I. Libergier High School

Practical Exercise 4: Growing Saintpaulia *in vitro*

Introduction:

In recent years, *in vitro* cultures have represented a revolution in horticulture and agriculture. *In vitro* cultivation is the cultivation of any plant organism under sterile conditions for the purpose of its reproduction under optimal and controlled conditions.

Principle

Take a number of cells and place them in a suitable place to multiply. It is possible to fragment this amount to clone the plant. Sterile conditions are essential throughout the growing period to prevent infection by microorganisms.

OBJECTIVE

Observe cellular dedifferentiation and then the differentiation of the plant cells and demonstrate the role of growth regulators (or phytohormones) in the culture.

WORKING PROCEDURE See Annex 1

Day D

Start of leaf blade cultivation in the SP1 environment

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D + 6 weeks (approx.)

Transition to the SP2 root environment

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D + 15 weeks (approx.)

Transition to compost

MATERIALS AND SOLUTIONS

- 1 adult leaf of saintpaulia
- 1 cultivation flask with 20 ml of sterile inclined SP1
- 3 100 ml empty, non-sterile jars (for the thinner, use a disinfection solution and alcohol)
- 4 100 ml jars with sterile distilled water for rinsing
- 1 sterile scalpel (or cutter)
- 1 sterile round-nose forceps
- 1 sterile scissors
- 1 sterile Petri dish
- 1 sterile square of filter paper
- 2 cotton swabs
- 1 protective headwear

- 1 disposable facemask

For the class

- 1 bottle of dishwashing detergent
- 70° alcohol to sterilize tools during use
- Disinfection solution: 12° chlorometric potassium hypochlorite
- 1 tray for collecting dissecting tools after the end of practical exercises
- 1 cultivation chamber (providing 16 to 18 hours of light per day)

WORK AREA PREPARATION

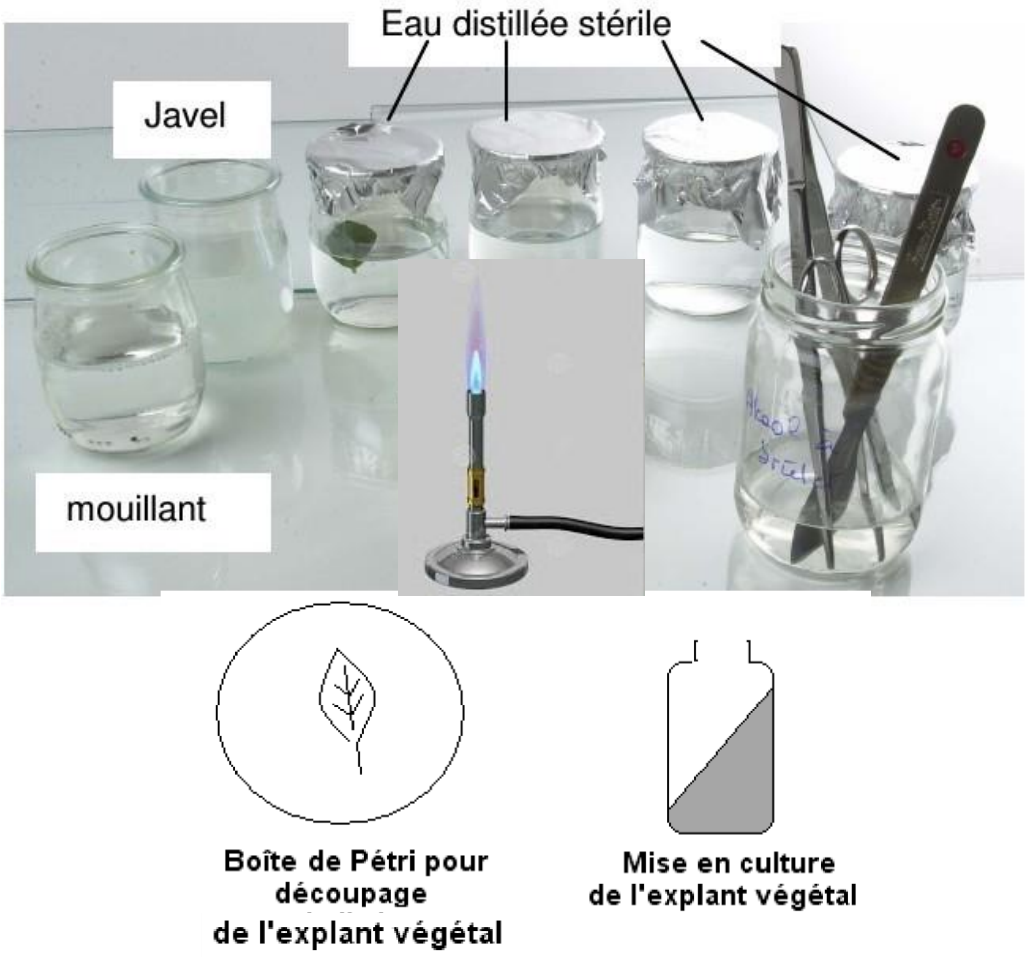


Figure legend:
Eau distillée stérile = Sterile distilled water
Javel = potassium chloride (Javellian lye), mouillant = wetting agent
Below: A Petri dish for slicing plant explants; Plant explant cultivation

– Before handling, wash your hands and forearms thoroughly with soap.
– Clean the work surface with potassium hypochlorite.
– Prepare a sterile zone using disinfectants and materials for the recovery of plant fragments:
➢ place the jar with alcohol to the right of the Bunsen burner (for right-handed users) where the tools will be placed (not too close!)
➢ place a sterile Petri dish for plant cutting in front of the Bunsen burner.

➢ place the flask with the SP1 cultivation environment to the left of the Bunsen burner.

➢ place the disinfection jars behind the Bunsen burner: one with the wetting agent (approx. 100 ml of water + 1 drop of dishwashing detergent), one with 12° potassium hypochlorite and 4 with sterile water.

– Put on your protective headwear and facemask.

PLANT TREATMENT

- *Do not damage the leaf blade by pressing it with your fingers or forceps: use a wide spatula.*
- *Plants used for cultivation must be disinfected and handled in a sterile manner.*

Caution: Never touch the ends of tools or plants with your hands, do not talk or create a draft, and properly close all doors and windows when handling the plants.

– Cut off a healthy leaf with its stem.

– Draw the leaf and include the following parameters: leafblade, leafstalk, nervation (use the "create a trace drawing" technical sheet).

– Hold the leaf by the leafstalk, rinse it under tap water for several seconds and then place it on an absorbent paper.

– Submerge the rinsed leafs for 2 minutes into the wetting agent.

– Rinse with potassium hypochlorite for 4 minutes (stop if translucent spots appear).

– Rinse for 5 minutes in 4 consecutive sterile distilled water baths.

– Let the leaf drip dry and place it in a sterile Petri dish.

– In the Petri dish, cut off two 2 cm pieces on each side with a scalpel, one at the base of the leaf (near the leafstalk); these squares are called "explants".

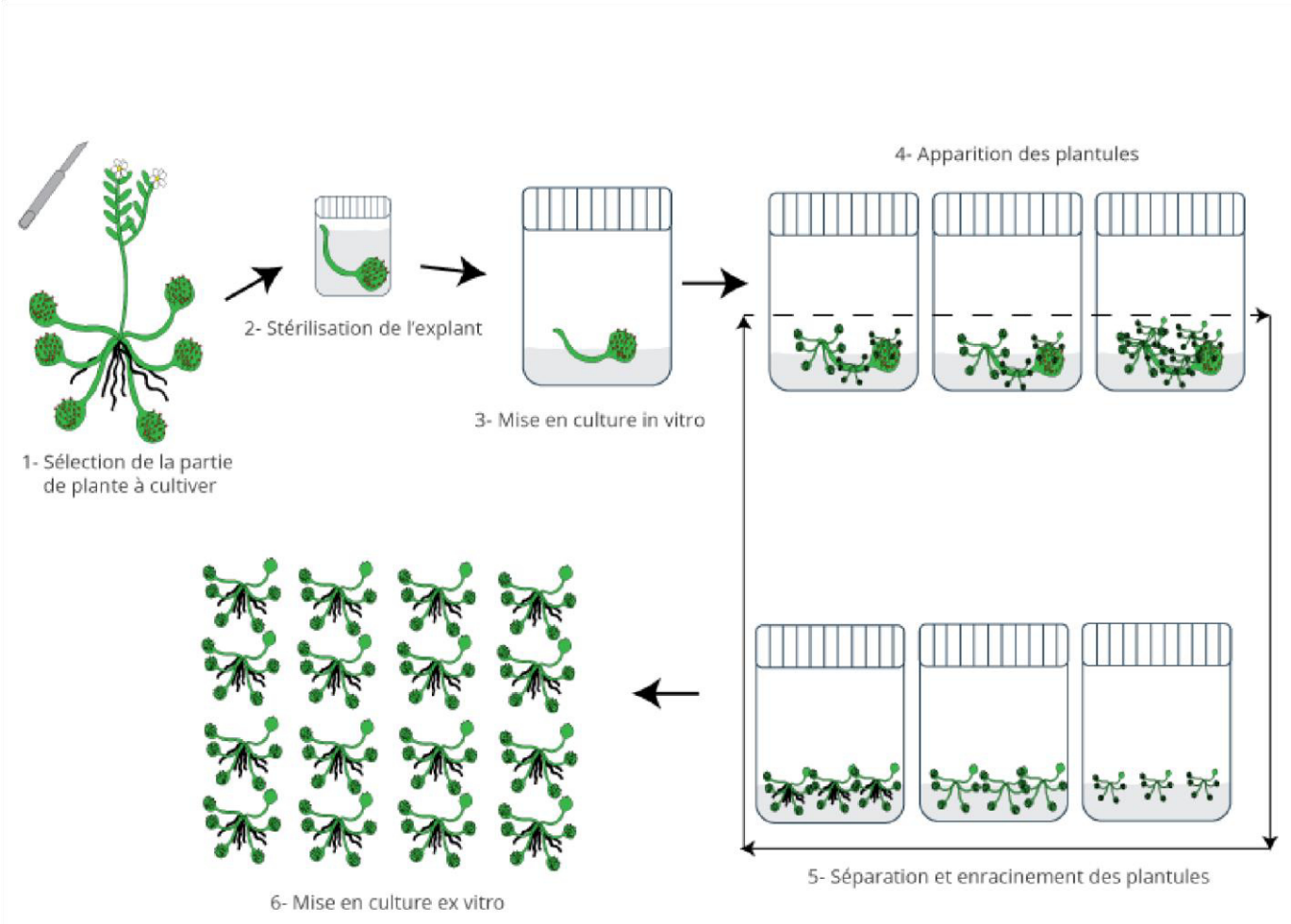
– Open the SP1 environment flask and place the stopper on a piece of paper saturated with potassium hypochlorite.

– Use the forceps to insert the explant with the underside down into the environment and then apply slight pressure on the leaf fragment. Do the same with the other explant.

– Wipe the opening of the flask with a cotton swab saturated with 12° potassium hypochlorite and close the flask.

Store the flasks in 20-25°C, under the light of a cultivation chamber **(16 to 18h of light every day)**

ANNEX 1
THE *IN VITRO* CULTIVATION STAGE



Legend:
1 – Selecting the plant part for cultivation
2 – Explant sterilization
3 – Storing for cultivation
4 – Emergence of sprouts
5 – Separation and rooting of sprouts
6 – Storing for cultivation ex vitro

ANNEX 2
COMPOSITION OF CULTIVATION ENVIRONMENTS USED IN THE MICROPROPAGATION OF SAINTPAULIA

ENVIRONMENT mg/l	SP 1	SP 2
Macroelements		
KNO3	1900	950
NH4NO3	1650	825
MgSO4, 7 H2O	370	185
CaCl2, 2H2O	440	220
KH2PO4	170	85
Microelements		
Mn SO4 4H2O	22.3	11.15
ZnSO4, 7H2O	8.6	4.3
H3BO3	6.2	3.1
KI	0.83	0.415
Na2MoO4, 2H2O	0.25	0.125
CuSO4, 5H2O	0.025	0.0125
CoCl2, 6H2O	0.025	0.0125
FeNa EDTA	36.7	18.4
Vitamins and amino acids		
mesoinositol	100	100
pyridoxin (HCl)	0.5	0.5
nicotinic acid	0.5	0.5
tiamin (HCl)	0.5	0.5
calcium pantothenate	0.5	0.5
biotin	0.01	0.01
Sucrose	20,000	20,000
agar	8,000	8,000
pH	5.7	5.7
AIA	0.2	
BAP	0.2	

Day 2: micropropagation and rooting of saintpaulia

MATERIALS AND PREPARATION

- Disinfection solution: 12° chlorimetric potassium hypochlorite
- Facemasks
- Protective headwear
- Sterile swabs on a wooden skewer
- Non-sterile 100 ml jar (for the disinfection solution)
- Cases with disinfection tools (scalpel, tweezers, scissors)
- A mini greenhouse

CULTIVATION ENVIRONMENT

Flasks with a wide opening containing 100 ml of sterile, vertically cooled SP2 environment.

MANIPULATION

Several small green leaves have developed in the SP1 environment.
During this stage, we can:

- split the tuft, proceed to micropropagation,
- move the leaves to the SP2 environment for rooting.

- Open the flask with the SP1 environment containing the tuft in a sterile manner.
- Carefully pull out the tuft using sterile forceps, place it on a Petri dish and split it.
- Wipe the inside of the stopper and the neck of the SP2 environment flask with a cotton swab saturated with disinfectant. Insert 1 or 2 tufts into the SP2 environment.
- Disinfect the perimeter of the stopper and the neck of the flask again and close it.
- Place the flask or flasks into 20-25°C, into the light of the cultivation chamber (16 to 18h of light every day).

The tuft and the leaves will grow, creating roots. After 4 to 6 weeks, you can move the sprout to a pot and put it in a greenhouse.

Day 3: transition to compost

MATERIAL

• 1 tray with fertilizing slurry (a mix of compost and water with a thick, dough-like consistency)
• Cups containing the moist compost
• Half of a PET bottle with cap

– Remove the sprout with the agar adhering to the roots to prevent damage.
– Remove the agar with a trickle of water and a gentle shake.
– Soak the roots in the slurry.
– Place the sprout into the compost cup.
– Cover with the PET bottle and place it into the mini greenhouse (with 20°C and lighting).

Prevent the compost from drying out.

ACCLIMATIZATION

Once the sprout is strong enough, let it grow in open air. If you're using a bottle to cover the pots, you can:
- first take the cap off the bottle,
- take off the bottle itself after eight to ten days.

Prevent the compost from drying out and keep it out of direct sunlight.

FINAL REPORT

1. Clarify the importance of the wetting agent.
2. Analyze the composition of the used environments (**see Annex 2**) and compare them.
3. Chronologically summarize the different *in vitro* cultivation procedures with a series of diagrams.
4. Photographically document the evolution of the culture over time in a table.