The use of growth regulating chemicals to promote rooting when using asexual propagation techniques is widespread. The class of compounds identified as auxins are the most predominant chemicals used to enhance rooting; the three most common auxins used for rooting are: indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and α-Napthalene acetic acid (NAA). The concentration, method of treatment, length of contact, and species being rooted all influence the effectiveness of the treatment. This laboratory exercise examines these parameters using herbaceous stem cuttings.

Objectives

• To determine the effects of different auxins on root initiation.
• To determine the influence of the method of application on the promotion of root initiation.
• To examine the effects of the length of time exposed to IBA on rooting

Materials

• Unrooted cuttings of *Dendranthema* (mums) or other easy to root herbaceous plant material.
• Propagation knives
• Pencils
• Labels
• Flats
• Media
• 50 ml beakers
• Auxin at various concentrations and application methods.

Procedures

Experimental design: Treatments

This part of the laboratory consists of three sections, each examining a different aspect of the cutting/auxin interaction.

Part 1 - Effect of Auxin Compound: comparison of three chemicals that exhibit auxin activity. The physical application of auxin is the quick dip method for all three chemicals. There will be 4 sets of cuttings in this evaluation:

a) Control - no auxin
b) IAA - 1000 ppm quick dip
c) IBA - 1000 ppm quick dip
d) NAA - 1000 ppm quick dip

Part 2 - Method of application: comparing 3 methods of applying IBA to the unrooted cuttings:

a) Control - (will use 1a above)
b) IBA - 1000 ppm quick dip (will use 1c above)
c) IBA - 1000 ppm powder dip (Hormodin 1®)
d) IBA - 1000 ppm dust (Hormodin 1® applied with a duster)
Part 3 - Duration of application: comparing length of exposure of the cut surface of the mums to auxin

a) Control - (will use 1a above)
b) IBA - 2500 ppm dip for 5 seconds
c) IBA - 2500 ppm dip for 30 seconds
d) IBA - 2500 ppm dip for 1 minute
e) IBA - 2500 ppm dip for 5 minutes
f) IBA - 2500 ppm dip for 10 minutes

Steps to follow

1. Work in pairs as usual.

2. Organize your work area with all the materials you'll need. Make sure you know where in the lab area the different stations for activity are located.

3. Prepare labels for each treatment. Make sure to use an indelible ink marker! Since there are a total of 11 different treatments (with the doubling up of controls), you will need 11 labels, each indicating what type of treatment the cuttings were exposed to.

4. Obtain 1 flat with propagation medium and place it near your work area.

5. Select 5 cuttings per treatment; since there are 11 treatments, you need at least 55 cuttings for this part of the laboratory. (Use only one control treatment and one IBA - 1000 ppm quick dip. These can be used for each experimental comparison where appropriate.)

6. Re-cut the base of each cutting and strip the leaves from the lowest node.

7. Treat each group of 5 cuttings with the appropriate treatment and stick into predibbled holes in the medium in the propagation flat. Be efficient in your use of space and place the cuttings in easily identifiable rows.

**NOTE:** For treatment #3, place 20 cuttings in the flask containing 2500 ppm IBA. Start timing immediately and 30 seconds later remove 5 cuttings. These are the 3c treatment. One minute after placing the cuttings in IBA, remove another 5 (treatment 3d). After five minutes remove 5 more (treatment 3e) and after 10 min, remove the last 5 cuttings (treatment 3f). While waiting for treatments 3e and 3f, you can do treatment 3b which is a 5 second dip in the IBA solution.

8. After sticking the cuttings, make sure you firm the medium around the base of the cutting.

At the end of this activity you should end up with 11 groups of cuttings neatly organized in a flat.

9. Place under mist in the misthouse. Visit your cuttings at least twice during the next two weeks to insure all is well with them.

Data will be collected in lab in 2 weeks.
Procedures to be followed two weeks after the cuttings were stuck in media.

Data collection and results

Carefully remove each group of rooted cuttings and rinse the medium off in a water bucket provided. DO NOT USE THE SINK TO RINSE MEDIUM! Place the 5 cuttings from each treatment in a piece of moist newspaper and use the label to keep the cuttings grouped together.

Group the treatments for each part of the experiment. (Remember we are using the single control treatment for each part, as well as the IBA 1000 ppm quick dip).

Evaluate the cuttings based on a 1-5 subjective scale for degree of rooting and record your results for each cutting. Give a rating of 1 to a cutting with no roots and a rating of 5 to the cutting with the most abundant root system. Look over the range of rooting responses across all 3 treatments before you decide on your scale. Enter your data or the sample tables provided below. Prepare an appropriate presentation and summary of your data.

Discussion and Conclusions

Compare your results for the type of auxin used, the method of application, and length of treatment. How would these results affect your approach to using root promoting chemicals for cutting propagation of different species?

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CUTTING NUMBER</th>
<th>AVERAGE VISUAL SCALE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Type of auxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
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</tr>
<tr>
<td>IBA - 1000 PPM DIP</td>
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<tr>
<td>IAA - 1000 PPM DIP</td>
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<tr>
<td>NAA - 1000 PPM DIP</td>
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| Method of application |     |     |     |     |
|-----------------------|     |     |     |     |
| IBA - 1000 PPM Dust   |     |     |     |     |
| IBA - 1000 PPM Powder |     |     |     |     |

| Exposure to auxin     |     |     |     |     |
|-----------------------|     |     |     |     |
| IBA - 2500 PPM 5 sec  |     |     |     |     |
| IBA - 2500 PPM 30 sec |     |     |     |     |
| IBA - 2500 PPM 1 min  |     |     |     |     |
| IBA - 2500 PPM 5 min  |     |     |     |     |
| IBA - 2500 PPM 10 min |     |     |     |     |