



Ministry of
Foreign Affairs
Republic of Latvia



IN-VITRO POTATO SEED GROWING TECHNOLOGY

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INTRODUCTION

❖ History of biotechnology

- ❖ Used since ancient times in daily needs (bread making, yogurt, beer, wine, vinegar).
- ❖ Took shape as an independent science in the second half of the 20th century.

❖ ♦ Current importance

- ❖ Helps to solve urgent global challenges:

- ❖ Ecology
- ❖ Food security
- ❖ Medicine

❖ I. Basic rules

- ❖ Change outer clothing and shoes before entering the lab.
- ❖ Wash hands thoroughly with soap up to the wrist.
- ❖ Immediately remove any contaminated sources (bacteria, fungi, yeasts) from the lab.
- ❖ Cover all equipment to prevent dust and spores.
- ❖ Move around the lab only when necessary.



- **Laboratory organization**
- **A) preparation room**
- Tissue culture conducted in separate areas.
- Contains equipment for medium preparation (autoclave, oven, refrigerator)
- Must have shelves for chemicals and clean glassware.
- Key equipment includes:
 - Workbench
 - Chemical agents
 - Glass containers
 - Heating plate and magnetic stirrer
 - Ph meter
 - Water purifier
 - Autoclave



pH Meter



Workbench



Heating plate



Chemical agents



Autoclave



Water purrifier



Magnetic stirrer



Glass container

- **B) storage room for equipment**

- Area for storing sterile test tubes and sterilized water.
- Reduces contamination risk.
- Keep glass containers covered to prevent dust.
- Store chemicals in a lockable cabinet.

- **C) sterile room**

- Used for tissue transplantation and reproduction.
- Organized with laminar airflow to reduce contamination.
- Must have ventilation to prevent dust entry.
- Store sterilized tools and chemicals for a comfortable working environment.

- **D) tissue culture chamber**

- Incubator for growing plant specimens.
- Single incubator sufficient for small samples; multi-level shelves for commercial purposes.
- Must control temperature, light intensity, photoperiod, and humidity.
- Optimal conditions: 10-27°C, 16 hours light, 8 hours dark.

- **Management of laboratory work**
- **Work manager**
 - Oversees laboratory operations.
 - Responsible for planning, organizing, and checking technical equipment.
 - Must have a background in fundamental plant biology (physiology, biochemistry, molecular biology).
- **Laboratory staff**
 - Assists the manager in daily operations.
 - Must track the number of seedlings, their location, and development stage.
 - Maintains an inventory of equipment and ensures it is functional.
 - Creates and follows a preventive maintenance plan (monthly, annual).
 - Responsible for purchasing experimental tools.
 - Classifies chemicals into toxic and non-toxic groups and manages their storage.
 - Organizes the use of laminar airflow shelf.



Practical skills

- Conduct inventory and create a database for rooms and equipment.
- Use appropriate forms for documentation.
- Develop a preventive maintenance plan for equipment.
- Maintain daily inventory of chemicals and supplies to avoid shortages.

Scientific staff

- Users include scientists and laboratory workers.
- Must be familiar with laboratory rules set by the manager or technician.



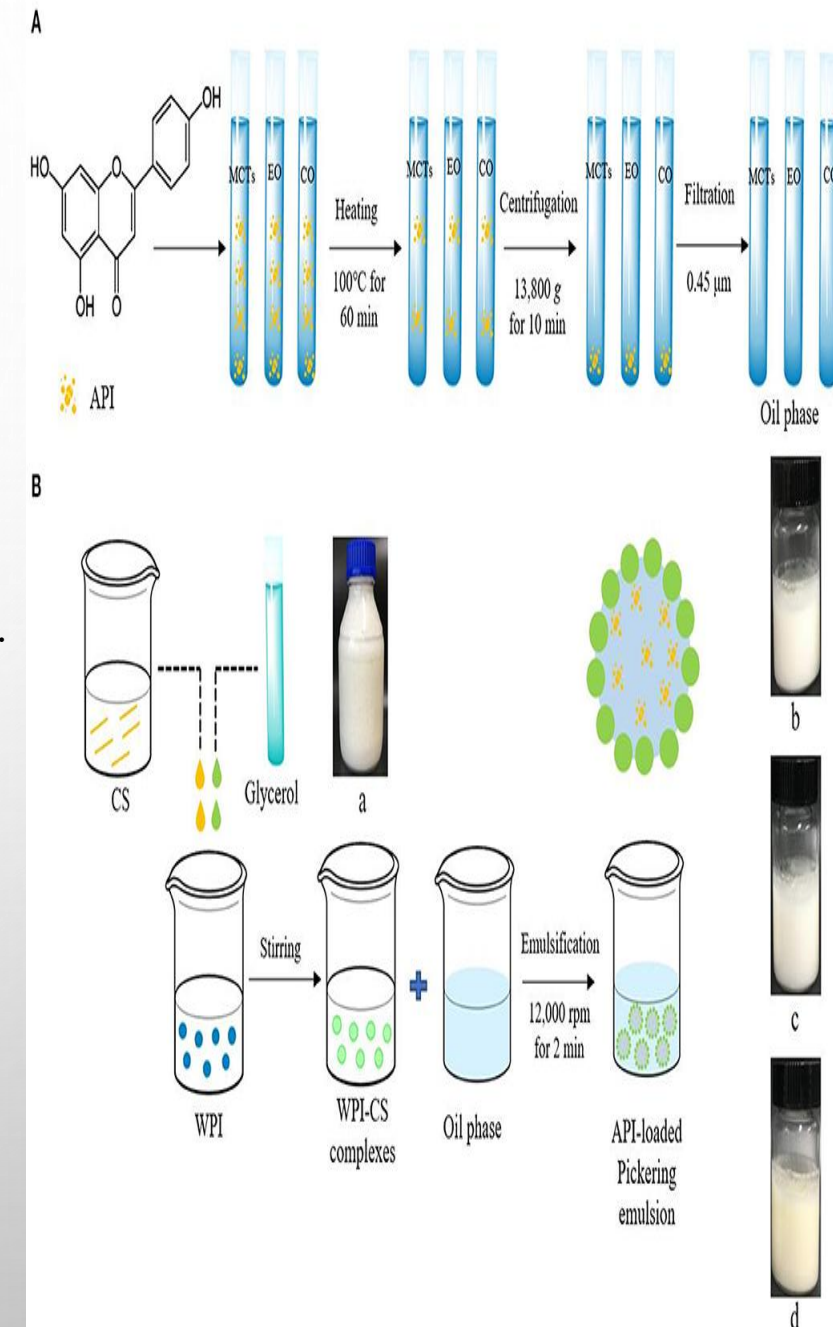
➤ **Laboratory safety**

- **Training requirements:** Before starting work in the laboratory, each person must participate in training.
- Participants will receive instructions on how to use large equipment (desalinizer, sterilizer, scales, pH meter, laminar air flow rack).
- Specific recommendations will be made for the disposal of various types of waste (used blades, broken glassware, toxic chemical waste, and household waste).
- The location and operation of the first aid kit, eyewash station, and portable fire extinguishers should be demonstrated to everyone.
- **Practical skills :** Never slurp liquids out of bottles; use only a pipette (dropper) for instillation.
- Extreme caution is required when working with alkalis and acids; always wear gloves and a face mask.
- Avoid working with alcohol near an open flame.
- Unplug electrical appliances that will not be used for long periods of time.
- Open the autoclave (steam sterilization machine) only when the temperature is below 100°C (when the pressure drops to 0).
- Toxic chemicals must be stored in a well-ventilated area.
- The use of the laboratory as a dining room is strictly prohibited; no eating, drinking, or smoking is allowed.
- Smoking is prohibited near or around the laboratory where smoke may enter.



Preparation of growing medium: Importance of growing medium: Generally consists of:

- Inorganic salts
 - Vitamins
 - Growth regulators
 - Carbon source
-
- Additional components (antibiotics, amino acids) may be added.
 - Gelatinizing agents (agar, phytoGel) are used for rigidity.
 - **Experimental work: preparation of basic solution (ae)**
 - **Notes entry:** record necessary information in the "base solutions" column (refer to table 1).
 - **Macronutrient salts:**
 - Dissolve each in 800 ml of distilled water in separate containers.
 - Calibrate to 1 liter; label with chemical formula, concentration, and preparation date.
 - **Micronutrient salts:**
 - Dissolve each in 900 ml of distilled water one at a time.
 - Dilute to 1 liter; label appropriately (e.g., Stock solution, DKW nutrient medium).
 - **Storage:**
 - Fenaedta must be stored in a gold-colored or foil-wrapped container.
 - Store stock solutions in the dark at +4°C (refrigerator).
 - Ensure labels include concentration, preparation date, and preparer's initials.



- **Sterilization methods**

- **Overview**

- Several methods of sterilizing the nutrient medium and tools (surgical forceps, scalpel, needle, forceps, etc.) Are used in plant tissue culture:

- **1. Sterilization using an autoclave**

- **Description:**

- Most laboratories sterilize culture medium and equipment by autoclaving, where they are heated with steam under pressure.

- **Standard conditions:**

- Maintain a temperature of 121°C for 15-20 minutes.
- Larger amounts of nutrient media will take longer to sterilize.

- **Practical skills:**

- For complete sterilization, the temperature of the feed medium must rise to 121°C and be held at that temperature for at least 15 minutes.
- Preheating mixed solutions can reduce the time it takes for the autoclave to reach 121°C.
- Ensure that the air inside the autoclave is fully evacuated to allow steam to treat all materials effectively.



- **2. Sterilization by dry heat**

- **Method:**

- Glassware and metal utensils can be sterilized by keeping them in an oven for 4 hours at 180-200°C.

- **3. Heating of working equipment in fire**

- **Chemical sterilization:**

- Needle tools are usually soaked in 95% alcohol and then sterilized by flaming.
- Hydrogen peroxide is preferred as a better sterilizing agent.

- **4. Glass bead sterilizer**

- **Method:**

- Instruments such as forceps and scalpels can be sterilized by heating them for 15 seconds in an electric glass bead sterilizer.

- **5. Filtering**

- **Note:**

- Effective drugs that cannot withstand strong heat should be sterilized by filtration and put into the autoclave only when the internal temperature drops below 40°C.



- **Laminar air sterilization**

- **Overview**

- A completely sterile or germ-free operating environment is critical for the success of in vitro tissue culture.
- All work processes must be carried out in a sterile environment using a laminar airflow cabinet.

- **Laminar airflow cabinet**

- **Function:**

- The primary filter captures larger particles in the incoming air.
- A highly efficient HEPA filter eliminates smaller dust particles.

- **Maintenance:**

- Inspect the filter regularly (at least once a year).
- Sterilize and wrap all tools and glassware in aluminum foil or place them in a metal box, only to be opened during operation in the cabinet.

- **Preparation before work**

- Start the laminar air flow cabinet at least 30 minutes before work.
- Disinfect the surface with 70-90% ethanol or isopropanol.
- Place all nutrient media, tools, glassware, and microscopes in the cabinet during air circulation to remove foreign particles.

Practical skills

To avoid contamination when working with a laminar air flow cabinet:

- Wash hands thoroughly before starting work.
- Wipe the cabinet surface regularly with 70-90% alcohol.
- Sterilize tools like forceps and scalpels by igniting or heating them, then allow them to cool.
- Be cautious with alcohol as it catches fire easily.
- Avoid moving hands over exposed sterilized specimens or culture medium containers.
- Disinfect hands regularly.
- Ignite the mouth of containers before opening and close immediately after use.
- Minimize talking over the laminar air flow cabinet.
- Remove all unwanted materials from the airflow path.
- Roll up lab coat sleeves and hide hair with a hat.

Sterilization of plant tissue surfaces

Sterilization of plant tissue surfaces

Contaminating microorganisms

- All plant parts and tissues have microorganisms on their surfaces that must be treated with a sterilizing chemical.

Disinfectants table

Disinfectants	Concentration	Contact Time	Result
Sodium hypochlorite	0.5 to 5%	5' to 30'	Very good
Calcium hypochlorite	3 to 10%	5' to 30'	Very good
Mercuric chloride	0.1 to 1%	1' to 15'	Satisfactory
Hydrogen peroxide	3 to 10%	5' to 15'	Good
Bromine Water	1 to 2%	2' to 10'	Very good
Silver nitrate	1%	5' to 30'	Good
Kohrsolin	3%	15' to 20'	Good
Ethanol	70%	5 sec to 5'	Good



- **Recommended sterilization conditions**
thin leaf: 1.00% naocl for 12-15 minutes
thick leaf: 1.50% naocl for 15-20 minutes
onion: 1.50% naocl for 30 minutes
core: 1.5 to 2.0% naocl for 25-30 minutes
seed: 2.00% naocl for 30 minutes

washing before disinfection

thoroughly wash plant samples under running water for 1 hour before disinfection.

Sterilization methods

(A) one-time processing:

70% ethanol for 5 seconds.

1% naocl + non-ionic soap for 15 minutes.

Rinse with sterile distilled water three times for 5 minutes each.

(B) generalized sterilization (2-fold treatment):

70% ethanol for 5 seconds.

1% naocl + non-ionic soap for 15 minutes.

Rinse once in sterile distilled water for 5 minutes.

3% kohrsolin for 15 minutes.

Rinse three times in sterile distilled water for 5 minutes each.

(C) generalized sterilization (3-fold processing):

70% ethanol for 5 seconds.

7% benlate for 30 minutes.

Rinse once in sterile distilled water for 5 minutes.

- 1% naocl + non-ionic soap for 15 minutes.
- Rinse once in sterile distilled water for 5 minutes.
- 3% kohrsolin for 15 minutes.
- Rinse three times in sterile distilled water for 5-10 minutes each.
- **(D) sterilization of dormant plant variety:**
 - Wash with antibacterial soap, dry in air.
 - Immerse in 70% ethyl alcohol for 1 minute.
 - Bleach buffer solution: mix 300 ml water with 5 grams of K_2O_4 and add 42 ml bleach to make 500 ml.
 - Place on a rotary shaker for 1 hour.
 - Rinse at least twice with sterile water.
 - Cut the meristem by peeling off the bud sheath.
- **(E) sterilization of vegetative (growing) tree sample:**
 - Wash with antibacterial soap and air dry.
 - Mix 2.8 grams of nadcc and 30 ml of RRM per 1 liter of water.
 - Place in a rotary shaker for 1.5 to 4 hours, depending on material hardness.

Practical skills in plant tissue culturesafety precautions

- **Sulema (mercuric chloride):**

- A poisonous substance; handle with care.

- **Lyapis (silver nitrate):**

- Requires the same level of caution.

- **Waste disposal:**

- Collect waste from toxic drugs in a separate container.

Work process :1.Preparation:

1. Organize the desk according to the instructor's instructions.
2. Cut stems from potato tubers and place them in a beaker.
3. Cover the beaker's mouth with gauze and secure it with a rubber band.

2.Washing:

1. Rinse the beaker under running water to remove dust and mud.
2. Wipe and dry with a paper towel.

Cutting explants:

1. Cut the tuber stems into explants with 2-3 joints each on a laminar airflow work table.
2. Quickly immerse explants in 70% alcohol for 3 seconds.

Disinfection:

1. Transfer explants to 1% naocl and soak for 15-20 minutes.
2. Agitate the container to ensure thorough mixing with the disinfectant.
3. Rinse in sterile distilled water.
4. Soak in 3% kohrsolin for 15 minutes.
5. Rinse three times in sterile distilled water for 5-7 minutes each.

Preparation for transplanting:

1. After rinsing, place explants on a petri dish or sterile paper.
2. Using sterile forceps and a scalpel, cut out both thirds of the explants, leaving one axillary bud on each.
3. Sterilize tools by flame after each dissection.

• Transplanting:

- Light the neck of the tissue culture vial before opening it.
- Transplant single-joint explants into sms medium, ensuring proper placement of the base onto the medium solidified with agar.
- Ignite the test tube again before capping it.
- Repeat the process until all explants are planted (16-18 times).

• Labeling:

- Label test tubes with plant species name, nutrient medium, age, and worker's initials.

• Incubation:

- Transfer transplanted plant tissues to an incubator (or grow room) at 20°C for 14 hours.

Practical skills:

(a) in order to determine whether endophytic microorganisms (such as bacteria, fungi, yeast) have fallen on similar materials during tissue development, two types of nutrient media can be used, they are:

(i) bactonutrient agar with 1% glucose and 0.5% yeast extract, ph5.8

(ii) LPGA: 1% glucose + 0.5% yeast extract +0.5% bacto peptone +0.8% agar, ph 5.8.

(B) propagation in a test tube

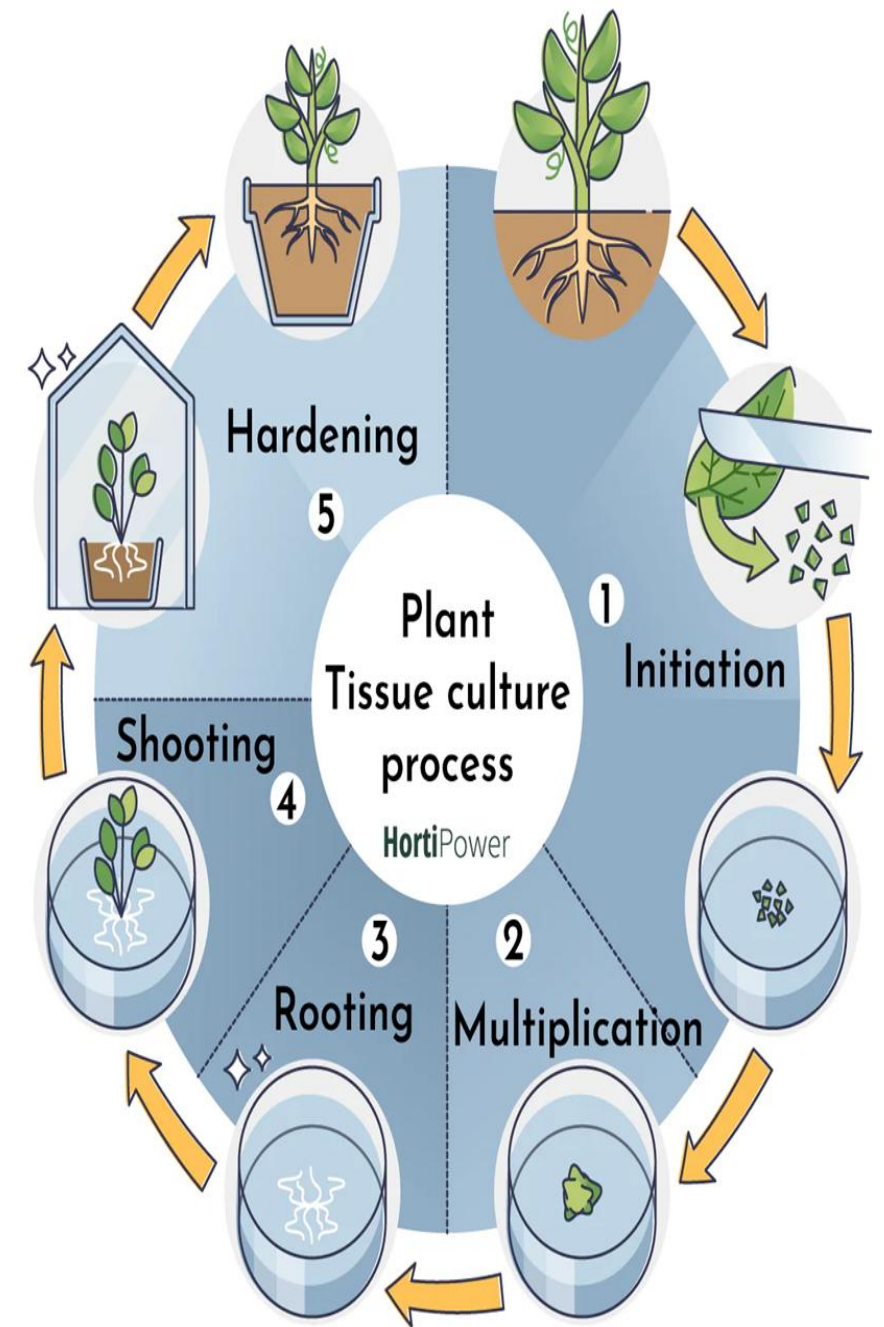
the purpose of this experiment is to acquaint laboratory personnel (laboratorians, students, etc.) With the proper and effective method of cell propagation in a test tube.

Experiment 4:

placement of large-scale potato (var. S. Tuberosum ssp.) In a test tube.

Materials needed for the experiment:

- plant material (sprouting potatoes)
- laboratory beakers, beakers, measuring glass bottles (100 ml)
- glass bottles with blue caps (250 ml)
- clamps
- scalpels
- knives
- 70% alcohol
- naocl solution and a few drops of additional moisturizing agent (non-ionic soap)
- 3% kohrsoline
- sterile distilled water (SDS)
- CMS - glass test tubes with potato growing medium
- sterilized petri dish (or sterile paper)
- paper towel
- shortcuts



Work process:

1. Start the laminar air flow cabinet 20-30 minutes before starting work.
2. Wipe the surface of the air flow cabinet with 70% alcohol.
3. Organize the desk following the instructor's instructions.
4. Use a checklist to make sure you have everything you need to get started.
5. Disinfect your hands together with the wrist using 70% alcohol (or other disinfectant).
6. Take the test tube with potato tissue in your left hand.
7. Open the cap of the test tube with your right index finger.
8. Remove the microplant (tissue) using sterile forceps and place in a petri dish (or on a sterile paper towel).
9. Cut the tissue into 4-5 unicellular pieces (explants) with a sterile scalpel while holding the tissue with sterile forceps.
10. Cut the microplant in thirds and place the monocot pieces in DKW nutrient medium using forceps.
11. Close the lid of the test tube containing the tissue.
12. Repeat this process (6-11 times) until you have used all the plant material for the experiment.
13. After appropriately labeling the tubes (name of the plant, name of the nutrient medium, date and initials of the worker), they are placed in a rack and placed in an incubator or growth room under a temperature of 20°C with a light regime of 17 hours.



- **Experiment 5:** growing potato plants in a test tube.
Materials needed for the experiment:
 - cultivated potato plant tissues (4-5 weeks of development)
 - DKW nutrient medium
 - clamps (20 cm)
 - scalpels (2 pcs.)
 - No. 10 blades
 - sterilized petri dish (two sterile paper towels)
 - 95% alcohol (for igniting tools)
 - 70% alcohol (for surface disinfection)
 - base for test tubes
 - paper towel
 - shortcuts
 - black pen

propagation of plants from cells

(a) placing in a test tube

purpose: the purpose of this experiment is to familiarize tissue workers (laboratorians, students, etc.) With tissues free of microorganisms (such as bacteria, fungi, yeast) and contaminating foreign materials.



Experiment 6:

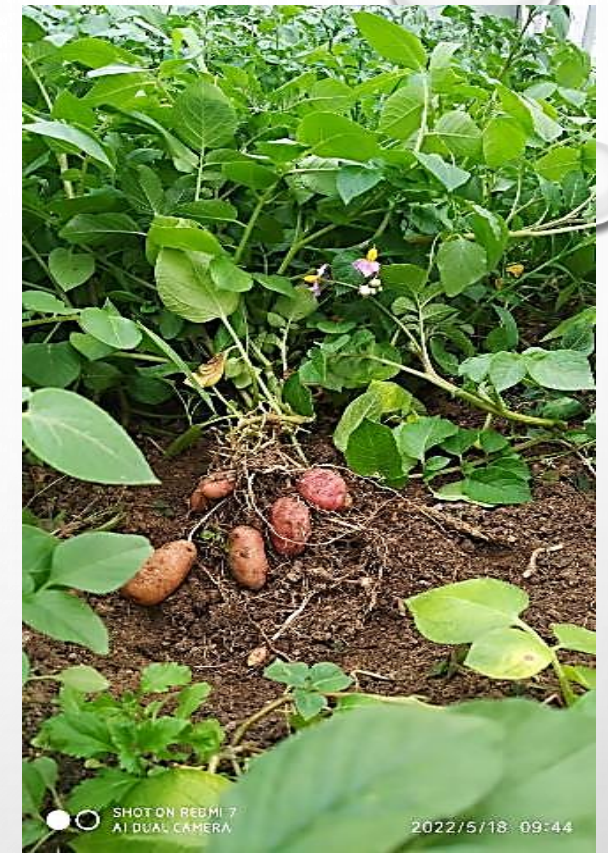
transplantation of potato plants grown in a test tube by adaptation to greenhouse conditions.

Materials needed for the experiment

- samples of potatoes rooted in a test tube (4-5 weeks ripe)
- for planting, a mixture of ordinary soil, perlite or sterilized peat/compost/sand (1/1/1)
 - plastic bag
- nutrient solution (1/2 MS nutrient medium without sucrose)
 - trays







In our research institute, potatoes are being cultivated using the in vitro method.

THANK YOU FOR YOUR ATTENTION!

