Edexcel B Biology A-Level

Core Practical 14

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

Gibberellins are a type of **plant hormone**, the effects of which include the **stimulation** of **elongation at cell internodes, the growth of fruit, and germination**. During germination, the **starch** stored in the **endosperm** is converted to **glucose**. Gibberellins are secreted by the **embryo**, which has been **activated** by the absorption of water. They diffuse to the **aleurone layer** of the **endosperm**, which stimulates the aleurone layer to produce **amylase**. Amylase then **hydrolyses the starch** (amylose and/or amylopectin) into glucose.

Equipment

- Cereal grains
- Sodium hypochlorite solution
- Muslin
- Beaker
- Scalpel
- Forceps
- Sterile containers to soak the grains
- Tile
- 1M gibberellic acid
- Distilled water
- Petri dishes
- Adhesive tape
- Potassium iodide solution
- Measuring cylinder

Method

- **Dilute the stock gibberellin solution** to several set concentrations. Place in labelled sample bottles.
- Collect the required number of seeds and pull any husks off the grains. Cut the seeds in half (one half should contain the **embryo** and the other half should contain the **endosperm**). Discard the half containing the embryo.
- **Sterilise** the half containing the endosperm by placing it in th**e sodium hypochlorite solution** and leaving for 3 minutes.
- Wash the seeds through distilled water 5 times until there is **no smell of bleach**.
- Place the **seed halves in the gibberellin solution** and leave for 12-48 hours. Leave lids of sample bottles loose to so prevent conditions becoming anoxic.
- Use sterile forceps to move seed halves onto a **sterile petri dish with starch agar**.
- Partially tape the lids (to **prevent conditions becoming anoxic**). Leave for 12-48 hours.
- Pour **potassium iodide** onto the plates.
- Measure the **zone of inhibition**/'clear zone; around the seed half.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Biohazard	Allergies; soil bacteria; contamination	Wash hands after practical	Seek assistance	Low
Cuts from sharp object	Take care when handling glass objects; keep away from the edge of the desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low	Cuts from sharp object
Sodium hypochlorite/gibberellic acid	Irritant	Wear eye protection and gloves	Wash eyes and skin with cold water	Low
Scalpel	Cuts from sharp object	Cut away from fingers;use forceps to hold sample whilst cutting	Elevate cuts; apply pressure; seek medical assistance	Low

Graph

- Plot a graph of the **gibberellin concentration** against **area of zone of inhibition.**

Conclusion

- As gibberellin concentration increased, **the amount of amylase** produced by the aleurone layer increased, so **area of starch** from the agar around the seed half digested also increased.
- **Iodine stains starch blue-black**, so the area where starch had been digested appeared as a **clear area** around the seed half.