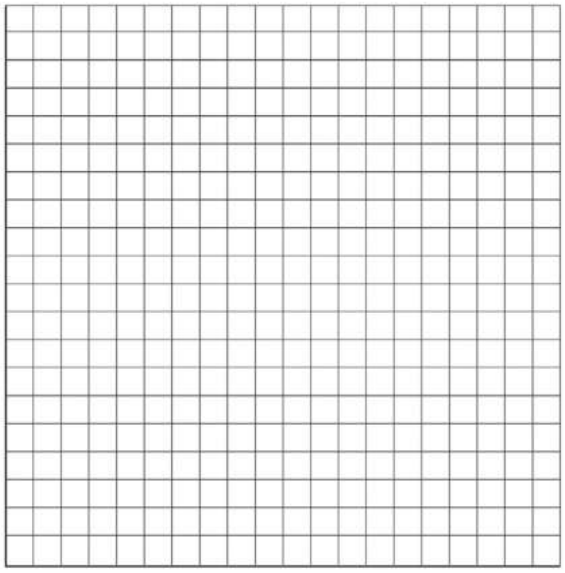
Table 2: Class Summary Data Statistical Analysis of Basil Rooting

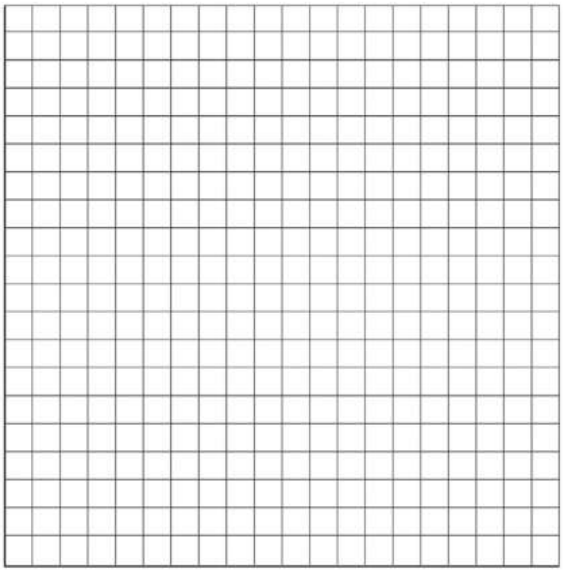
Group	Number of days until formation of callus			Number of days until first ARs form			Final Number of Adventitious Roots			Final Average Length of 3 Longest ARs		
	W	RS	A	W	RS	A	W	RS	A	W	RS	A
1												
2												
3												
4												
5												
6												
7												
8												
AVG												
SD												
2 SEM												

DATA ANALYSIS:

After recording the class data set presented in class you will use the Google Sheet available in BrightSpace to perform statistical analysis of your data. Once you have completed Table 2, create a graph to summarize the data.

1. Create two graphs to summarize the data you have collected. You will create graphs for 2 of the 4 types of data collected that were assigned to you by your teacher.





2. What type of trends or patterns did you observe in your observations and data?

3. Based on your analysis of the class data, was there a difference in the rooting process in the experimental groups vs. the negative control? Describe any differences indicated by analyzing the data.

4. Make a claim about the effectiveness of commercial root stimulator and the use of aloe as a possible replacement for a commercial root stimulator.

https://academic.oup.com/plphys/article/170/2/603/6114063?login=false

Notes:

https://www.hydrodynamicsintl.com/product/clonex-rooting-gel/ Rooting gel most like aloe latex?

https://extension.oregonstate.edu/gardening/techniques/how-hormones-growth-regulators-affect-your-plants

Ferti · lome root stimulator = 0.0004% IBA

Mix 3.5 tbsp in 1 gallon water (52ml/3.8L) OR 15mL / L solution

References

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Rooting Observation Sheet