Science &Plants for Schools

TEACHING AND LEARNING NOTES

KEY STAGE 4 RESOURCES [TIME REQUIRED = ONE HOUR+]
Starter activity: Case study and questions
Foundation activity: Micropropagation of cauliflowers
Higher activity: The millennium seed bank
Extension activity: Food security
Plenary activity: To conserve or not to conserve?

AIMS

Careers education

Motivate and engage young people so more of them want to continue to study science and make it their career.

How science works

Pupils should be taught to:

- collect data from primary and secondary sources, including using ICT sources and tools
- work accurately and safely, individually and with others, when collecting first-hand data
- recall, analyse, interpret, apply and question scientific information or ideas.

Environment, Earth and universe

Pupils will understand that the effects of human activity on the environment can be assessed using living and non-living indicators.

KEY VOCABULARY

micropropagation · tissue culture · totipotency · biodiversity · extinction · clone · seed bank

STARTER ACTIVITY: CAREER CASE STUDY AND QUESTIONS

Ask pupils to read through the career case study in the starter activity worksheet. They may then discuss the questions in small groups, noting their answers for a brief class discussion. Use this to establish what Jonathan does and why he does it. A job profile for a biotechnologist may be found at

https://nationalcareersservice.direct.gov.uk/advice/planning/jobprofiles/Pages/biotechnologist.aspx

Answers

a) Possible reasons include deforestation due to logging and clearing space for agriculture or housing, collectors, volcanic eruptions, climate change and global warming causing sea levels to rise.
b) To destroy bacteria and fungi to prevent the germinating seeds from rotting.
c) Students may mention a variety of skills with which they should be familiar from the criteria by which they are assessed. Jonathan also mentions an eye for detail, dexterity and patience.
d) Suggestions might include the ability to save endangered species and to travel to exotic locations.

FOUNDATION ACTIVITY: MICROPROPAGATION OF CAULIFLOWERS

The worksheet introduces the concepts and principles of micropropagation, tissue culture, totipotency, cloning and aseptic technique. Pupils may benefit from additional help with these concepts which may be discussed after the practical has been carried out. The emphasis in this practical should lie with aseptic technique – the most common problem is contamination and decay of the explants before they mature. You may reinforce what is meant by 'aseptic technique' and demonstrate procedures. The use of SDICN significantly reduces the problem of contamination – this can be related to bleaching of seeds by Jonathan.

Further information, including diagrams of the protocol and video guidance, can be found in the SAPS resource Cauliflower Cloning: https://www.saps.org.uk/cauliflower A kit for this practical is available from the National Centre for Biotechnology Education (NCBE) https://www.ncbe.reading.ac.uk/plant-tissue-culture/

Answers

a) The appearance of new tissues and organs should be described, including roots and green leaves.

b) Explanations should relate to totipotency.

c) The common problems are likely to relate to the growth of bacteria or moulds and the decay of the explants if the procedures have not been followed carefully. Improved aseptic technique could overcome them.

d) The use of nutrient gels to maximize germination rates and growth, and the use of micropropagation to produce numerous clones should be explained.

e) Suggestions might include being able to clone the most productive individuals or those that can only reproduce asexually, or be able to reproduce useful plants which have poor seed germination. If a new successful variety is obtained, large numbers can be generated rapidly by cloning.

HIGHER ACTIVITY: THE MILLENIUM SEED BANK

Pupils who complete the practical in good time be given the higher activity to start and it may be set in whole or part as homework. Pupils could be encouraged to approach the answers to the questions in greater depth if they use the Q&A web site of Jonathan Drori.

Answers

a) It is where the seeds of a very large number of plant species are kept in long term cold storage. It is at the Kew Royal Botanic Gardens, Wakehurst Place, West Sussex.

b) It provides a backup when conservation efforts in the wild have failed.

c) More than two billion.

d) 10%.

e) Stops metabolic processes and germination without killing them.

f) Supplies seeds to researchers.

g) Many plant species are threatened by extinction in the wild. Ecosystems with greater biodiversity are more able to resist loss of species. Conservation of seeds allows the eventual return of species to habitats.

EXTENSION ACTIVITY: FOOD SECURITY

The article demonstrates the use of a gene from a wild species to improve wheat to cope with environmental change.

Students could work in small groups and report their answers, or the task could be set as homework. The use of gels can be used in the design of an investigation of the effects of salinity on seed germination. If time and resources permit, students could undertake project work based on their design.

Answers

a) The accumulation of salts in soil.

b) Irrigation and flooding.

c) They have produced a salt-tolerant wheat variety.

d) They used traditional selective breeding techniques to introduce the gene for salt-tolerance from a wild wheat ancestor into the wheat.

e) If the wild species had become extinct the gene would have been lost and could not have been used to produce the salt-tolerant wheat.

f) Plans should vary the salt content of the gel but keep all other factors constant. Sufficient numbers of seeds should be germinated, to allow averages to be estimated, to allow for individual variation.

PLENARY ACTIVITY: TO CONSERVE OR NOT TO CONSERVE?

A wide range of potential arguments are possible, hopefully pupils will come down on the side of conservation. They should be encouraged to consider humans as part of the biosphere and responsible for the maintenance of life on Earth, which requires the resilience and ability to adapt that can only come through maximising biodiversity. They should be aware that all humans depend on plants for their existence.

TECHNICIAN NOTES

FOUNDATION ACTIVITY: MICROPROPAGATION OF CAULIFLOWERS

This method of cauliflower cloning uses the sterilising agent Sodium Dichloroisocyanurate (SDICN) to sterilise the cauliflower explants. This bleaching agent is commonly used to sterilise babies' bottles and for emergency drinking water purification. Due to its gentle action the sterilant does not need to be rinsed off the plant material before adding the explants to the media. The agar plant growth media in this method also contains low levels of the SDICN to help maintain a clean culture. The combination of no rinsing and a background level of sterilant greatly reduces contamination over previous methods of plant cloning in schools.

The technique is based on a protocol developed by plant scientists at the Royal Botanic Gardens, Kew, and is used in their conservation programmes, allowing critically endangered plant material to be cloned in the field, rather than waiting until a 'clean lab' can be found to rescue plant material.

Further information, including diagrams of the protocol and video guidance, can be found in the SAPS resource Cauliflower Cloning: https://www.saps.org.uk/cauliflower A kit for this practical is also available from the National Centre for Biotechnology Education (NCBE) https://www.ncbe.reading.ac.uk/plant-tissue-culture/

Equipment and materials

Each student/pair requires:

- 'Diluvials' or small sterilised glass jars containing medium (MS, 20g/l sucrose, 2.5mg/l Kinetin, 0.032% SDICN – see media prep notes)
- White ceramic tiles/chopping board
- Forceps
- Scalpel
- 0.5% Solution Sodium Dichloroisocyanurate (SDICN) in a small glass jar with a cap (for sterilising forceps)
- 10ml 0.5% SDICN solution in a Universal bottle (28 cm^3 glass bottle) with a screw cap. (1 x 4g Milton tablet in 160 cm^3 DI water, 2 in 320 cm^3, 4 in 640 cm^3 or 5 in 800 cm^3 – see media prep notes)
- Petri dish
- Safety glasses and disposable gloves
- Lab coat

Students/pairs require access to:

- 70% ethanol for wiping down surfaces
- Paper towels
- Cauliflower curd (the white 'floret' part) cut into 10 mm^3 pieces. Curd should be taken from a fresh, whole cauliflower, not ready-prepared cauliflower pieces.
- Glass or plastic beaker for waste solutions

Summary of method

Pupils work using aseptic technique to prepare explants from pieces of cauliflower curd and transfer them to vials of nutrient gel containing growth factors. Forceps and explants are sterilised using SDICN solution to reduce the risk of contamination by bacteria or fungi.

Safety

Risk assessments should be prepared for students and also for staff involved with media preparation.

Students

- Safety Glasses to be worn at all times
- Sodium Dichloroisocyanurate is toxic and a bleach that removes colour from clothing
- Wear a protective apron/lab coat and gloves when handling bottles containing the sterilant as caps may leak
- Do not inhale chlorine vapours from SDICN.
- Beware sharp instruments.

Technician/teacher

In addition to above, when preparing media beware of hot liquids when using the microwave

- Follow your local guidance (e.g. CLEAPSS or SSERC) when weighing chemicals: wear a mask and gloves

- Ensure heated medium is cooled to 45 °C before adding stock solution of Sodium Dichloroisocyanurate, ideally in a fume cupboard, and avoid inhaling vapours released.
- Pour the medium into vials in a well ventilated area. However, avoid draughts to reduce contamination of agar whilst pouring.
- Dispose of scalpel blades in sharps bin or equivalent.

Aseptic Technique

- All glassware should be kept scrupulously clean and, after washing with hot, soapy water, double-rinsed with distilled water and allowed to dry.
- Wash hands and arms thoroughly before procedures.
- Wear a lab coat or a similar protective garment.
- Wipe the bench/tiles with 70% alcohol.
- Don't bend over the plant material or the containers you are working with.
- Keep movements as smooth as possible to prevent the creation of eddies.
- Instruments should be kept sterile by standing in the SDICN solution. It's advisable to have sterilised the class set of forceps and scalpels in advance in a SDICN solution for at least 20 minutes.

Media Preparation - see video at https://www.youtube.com/watch?v=Nw3ZQGBnsWc

Plant tissue culture medium is normally autoclaved under pressure (15 psi at 121 °C for 15 minutes) in order to sterilise. This practical is based on a method developed at the Conservation Biotechnology Unit, Royal Botanic Gardens, Kew for media made without an autoclave, using just a microwave and water sterilising tablets (active ingredient sodium dichloroisocyanurate). This sterilant is added to the media which has the benefit both of simplifying media preparation and substantially reducing contamination of cultures as it persists in the medium.

'Milton' sterilising tablets (not the ready made 'Milton' solution available in bottles) must be used for this practical. The tablets contain a different bleaching agent to the ready made solution. Unbranded water sterilising tablets are also available from supermarkets and chemists and are suitable for this medium. Milton tablets are commonly sold in packs of 28 tablets (112 g). Each tablet is 4 g, but contains 19.5% m/m (by mass) of the sterilant SDICN. Therefore each tablet contains ~800 mg (0.8 g) SDICN. Check your pack if using own brand alternatives, which are often sold in smaller tablets. The 0.5% solution we are using is stronger than that advised on the pack. Adding 20 cm^3 of 0.5% SDICN solution to your Agar gives an end content of 0.032%. **Plain Agar powder** should be used, not Nutrient Agar powder, to make up this medium.

For 1 dm^3 of plant tissue culture media (makes around 100 vials of media):

Chemicals:

- 4.44 g Murashige and Skoog (MS) medium (Melford Labs M0222.0001 – Store in Fridge) (unless using MS medium + agar from NCBE)
- 20 g Granulated cane sugar
- 7 g Agar (2 x 3.5 g) OR 2 x MS medium + agar packets from NCBE
- Kinetin stock solution (10 mg (0.01 g) dissolved in 10 cm^3 70% ethanol) OR 2 x 1.25ml Kinetin solution as purchased from the NCBE
- Sodium Dichloroisocyanurate (1 x 'Milton' (800 mg SDICN) tablet in 100 cm^3 distilled water)
- 0.1M Hydrochloric acid and 0.1M potassium hydroxide solution (for adjusting pH)

Equipment:

- Microwave
- pH meter
- Chemical balance
- Diluvials (see supplier info following)
- Pipettes for Kinetin stock solution and SDICN solution
- A large glass beaker or jug (suitable for use in a microwave)
- Smaller glass beaker or jug for pouring media
- A stirring rod
- Safety glasses, gloves

Making up Media

- Weigh the individual components of the medium carefully.
- Measure 960 cm^3 water into a large beaker or jug (liquid should fill the vessel no more than half-full).
- Add the powdered medium stirring constantly until it has dissolved completely. (Exclude this step if using MS + agar from NCBE.)
- Once the mineral salts have dissolved, add the sucrose and stir until it has dissolved.
- Add 2.5 cm^3 kinetin using a pipette (2.5 mg/dm^3 kinetin from stock solution of 10 mg/10 cm^3, dissolved in ethanol).
- Measure the pH either using pH paper or a pH meter.
- Adjust pH to pH 5.7 using 0.1M Potassium hydroxide.
- Add the agar (or MS medium + agar from NCBE) and stir well.
- Divide media into 500ml batches.
- Heat the medium to dissolve the agar:
  - Cover the jug with cling-film, pierced to prevent build-up of steam.
  - Cook on high power for half of the recommended time, stir, re-cover and heat again for the remaining time. Recommended times for heating will vary with the power of the microwave (e.g. 8 minutes per dm^3 for a 850 W microwave).
  - When the agar is completely dissolved, allow to cool to around 45 ^oC (leave hot enough to pour before setting but cool enough to reduce chlorine release.
  - Add sterilant.
  - In a well ventilated area or fume hood if available add 20 cm^3 of 800 mg/100 cm^3 stock solution of sodium dichloroisocyanurate (SDICN) to 500 cm^3 heated and cooled medium, stir well (this gives a concentration of 0.032%) Do not breathe in chlorine vapours released.
- Dispense medium immediately:

Dispense medium

- Wipe down lab surface or tray with 70% alcohol or a bleach solution.
- Remove lids from Diluvials and place inner side downwards on surface.
- Pour media into Diluvials (approximately 10 cm^3 per vial) and leave to cool with lids ajar (about 1 hour).
- When medium is cooled and set (about 1 hour) replace lids and label vials.

- Store in fridge until required (will last about 6 weeks).

It is possible to grow your cloned cauliflower into a normal plant from the vials of media. First, they need transferring to a medium with no Kinetin (to promote extra root growth). Then, when rooted, rinse off all the gel and pot in some free-draining seedling compost. Keep moist, preferably covered with a bag for the first week or so, as with conventional cuttings.

Suppliers

NCBE sell cauliflower cloning kits and refills for agar and MS medium (mixed) and kinetin at a much cheaper price than other suppliers. Be aware that these refills are for half the quantity stated in this protocol so you will need to purchase two of each. The MS medium comes ready-mixed with the agar and the kinetin is already dissolved in ethanol at the correct concentration.

https://www.ncbe.reading.ac.uk/plant-tissue-culture/

https://www.store.reading.ac.uk/product-catalogue/national-centre-for-biotechnology-education/spare-parts/kinetin-solution-125-ml

https://www.store.reading.ac.uk/product-catalogue/national-centre-for-biotechnology-education/spare-parts/ms-medium-agar

Alpha Laboratories supply polystyrene Coulter counter cups. These 'Diluvials', because they are manufactured under clean room conditions, are sterile although not sold as such. At **£75.00 per 500** (as of 2023) plus postage they are cheaper than vials sold as sterile. You may want to consider sharing a load with a nearby school. https://www.alphalabs.co.uk/cv4010

The Consumables Company also sell Coulter counter cups from **£8 for 50** www.theconsumablescompany.com/shop/25ml-coulter-counter-snap-cap-container-polystyrene-with-lidps-50-pack/ and from **£25 for 250** https://www.theconsumablescompany.com/shop/25ml-coulter-counter-snap-cap-container-polystyrene-with-lid-250-pack/ (as of 2023)

Pre-sterilised Universal glass jars (28 cm^3) and lids could be used as an alternative to these, although inserting the explants is more difficult.

Melford Laboratories Ltd are the UK distributor for a wide range of plant tissue culture media components and products from Duchefa (https://www.duchefa-biochemie.com/)

MS medium (M0222.0001): https://www.melford.co.uk/products/duchefa/plant-tissue-culture-media/duchefa-murashige-skoog-medium-including-vitamins-1-l.html

Kinetin (K0905): https://www.melford.co.uk/products/duchefa/growth-regulators/duchefa-kinetin-1-g.html

MS Media and Kinetin are also available through some standard school suppliers.

Jonathan Kendon

Conservation Biotechnologist

Science &Plants for Schools

CAREER CASE STUDY

Jonathan is a plant scientist at the Royal Botanic Gardens at Kew. He works in the Conservation Biotechnology Department with three other scientists. They provide 'intensive care' for orchids, ferns and other plants threatened with extinction by such things as deforestation, climate change, collectors and volcanic eruptions.

Pointing at a shelf of Madagascan orchids, Jonathan said: "I don't think it's an exaggeration to say that there are more of these plants in front of us than there are left in the wild."

Logging has meant that the orchid *Paralophia epiphytica* is almost extinct in the wild, but Jonathan has grown 300 specimens from wild seeds collected in 2006. When it is safe, they will be reintroduced into their natural habitat.

He also has a few yellow orchids (*Encyclia caicensis*) which he is trying to save after realising that they only grow on beaches in the Turks and Caicos Islands. There they are threatened by rises in sea-level and holiday resorts.

The seeds of orchids are very small, weigh only a few millionths of a gram and are very difficult to grow. Jonathan has developed special techniques. In the lab he cleans them, and bleaches them to kill any bacteria and fungi. Then he places them on a nutrient rich gel to which he adds ingredients to try to make them germinate.

He says, "I've discovered that adding pineapple juice can give some species of orchid a boost." If any seeds fail to germinate or grow poorly, he tweaks the ingredients until they improve. Other ingredients he uses include sugar, mashed bananas and coconut water.

Many of the plants grow very slowly. He has nurtured one small dark green plant from a single seed that he collected from the mountains in Nepal in 1981. No-one knows what it is and Jonathan will only be able to identify it when it flowers, which could take another five years.

"Patience, an eye for detail and good dexterity are what you need to do my job," he says.

Jonathan studied Plant Sciences at Durham University and then did a Masters degree in Plant Conservation at the University of Sussex. He started at Kew by doing four months of work experience in the Micropropagation Unit (now called the Conservation Biotechnology Department).

Jonathan told New Scientist, "It was unpaid so it was tough, but if you plug away and you're determined and passionate, it stands you in good stead for eventually getting a paid job."

You can read the full New Scientist article about Jonathan at:

https://www.newscientist.com/article/mg20827830-800-dream-job-rare-plant-resuscitator/

QUESTIONS

Use the information above to answer these questions:

a) Why are plants like the orchids grown by Jonathan threatened with extinction? Try to give as full an explanation as you can.

The technique used by Jonathan is called micropropagation. Micropropagation involves growing plants from seed or small pieces of tissue under sterile conditions in a laboratory on specially selected media.

b) Why is bleach used by Jonathan in his micropropagation technique?

c) What skills and qualities do you think Jonathan needs to be able to do his job?

d) Suggest some of the things that you think Jonathan may like about his job.

You can find out more about people that work at Kew in their website.

You will study micropropagation in the investigation: ***Micropropagation of cauliflowers***.

MICROPROPAGATION OF CAULIFLOWERS

Jonathan Kendon uses micropropagation techniques to help to increase the numbers of rare plants by germinating seeds. You are going to use these techniques for the tissue culture of small pieces of cauliflower to produce whole new plants complete with leaves and roots. The small pieces of tissue are called explants and can be used to produce clones - each new plant has an identical genetic makeup to the original plant.

This is possible because many plant cells are totipotent – that means that each cell has the ability to regenerate all the different types of cell found in an entire plant.

The method that you will use has been developed by the plant scientists at the Royal Botanic Gardens at Kew. It has been adapted from one that they use in the field anywhere in the world where a 'super-clean' laboratory is not available. It is often used by them to clone endangered plant species.

An important part of the process is keeping the culture free from bacteria and fungi. You will use sterilised equipment and growing media (the gel used in your culture) and aseptic technique – methods which reduce the chance of contamination. This method uses the bleaching agent sodium dichloroisocyanurate (SDICN) to sterilise the cauliflower explants. It is often used to sterilise babies' bottles and to purify emergency drinking water.

SAFETY

- Safety Glasses to be worn at all times.
- Sodium Dichloroisocyanurate (SDICN) is toxic and a bleach that removes colour from clothing.
- Do not inhale the chlorine vapours from the SDICN.
- Wear a protective apron/lab coat and gloves when handling bottles containing the sterilant as caps may leak.
- Beware of sharp instruments.

EQUIPMENT

Safety glasses and disposable gloves · Lab coat or apron · Diluvials or small sterilised glass jars containing medium · White ceramic tiles / chopping board · Forceps · Scalpel · 0.5% SDICN in small glass jar or pot (for sterilising forceps) · 10 cm^3 0.5% SDICN solution in Universal bottle (28 cm^3 glass bottle) with screw cap · Petri dish · 70% ethanol for wiping down surfaces · paper towels · Cauliflower curd (the white 'floret' part) cut into 10 mm^3 pieces · Glass or plastic beaker for waste solutions

PROCEDURE

Cleanliness is very important. Before you start work, wash your hands thoroughly with soap and water. Try not to lean over your working area, to minimise contamination.

01) Place your forceps in a pot or jar of sterilising solution (labelled SDICN).

02) Clean the bench and wipe down the surface with a small amount of 70% ethanol on a paper towel – your teacher may have already done this for you.

03) Collect a small (10-15 mm^3) 'mini-floret' of cauliflower and place in a petri dish.

04) Using a scalpel carefully cut the mini-floret lengthways into small 3-5 mm^3 pieces. These are your 'explants'.

05) Sterilise the explants by picking them up with the pre-sterilised forceps and place them in the bottle of SDICN. Put the lid on, and put the forceps back in their pot.

06) Every 2-3 minutes swirl the bottle with the explants gently for 5 seconds. Repeat until 15 minutes have passed.

07) Carefully strain the liquid from the bottle into a waste beaker. Use the forceps to stop the explants falling into the beaker. Put the forceps in the bottle with the explants.

08) Take the lid off a vial containing agar plant growth medium. Put the lid face down on a clean tile. To minimise contamination, do not lean over your working area.

09) Use the forceps to pick up an explant from a bottle and transfer it to the agar vial, pressing the stalk end into the medium slightly. Replace the cap and use a permanent marker to label with your name and the date.

10) Repeat for other explants if more diluvials are available.

11) Incubate in a warm lab near to a window or light bank. Examine each culture weekly – greening of the explant and growth should be visible within 10 days.

INTERPRETING YOUR RESULTS

a) Describe how the appearance of the explants changes.
b) Explain the changes you have observed.
c) Did you encounter any problems? If so, suggest how they might be avoided if you repeated the investigation.

MORE ABOUT MICROPROPAGATION

d) Why might you use micropropagation to conserve rare and endangered species of plants?
e) What are the advantages of using this method to cultivate new plants?

THE MILLENNIUM SEED BANK

You will need a computer with an internet connection. If the suggested website is no longer available, search for other sources of information to answer the questions.

Watch the video on the Millennium Seed Bank at http://blog.ted.com/step-inside-the-millennium-seed-bank-video/ and answer the questions.

You may need to do some further research, for example more information can be found in Scenes from the Millennium Seed Bank: Q&A with Jonathan Drori at http://blog.ted.com/more_news_from/

a) What is the Millennium Seed Bank and where is it?

b) In what way is the seed bank a backup?

c) How many seeds are presently in the bank?

d) What proportion of plant diversity is presently held in the bank?

e) What effect does refrigeration have on the seeds that makes it possible to store them?

f) What does the bank do besides storing seeds?

g) Why is the bank necessary?

FOOD SECURITY

You will need a computer with an internet connection. If the suggested website is no longer available, search for other sources of information to answer the questions.

Read the article from the Guardian dated 12 March 2012, '*Food security: our daily bread*', at http://www.guardian.co.uk/commentisfree/2012/mar/12/food-security-our-daily-bread and answer the questions.

a) What is salination of soil?

b) What causes salination of soil and why is it a major problem?

c) What have the plant scientists in Australia done that will help with the problem of salination?

d) How did they produce their new plant variety?

e) How does this illustrate the value of conservation of plant species to the human species?

The methods used by Jonathan Kendon and other plant scientists at Kew allow seeds to be germinated on gels containing a variety of substances. For example, Jonathan has discovered that adding pineapple juice can aid the germination of some orchid seeds. Your task is to modify the technique to investigate the ability of seeds from different species, or varieties of the same species, to germinate in saline conditions.

f) Write a plan in outline to use gels to find a plant species (or variety of a species) that can germinate in saline conditions. Explain how you will alter or change variables to compare different seeds. You do not need to give details of recipes or state concentrations you would use.

g) If you can, run a pilot experiment to test your design.

TO CONSERVE OR NOT TO CONSERVE?

Some people and politicians are of the opinion that conservation efforts are futile and a waste of time and money. They consider that extinction is a natural process with 'survival of the fittest' and that humans can survive with only a few essential plant species.

WHAT DO YOU THINK?

In a small group discuss the issues and report back to your class why you think such people are right or wrong.