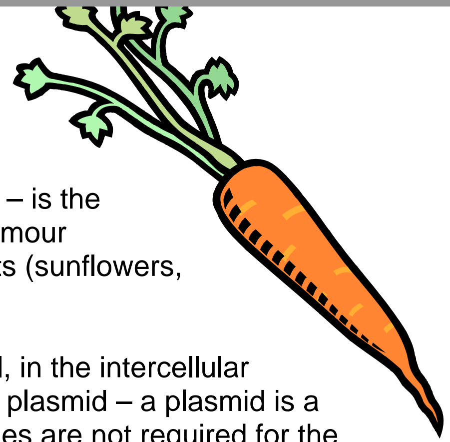
Cancer Growth in Plants

TEACHER WORKSHEET
YEARS 6-12



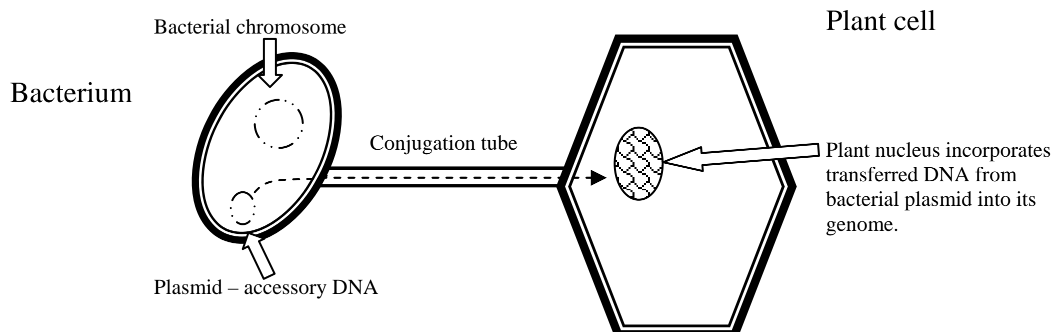
OBJECTIVE

1. Observe growth of crown gall on carrot slices

BACKGROUND INFORMATION

The Gram negative soil bacterium – Agrobacterium tumefaciens L. – is the causative agent of crown gall disease. Crown gall is a malignant tumour occurring on the stems and leaves of infected dicotyledonous plants (sunflowers, tomatoes etc.).

In infected plants, the bacterium remains outside the host plant cell, in the intercellular spaces of the plant. The bacterium contains a tumour-inducing (Ti) plasmid – a plasmid is a small ring of DNA. Plasmids have only a few genes, and these genes are not required for the survival and reproduction of the bacterium under normal conditions.



Upon infection of a plant with the bacterium, defence chemicals released from the plant activate virulence genes on the Ti plasmid. The bacterium initiates a conjugation tube by which it transfers the segment of activated genes on the Ti plasmid (called transferred DNA or T-DNA) into the host plant cell. The host cell subsequently incorporates the transferred DNA into the plant's genome. T-DNA contains genes for the syntheses of opines (chemicals used as sources of carbon and nitrogen) and phytohormones. Over-production of these phytohormones (auxin and cytokinin) results in uncontrolled plant cell growth and hence the development of a tumour.

While tumors are often lethal in animals because the cancerous cells metastasize, or migrate, around the body and grow in places they shouldn't, they're rarely deadly to a plant, whose cells are constrained by a rigid cell wall that doesn't allow them to wander enough to interfere with vital cell functions.

The crown gall will appear as a concentric ring of a creamy-to-yellowish lumpy growth surrounding the pith of the carrot slice. Under the microscope, cells comprising the gall appear smaller and disorganised; whereas, the cells of uninfected, non-malignant carrot tissue are organized with a definitive morphology. Xylem tissue will be noted in the uninfected carrot tissue.

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WHAT YOU WILL NEED

- Knife
- Forceps
- Vegetable peeler
- 70% alcohol (isocol will do)
- 250mL beaker x 2
- Bleach
- 1.5% bacto-agar
- Petri dish x 2
- Micropipette (capable of 10µL)
- Micropipette tips
- Ice-cream container or large canister
- Tissues, kimwipes or paper towel
- Broth of Agrobacterium tumefaciens grown in tryptic soy broth at 26°C
- Methylene blue
  Microscope slides
- Transmission light microscope

Note: Because the plasmid does not replicate above 37°C care should be taken to avoid elevated temperatures throughout the entire experiment.

WHAT TO DO

1. Cover the knife, forceps and vegetable peeler in a container with 70% alcohol.
2. Prepare a petri dish with agar.
3. Wash and peel a fresh carrot with the sterilised vegetable peeler.
4. With the sterilised knife remove carrot ends to produce a 5cm carrot segment.
5. With sterilised forceps, place this carrot segment into a vessel containing 6.4% bleach for about 4 minutes.
6. With sterilised forceps, pick up the carrot segment and let the excess bleach drain into the vessel.
7. To remove any residual bleach, soak the carrot segment in three changes fo sterile water (about 4 minutes each rinse).
8. Place the carrot segment into an empty, sterile petri dish and using a sterile scalpel, cut a 1cm slice from the top and bottom of the carrot segment; discard these slices because they were directly exposed to the bleach.
9. Using the scalpel, cut the carrot segment into slices about 5mm thick.
10. With sterile forceps, transfer a carrot slice to the petri dish with agar. Using a micropipette, inoculate 10 µL of a broth culture of A. tumefaciens onto the centre of the carrot sliced. Discard the tip.
11. Cover the petri dish and place it in a humidified chamber (e.g. a large, partially sealed ice-cream container with wet tissues on the bottom) housed in the dark within an incubator set at 26°C.

14-21 DAYS LATER

12. Examine the slices.
13. Using a sterile razor or scalpel, make a longitudinal cut through the gall and the plant tissue. From this, prepare a thin slice for microscopy.
14. Transfer the thin slice to a microscope slide and view. There is no need to add a coverslip.
15. For better contrast, add a few drops of methylene blue to the thin slice of carrot. After one minute, add a few drops of water to remove the excess stain and draw off the excess fluid with an absorbent towel.

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QUESTIONS

1. Describe the tumour's appearance on the carrot slice before its dissection.
2. After viewing the thin slice under the microscope, compare and contrast the tumour cells with the non-malignant cells.
3. How might the Ti plasmid be used to establish transgenic plants?

EXTENSION

1. Demonstrate crown gall development in the intact plant by inoculating a potted dicotyledonous plant (e.g. sunflower)
2. Demonstrate that the induction of plant cancer is due to genes on the plasmid, rather than to those on the bacterial chromosome by inoculating with a plasmidless strain.
3. Use gel electrophoresis to compare DNA isolated from plasmid and plasmidless strains.

CURRICULUM

Senior Biology Key Ideas

10. Malfunctioning in one system or part of a system may affect the whole organism.
22. In most organisms coded instructions within the DNA molecule account for their inherited characteristics.

REFERENCES

1. Black, S., Haugen, H. & Moore, R. (Ed.) 2000. Biology Labs That Work: The Best of How-To-Do-Its, Volume II. Virgi

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