Unit: Control and Regulation (H): Control of growth and development
Title: The effects of indole acetic acid (IAA) on root growth in mustard seedlings

TEACHER/LECTURER GUIDE

Type and purpose of activity

This experiment can be used to:

- provide evidence for the assessment of Outcome 3
  (For advice on marking Outcome 3 report please contact the SAPS Scotland office.)
- develop knowledge and understanding of the effect of IAA on rate of root growth
- develop problem solving skills and in particular Outcome 2 PC's:
  (b) Information is accurately processed, using calculations where appropriate
  (d) Experimental procedures are planned, designed and evaluated appropriately.

Background information

Different concentrations of IAA appear to have differing effects on root growth. These effects may vary from one species to another but generally at low concentrations e.g. 10^{-4}ppm, IAA stimulates root growth while at higher concentrations e.g. 1ppm, IAA inhibits root growth.

Mustard seedlings were chosen in this activity as they are relatively sturdy and have large root hairs at the top of the root. These make it easy to see where the root begins and thus root length can be measured accurately.

Classroom management

- Students should work in groups of two or three depending on the availability of equipment. Class results should be pooled making students aware of a larger sample size making averaged results more reliable. Students should have had experience of calculating % change prior to carrying out this experiment. The section of this experiment headed 'Supplementary Student Information' provides help with this calculation if necessary. % inhibition/stimulation should then be calculated for both group and class results and line graphs drawn.
- Estimated times: Day one - about 30 minutes. Day two - about one hour to measure root lengths and pool results.

Supply of materials

In order to satisfy the core skill in problem solving, students will be required to 'identify and obtain resources' required for themselves. Further advice on supply of material is given in the technical guide.

Extension work

- Compare the effects of IAA on different seeds.
- Measure the response of IAA in shoots.
- Vary light intensity or temperature during the two day growing period.
- Use GA instead of IAA.
- Using no plant growth substances, vary the pH of the bathing solution.
- Add chemicals thought to have an effect on germination to the bathing medium e.g. heather ash or fruit juices.
- Repeat germination several times without changing the bathing medium. Some seeds secrete chemicals when germinating to prevent other seeds from germinating. Any inhibition in % germination or rate of growth could be used to demonstrate intraspecific or interspecific competition.
- Investigate how crucial the initial volume of liquid is which is used to soak the filter papers.

Unit: Control and Regulation (H): Control of growth and development
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TECHNICAL GUIDE

Materials Required

Materials required by each student/group:

Day one of experiment

6 petri dishes
plastic 1 cm^3 dropper or 5 cm^3/10 cm^3 syringe
6 acetate grids to fit petri dish lids (see preparation)
6 filter papers to fit petri dish lids
mustard seeds (at least 60)

Day two of experiment

petri dishes in bottles as set up on day one
hand lens
blunt forcaps
paper towel

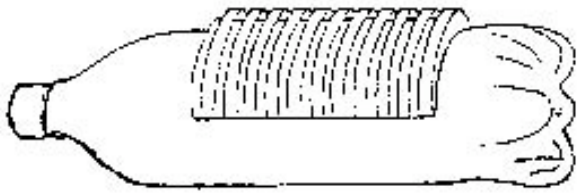
Materials to be shared:

At least six previously prepared bottles (see preparation).

A separate bottle is required for each IAA concentration (1ppm to 10^{-4}ppm) + a bottle containing distilled water.

Each bottle should be clearly labelled and contain about 200 cm^3 of the appropriate liquid. If bottles are round they will require some form of support.

Preparation of materials



- One and a half litre plastic juice bottles require to be cut as shown
- The section removed should be wide enough to fit eight or nine petri dishes. Both ends of the bottles should be packed with old petri dishes so that dishes set up by the students can be stacked vertically. Each bottle must be clearly labelled with a different IAA solution or control.
- A template for the acetate grids can be prepared by drawing round the base of a petri dish on a piece of graph paper. Six such circles can be fitted onto an A4 sheet. Acetates can then be made of the sheet and the circles cut out.
- Filter papers can be of basic quality and be about 85 mm diameter to fit petri dish lids.

Unit: Control and Regulation (H): Control of growth and development
Title: The effects of indole acetic acid (IAA) on root growth in mustard seedlings

IAA Solution

- IAA is difficult to dissolve in water. You can overcome this problem by first dissolving 0.1g IAA in a few drops of 95% ethanol and then adding 1 litre of distilled water. This makes a stock solution of 100ppm which can be stored in a dark refrigerator for up to a month.

- Take 3 cm^3 of this stock solution and add 297 cm^3 of distilled water to make the 1ppm IAA solution.

- Take 30 cm^3 of this stock solution and add 270 cm^3 of distilled water to make 10^{-1}ppm IAA solution.

- Repeat this procedure with each dilution to make dilutions of 10^{-2}ppm, 10^{-3}ppm and 10^{-4}ppm of IAA solution.

- Pour about 200 cm3 of each solution in its appropriately labelled bottle so as the liquid is about 1cm deep. During the experiment bottles with germinating seeds are best kept in the dark at 20-25ºC.

- After the experiment has been carried out, ensure all bottles and petri dishes are well cleaned. Sediments appear to affect results.

Supply of materials

It is not appropriate to provide all equipment and materials in e.g.a tray system for each student/group. Equipment and materials should be supplied in a way that students have to identify and obtain resources. Normal laboratory apparatus should not be made available in kits but should generally be available in the laboratory. Trays could be provided containing one type of specialist equipment or materials.

Unit: Control and Regulation (H): Control of growth and development
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STUDENT ACTIVITY GUIDE

Introduction

IAA belongs to a group of plant growth substances called AUXINS. They are extremely powerful substances even at low concentrations. The relationship between IAA concentration and rate of growth is not always a simple one. For example, the same concentration of IAA can have a different effect on different parts of a plant.

In the following experiment mustard seeds are grown in contact with different concentrations of IAA. You are trying to find out what effect these different concentrations have on root growth in the seedlings.

Preparing for the activity

Read through the following list of Equipment and materials, and Instructions on carrying out the activity.

Equipment and materials

1. What safety measures are you required to take?
2. Check availability of equipment and materials. Do you require any alternatives? Do you need to make up e.g. different concentrations of solutions?
3. Is there any equipment you are not familiar with? If so, ask your teacher/lecturer for help.

Analysis of activity

1. What is the aim of the activity?
2. What variable is being studied?
3. Which variables must be kept constant to ensure the validity of the results?
4. What measurements/observations are you going to make? What equipment will you use to make these?
5. What is the control in the activity? Are further control measures necessary?
6. Each set of results will need to be averaged. Do you know how to calculate an average?
7. As well as your group results you are going to collect whole class results and average them. What differences do you expect in the reliability of the two sets of results?
8. It is important not to cross contaminate solutions but you only have the one dropper/syringe. To prevent significant cross contamination will you start with the weakest or strongest solution allocated to yourself?

Recording of data

- Construct a table for recording your data (measurements/observations made during the activity). You should use a ruler, correct units, and construct the table so that you can record the root lengths in each dish.
- You will also need to construct a table to record
  a) the average root length for each dish in your group
  b) the average root length for dishes set up by other other groups
- You will then need to calculate the overall average root length for each IAA concentration used by the class.
- By comparing the IAA dishes with the control you will be able to calculate the %change caused by each concentration of the IAA. Help with this calculation is available in the section 'Supplementary Student Information'.
- You will then draw a line graph. IAA concentration (ppm) will be on the x-axis. %stimulation/inhibition will be on the y-axis. A graph of both your group results and the whole class results should be drawn.

Unit: Control and Regulation (H): Control of growth and development
Title: The effects of indole acetic acid (IAA) on root growth in mustard seedlings

Organising for the activity

In your groups decide how the activity will be managed by allocating tasks to each member. For Outcome 3 it is important that you play an active part in setting up the experiment and in collecting the results.

Before you start decide:

1. what liquid the control seeds will be in contact with
2. the order in which you will set up the petri dishes to prevent cross contamination.

Equipment and materials

Materials required by each student/group:

Day one of experiment
6 petri dishes
plastic 1 cm^3 dropper or 5 cm^3 /10 cm^3 syringe
6 acetate grids to fit petri dish lids
6 filter papers to fit petri dish lids
mustard seeds (at least 60)
distilled water
different concentrations of IAA solution (1ppm – 10^{-4}ppm)

Day two of experiment
petri dishes in bottles as set up on day one
blunt forceps
hand lens
paper towel

Materials to be shared:

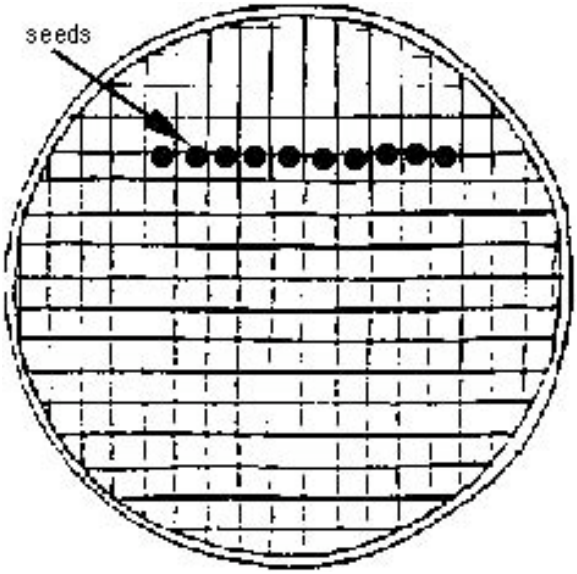
Several previously prepared bottles.
A separate bottle for each IAA concentration (1ppm to 10^{-4}ppm) + a bottle containing distilled water.
Each bottle should be clearly labelled and contain about 200 cm^3 of the appropriate liquid.

Unit: Control and Regulation (H): Control of growth and development
Title: The effects of indole acetic acid (IAA) on root growth in mustard seedlings

Instructions

Collect 6 petri dishes and the same number of circular acetate grids and filter papers.

Fit an acetate grid inside the lid of each petri dish.



Place a filter paper on top of this.

Label each lid with your initials and with control or the concentration of IAA as appropriate.

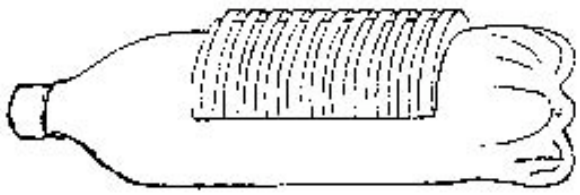
Starting with the control soak the filter paper with 3 or 4 cm^3 of the solution.

Place about 10 seeds on the damp filter paper at regular intervals along a grid line near one end of the petri dish.

Put the base of the petri dish in place.

Repeat this procedure with the other solutions.

Place each petri dish in its appropriately labelled plastic bottle, containing about 200 cm^3 of the IAA solution shown on its label.



When placed in its appropriate bottle your petri dishes should be vertical. The seeds should form a horizontal line near the 'top' of the dish.

All bottles will then be put in a warm dark place for 2 days. You should consider why these conditions are appropriate for this experiment.

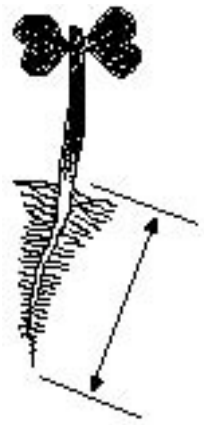
Unit: Control and Regulation (H): Control of growth and development
Title: The effects of indole acetic acid (IAA) on root growth in mustard seedlings

After two or three days

Collect your petri dishes from the bottles.

Use forceps to handle the seedlings when this is necessary.

The hand lens will help you see root hairs and thus enable you to decide where the root begins.



Discard any seedlings that look very different from usual for that dish e.g. exceptionally long, distorted growth, slow germination.

Measure the length of the remaining roots by counting the number of boxes each covers (if the root is not at right angles to the grid it can be moved using the forceps to make measuring easier). Make sure you know where the shoot ends and the root begins (there is usually a slight bulge where the two meet with root hairs obvious at the start of the root).

Record the results in a suitable table.

Concentration of IAA (ppm)	Length of seedling roots (number of boxes or mm)						Total length	Average length
	1	2	3	4	5	6		
0								
10^{-4}								
10^{-3}								
10^{-2}								
10^{-1}								
1								

Unit: Control and Regulation (H): Control of growth and development
Title: The effects of indole acetic acid (IAA) on root growth in mustard seedlings

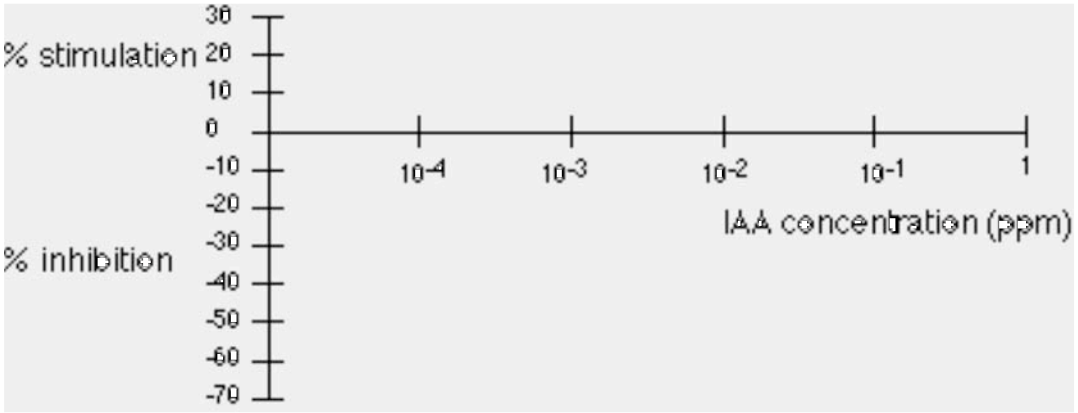
Calculate the average root length for each dish. Decide as a class how accurate you wish the averages to be. e.g. nearest whole number, to one decimal place, to two decimal places etc.

Collect the average root lengths from other groups and calculate the class average root length for each solution used.

Calculate the % stimulation/inhibition for each IAA concentration by comparing the average root length in IAA with the average root length in the control. Help with this calculation is available in the Supplementary Student Information: Calculating % Change.

Concentration of IAA (ppm)	Class average root length	% change	Seedling root growth stimulated or inhibited
0		----------	----------
10^{-4}			
10^{-3}			
10^{-2}			
10^{-1}			
1			

Present your results as a graph with suitable scales and axes labelled with quantities and units.



SUPPLEMENTARY STUDENT INFORMATION

Calculating % change

Use the formula: $$\% \text{ change} = \frac{\text{average root length in IAA - average root length in control}}{\text{average root length in control}} \times 100$$

If the % change has a positive value the roots have been stimulated by that concentration of IAA.
If the % change has a negative value the roots have been inhibited by that concentration of IAA.

e.g. average root length in 10^{-4} IAA = 16.2 boxes
average root length in water = 14.0 boxes

$$\% \text{ change} = \frac{16.2 - 14.0}{14.0} \times 100 = +15.71428 = +15.7\% \text{ to one decimal place.}$$

Has the root been stimulated or inhibited by this concentration of IAA?

e.g. average root length in 10^{-1} IAA = 10.2 boxes
average root length in water = 14.0 boxes

$$\% \text{ change} = \frac{10.2 - 14.0}{14.0} \times 100 = -27.14285 = -27.1 \% \text{ to one decimal place.}$$

Has the root been stimulated or inhibited by this concentration of IAA?

References

SAPS Student sheet 5 (1992) Investigating seed germination. *Osmosis*, **3**.

Latto J. and Wright H., (1995) Allelopathy in seeds. *Journal of Biological Education* , **29**(2), 123-128.

Lenz P., (1993) Inhibition of mustard seed germination by *Calluna* extract. *Journal of Biological Education*, **27**(2), 87-89.

This worksheet was devised by R. McAndrew, Queensferry High School, while working as a research and development teacher with the SAPS programme at the Royal Botanic Garden, Edinburgh.

Acknowledgements to: Rodger McAndrew, Queensferry High School. UK