Edexcel IAL Biology A Level

Core Practical 18

Investigate the production of amylase in germinating cereal grains.

Independent variable: Concentration of gibberellins solution
Dependent variable: Area of clear zone created

Background information

Cereal grains contain a store of starch which is **insoluble.** This needs to be transported to the embryo so it has a supply of energy for growth. In order to be transported across the grain, the starch needs to be made soluble which occurs when the developing embryo releases a hormones called **gibberellins** which stimulate other cells causing the release of **amylase** - the enzyme that digests starch.

In this experiment, the cereal grains will be soaked in different concentrations of gibberellins, thus causing **different amounts of amylase to be released** and so a different amount of starch digested. The starch digestion can be observed when the agar plates containing the soaked seeds are washed with potassium iodine in iodide solution which turns **blue black** in the presence of starch. Therefore, **the more starch that is digested, the larger the clear zone around the seeds will be created.**

Equipment list

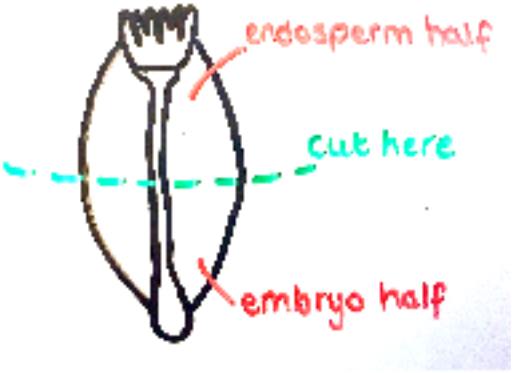
- Gibberellic acid solution ($1g/dm^3$)
- Distilled water
- Pipettes
- Cereal grains
- Small bottles
- Scalpel
- White tile
- Sodium hypochlorite solution
- Stopwatch
- Sterile water
- Muslin or gauze
- Sterile forceps or tweezers
- Petri dishes containing starch agar jelly
- Tape
- Marker pen
- Iodine in potassium iodide solution
- Ruler

Method

Before the experiment the different concentrations of gibberellins must be made up using varying volumes of distilled water and gibberellin acid.

Concentration (g/dm³)	Volume of gibberellic acid solution (dm³)	Volume of distilled water (dm³)
0.0	0	5
0.2	1	4
0.4	2	3
0.6	3	2
0.8	4	1
1.0	5	0

Day 1



1. Use a pipette to add the prepared solutions to each small sample bottle and label the bottle with its concentration, using a marker pen.
2. Collect the number of seeds you need for the experiment and use a scalpel to cut them in half as shown in the diagram. Discard the embryo halves.
3. Place the endosperm halves in a solution of sodium hydrochlorite for 5 minutes to sterilise them.
4. Now rinse the seeds 5 separate times through with sterilised water, draining them carefully using a muslin after each wash.
5. Use tweezers to place 3 seeds in each gibberellin solution and leave them to soak for 24-48 hours, place a lid on the solution bottles but leave it **slightly unscrewed to allow oxygen to enter.**

Day 2

6. After the seeds have been soaked take 6 Petri dishes and label each one with a different gibberellin concentration and use sterile tweezers or forceps to place the 3 seeds soaked in each bottle to the Petri dish labelled with the **same concentration.**
7. When placing the seeds in the Petri dish be sure to place them with the **cut face down.** Use adhesive tape to secure the lids of the dishes in place at 2 points.
8. Incubate the Petri dishes for 24-48 hours.

Day 3

9. Take the incubated plates and one at a time slightly open the lid and use a syringe to pour potassium iodine in potassium iodide solution on the surface of the agar plate.
10. Where **starch is present the solution will turn blue black**, where the **starch has been digested it will leave a clear zone.**
11. Measure the diameter of the clear zone created for each seed and record it in a suitable table.

Risk assessment

Hazard	Risk	Precaution
Glassware	Cuts from sharp objects	Take care when handling glass objects Keep away from edge of desk
Seeds	Potential allergic reaction	Wear gloves when handling Wash hands after the practical
Bleach	Irritant	Wear eye protection Use in well-ventilated area
Gibberellic acid	Irritates the skin and eyes	Wear eye protection and gloves Wash hands after use
Petri dishes	Biohazard	Dispose of safely, do not incubate above 30 degrees Use aseptic technique when handling and preparing them

Results table

Concentration of gibberellin solution (g/dm^3)	Diameter of seed 1 (mm)	Diameter of seed 2 (mm)	Diameter of seed 3 (mm)	Mean diameter of clear zone (mm)	Mean area of clear zone (mm^2)
0.0					
0.2					
0.4					
0.6					
0.8					
1.0					

To calculate the area of the clear zone use the formula:
Area = πr^2