Core practical 14: Investigate the effect of gibberellin on the production of amylase in germinating cereals using a starch agar assay

Objectives

- To investigate the effect of gibberellin concentration on amylase production in seeds
- To develop the skills of planning an investigation

Safety

- Gibberellic acid may cause irritation of skin and eyes. Wear eye protection and avoid skin contact. Wash your hands after use.
- 3% sodium hypochlorite solution (bleach) is an irritant. Wear eye protection. Use in a well-ventilated space.
- The incubated agar plates are a potential biohazard, though a hazard is unlikely with starch agar. Practise good aseptic technique and do not completely remove lids. Wash hands after handling.
- Wear eye protection when pouring the iodine in potassium iodide solution.
- Sharp scalpels may cause cuts. Hold seeds in forceps when cutting.

Specification links

- Practical techniques 1, 3, 8, 9
- CPAC 1a, 2a–2d, 4a, 4b

Procedure

A cereal grain contains a store of starch within the endosperm. During germination the starch must be made soluble so that it can be transported to the embryo to support the growth of the seedling. The embryo is much smaller than the endosperm and is situated at the more pointed end of the grain. The developing embryo releases gibberellins that act on a layer of cells on the outside of the endosperm, stimulating these cells to release the starch-digesting enzyme amylase. In this activity you will remove the embryo and investigate the effect of different concentrations of gibberellin on the production of amylase.

The production of amylase will be assessed using a starch agar assay. Cereal grains that have had the embryo removed are first soaked in gibberellic acid, then placed onto the starch agar plates and incubated. The agar plate is then flooded with iodine solution, which stains starch blue-black. The areas where starch has been digested will not stain. The size of the clear area around a cereal grain indicates the amount of amylase produced by the seed.

Planning

The basic procedure is outlined below but you will need to produce a plan before you start. This should provide detail and justify your decisions on how you will do the following.

- Set the levels of the independent variable (gibberellin concentration).

  You will be provided with a stock solution of 1 g dm^{-3}. The normal concentration in germinating seeds is in the order of 300 µg dm^{-3}. Only small volumes of each concentration are required; for example, 2 cm^3 will be enough to cover seeds in a small vial.
- Measure the dependent variable (size of the clear area).

  Will you measure diameter or area? How many dimensions will you measure? What equipment will you need?
- Control any other factors that are not accounted for.

It is important that a good sterile technique is used throughout this investigation to prevent the growth of microorganisms that could present a biohazard.

Day 1

1. Make up your gibberellin solutions as planned. Only small volumes (a few cm^3) of each are required. Place each solution in a small labelled sample bottle.
2. Collect the number of seeds that you require and pull any husks off the grains so the shape can be seen clearly.
3. Cut each seed across the line X–Y (see fig A) so that one half contains the embryo and the other the endosperm. Keep the two halves separate and discard the seed halves that contain the embryo.
4. Sterilise the remaining endosperm halves of the seeds by placing them in 3% sodium hypochlorite solution for 5 minutes.
5. Wash the seeds thoroughly, but quickly, through five changes of sterile water, draining carefully through muslin each time, until there is no smell of chlorine. Drain fully.
6. Using sterile forceps, place the seed halves into the gibberellin solutions. Leave for 12–48 hours with the screw tops slightly loose to allow oxygen to enter.

Day 2

7. Collect one sterile Petri dish containing starch agar for each of your planned concentrations and label them on the underside. Using sterile forceps, place a number of seed halves onto the agar with the cut face down.
8. Tape each lid with two pieces of tape and incubate for 24–48 hours.

Day 3

9. Remove the plates from the incubator. Opening the lids only slightly, pour a solution of iodine in potassium iodide all over the surface of the agar in each dish. Once stained, pour off into a waste container.
10. Measure the clear zone as planned and record your observations in a suitable table.

Notes on procedure

- Careful time planning will be needed for this practical. Set time aside well in advance of the practical lesson to introduce the task and allow students to write a plan. Steps 1–6, 7–8 and 9–10 need to be done in three separate sessions with 24–48 hours between them. Enzyme activity can be slowed down by refrigeration if an extra day is required.
- Students will ideally work in groups of three to five and will all share the time-consuming task of preparing seeds.
- When setting the levels of the independent variable (gibberellin concentration), students should plan for at least five concentrations with a good range, which should include the 'normal' value. Encourage students to make serial dilutions with the minimum concentration at least as low as 'natural' levels. Some thought will need to be given to provision of suitable volumetric glassware for initial dilution of the stock solution. This is an opportunity to practise serial dilutions.
- Given that the size of the clear area (the dependent variable) increases as the square of the dimension, differences will be larger with area than with diameter. Ideally the area should be calculated using the mean of more than one diameter. The equipment used may include a ruler, callipers or graph paper on acetate.
- Control any other factors that are not accounted for. Consider the size and number of seeds: more than five on each dish might be difficult to measure. Time and temperature of incubation should be around 24–48 hours at no more than 30 °C.
- This is an ideal opportunity to use the required mathematical skills for calculating correlation coefficients. Groups will be able to pool data for calculating correlation even if they investigate different concentrations. Students should plot a graph initially and discuss the suitability of the data for calculating Spearman's rank correlation coefficient. It is unlikely that data will show a linear relationship. When they write conclusions, students should use appropriate language, remembering that correlation does not imply causation.

Answers to questions

1. The embryos would produce gibberellin in unknown quantities, reducing the validity of the results.
2. Microorganisms such as fungi and bacteria may produce amylase, which would produce clear zones. If there had been contamination close to the seeds this would make the measured results invalid.
3. Gibberellin at certain concentrations stimulates the production of amylase by the aleurone layer around the endosperm. This hydrolyses starch to maltose. Iodine only stains the areas that contain starch.

Sample data

Gibberellin concentration/g dm^{-3}	Area of unstained zone/mm^2
1×10^{-6}	0
1×10^{-5}	20
1×10^{-4}	125
1×10^{-3}	210
1×10^{-2}	260
1×10^{-1}	256

table A Effect of gibberellin on production of amylase by wheat seeds.

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Safety

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All the maths you need

- Recognise and make use of appropriate units in calculations.
- Use an appropriate number of significant figures.
- Find arithmetic means.
- Understand simple probability.
- Use a scatter diagram to identify a correlation between two variables.
- Make order of magnitude calculations.
- Select and use a statistical test.
- Translate information between graphical, numerical and algebraic forms.
- Plot two variables from experimental or other data.
- Draw and use the slope of a tangent to a curve as a measure of rate of change.
- Calculate the circumferences, surface areas and volumes of regular shapes.

Equipment

- cereal grains
- 3% sodium hypochlorite bleach
- muslin
- small beaker
- scalpel
- forceps
- small sterile containers for soaking grains
- tile
- gibberellic acid 1 g dm^{-3} stock solution
- distilled water
- Petri dishes with starch agar
- adhesive tape
- iodine in potassium iodide solution
- volumetric glassware and measuring cylinders as required for dilutions of gibberellin
- marker pen

Diagram

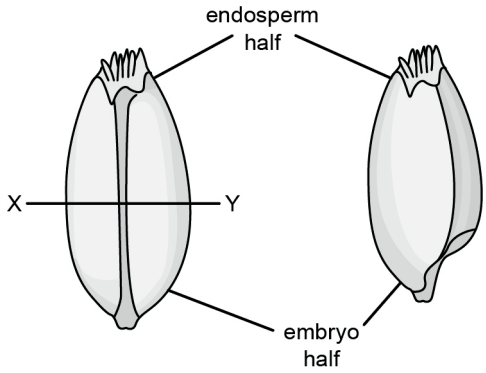

fig A Cereal grain (wheat) showing orientation of the embryo and endosperm halves from different aspects and the line of cut (X–Y).

Procedure

A cereal grain contains a store of starch within the endosperm. During germination the starch must be made soluble so that it can be transported to the embryo to support the growth of the seedling. The embryo is much smaller than the endosperm and is situated at the more pointed end of the grain. The developing embryo releases gibberellins that act on a layer of cells on the outside of the endosperm, stimulating these cells to release the starch-digesting enzyme amylase. In this activity you will remove the embryo and investigate the effect of different concentrations of gibberellin on the production of amylase.

The production of amylase will be assessed using a starch agar assay. Cereal grains that have had the embryo removed are first soaked in gibberellic acid, then placed onto the starch agar plates and incubated. The agar plate is then flooded with iodine solution, which stains starch blue-black. The areas where starch has been digested will not stain. The size of the clear area around a cereal grain indicates the amount of amylase produced by the seed.

Planning

The basic procedure is outlined below but you will need to produce a plan before you start. This should provide detail and justify your decisions on how you will do the following.

- Set the levels of the independent variable (gibberellin concentration).

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- Measure the dependent variable (size of the clear area).

  Will you measure diameter or area? How many dimensions will you measure? What equipment will you need?

- Control any other factors that are not accounted for.

It is important that a good sterile technique is used throughout this investigation to prevent the growth of microorganisms that could present a biohazard.

Day 1

1. Make up your gibberellin solutions as planned. Only small volumes (a few cm^3) of each are required. Place each solution in a small labelled sample bottle.
2. Collect the number of seeds that you require and pull any husks off the grains so the shape can be seen clearly.
3. Cut each seed across the line X–Y (see fig A) so that one half contains the embryo and the other the endosperm. Keep the two halves separate and discard the seed halves that contain the embryo.
4. Sterilise the remaining endosperm halves of the seeds by placing them in 3% sodium hypochlorite solution for 5 minutes.
5. Wash the seeds thoroughly, but quickly, through five changes of sterile water, draining carefully through muslin each time, until there is no smell of chlorine. Drain fully.

6. Using sterile forceps, place the seed halves into the gibberellin solutions. Leave for 12–48 hours with the screw tops slightly loose to allow oxygen to enter.

Day 2

7. Collect one sterile Petri dish containing starch agar for each of your planned concentrations and label them on the underside. Using sterile forceps, place a number of seed halves onto the agar with the cut face down.
8. Tape each lid with two pieces of tape and incubate for 24–48 hours.

Day 3

9. Remove the plates from the incubator. Opening the lids only slightly, pour a solution of iodine in potassium iodide all over the surface of the agar in each dish. Once stained, pour off into a waste container.
10. Measure the clear zone as planned and record your observations in a suitable table.

Analysis of results

1. Calculate a mean value of the amylase activity (for example, using the area of the clear zone) for each of your gibberellin concentrations.
2. Gather data from other groups and plot a scatter graph of size of clear zone against gibberellin concentration.
3. Write a summary of the trends shown in the graph, using numerical descriptors where possible.
4. Consider whether the class data are suitable for calculation of Spearman's rank correlation coefficient. Write a null hypothesis and an alternative hypothesis before carrying out the statistical analysis.
5. Write a conclusion using detailed scientific knowledge that might explain the results. Consider ideas such as receptors for gibberellin, secondary messengers, activation of genes and transcription.

Learning tip

- Make sure you understand the different roles of gibberellin as a hormone and amylase as an enzyme. Research the mechanism by which gibberellin stimulates the production of amylase in the target cells.

Questions

1. Why were the embryos removed from the seeds used in this investigation?
2. It was important to avoid contamination of the Petri dishes by microorganisms. Contamination would present a potential biohazard, but could also affect the validity of the results. Explain why this is the case.
3. Explain why clear areas appeared around some seeds.

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Safety

- Gibberellic acid may cause irritation of skin and eyes. Wear eye protection and avoid skin contact. Wash your hands after use.
- Undiluted sodium hypochlorite solution (bleach) is corrosive. Wear eye protection, use in a well-ventilated space and avoid skin contact. It reacts violently with acids and some other compounds. The 3% solution is an irritant.
- The incubated agar plates are a potential biohazard. Wash hands after handling, practise aseptic technique and follow current best practice for disposal.
- Wear eye protection and work in a well-ventilated room when making up the iodine in potassium iodide solution.

Equipment per student/group	Notes on equipment
cereal grains	Barley or wheat. 30 grains per group.
3% sodium hypochlorite solution (bleach)	Sodium hypochlorite (sodium chlorate(I)). This will be used to disinfect the seeds.
muslin	
small beaker	To hold seeds during sterilisation
scalpel	One per student
forceps	One per student
small sterile containers for soaking grains	Six per group. Provide small sample bottles, centrifuge tubes or similar containers with screw tops.
tile	One per student
gibberellic acid 1 g dm^{-3} stock solution	Put 0.1 g gibberellic acid (GA_3) into a 100 cm^3 volumetric flask and add 1 cm^3 of 95% ethanol to help dissolve the gibberellic acid. Add sufficient water to make 100 cm^3. Students will need only small amounts of this stock solution. A 1 g dm^{-3} solution is approximately a 3×10^{-3} mol dm^{-3} (3 mmol dm^{-3}) solution of gibberellic acid.
distilled water	This should be sterile.
Petri dishes with starch agar	Six dishes per group. These must be sterile. For six dishes of starch agar, add 2 g of agar to 100 cm^3 of cold 1% starch suspension and allow to stand for 10 minutes. Heat slowly until boiling then allow the starch agar to cool to about 60 °C and pour into sterile Petri dishes to a depth of approximately 3 mm. Leave to set. Store until required.

adhesive tape	
iodine in potassium iodide solution	Iodine in aqueous potassium iodide solution. Dissolve 6 g of potassium iodide in about 200 cm^3 of distilled water and then add 3 g of iodine crystals. Make the solution up to 1 dm^3 with distilled water. It is slow to dissolve.
volumetric glassware and measuring cylinders as required for dilutions of gibberellin	Dependent on students' plans
marker pen	One per group

Notes