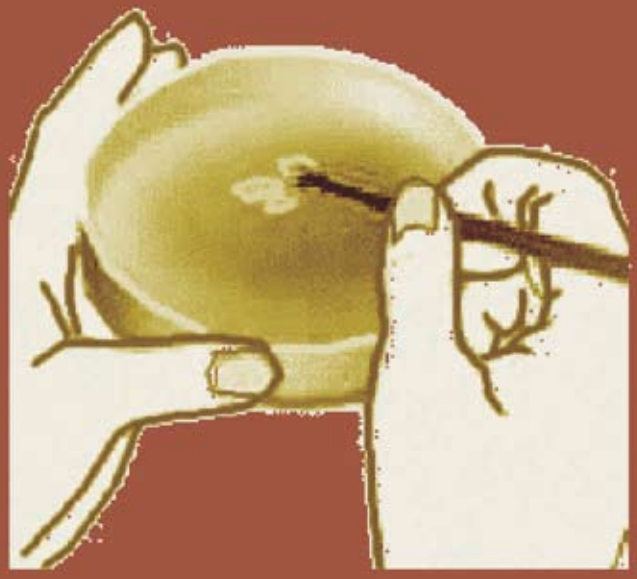
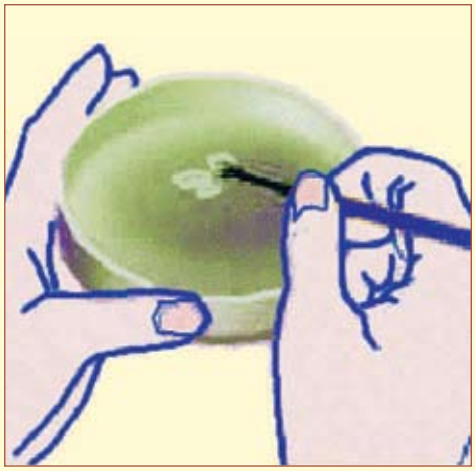
Scene Eight



Tissue culture

Wednesday morning

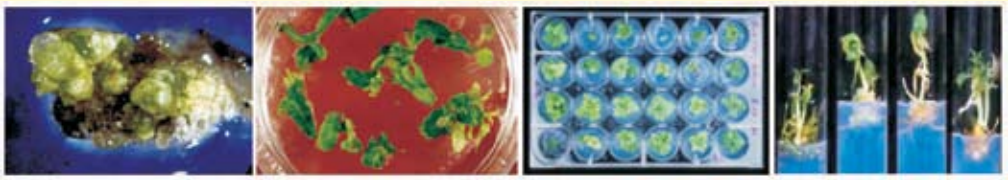
Scene Eight:	Wednesday morning
Place:	Classroom
Characters:	Students, teacher and Dr Mardea
Situation:	Dr Mardea talks with the students about tissue culture and cryopreservation



A petri dish used for plating cells in a nutrient medium in the form of gel

Photo source: G.D. Stone

Cassava plants growing from callus



Different stages of cell culture

Scene Eight:	Wednesday morning
Place:	Classroom
Characters:	Students, teacher and Dr Mardea
Situation:	Dr Mardea talks with the students about tissue culture and cryopreservation

Teacher:


Teacher

Good morning. I am sure you all remember something about PGR. Today we will learn more about plant tissue culture and cryopreservation from Dr Mardea.

Dr Mardea:


Dr Mardea

Hello students. Are you ready? Shall we talk about tissue culture techniques and how cryopreservation is essential, for us to use tissue culture as a biotechnology tool for preserving genetic resources?

Safwani:


Safwani

Yes, Dr Mardea. This is so interesting **(*with a bright smile*)**

Dhakiya:


Dhakiya

I can't wait to know the details **(*says in a thrilled voice*)**

Dr Mardea:


Dr Mardea

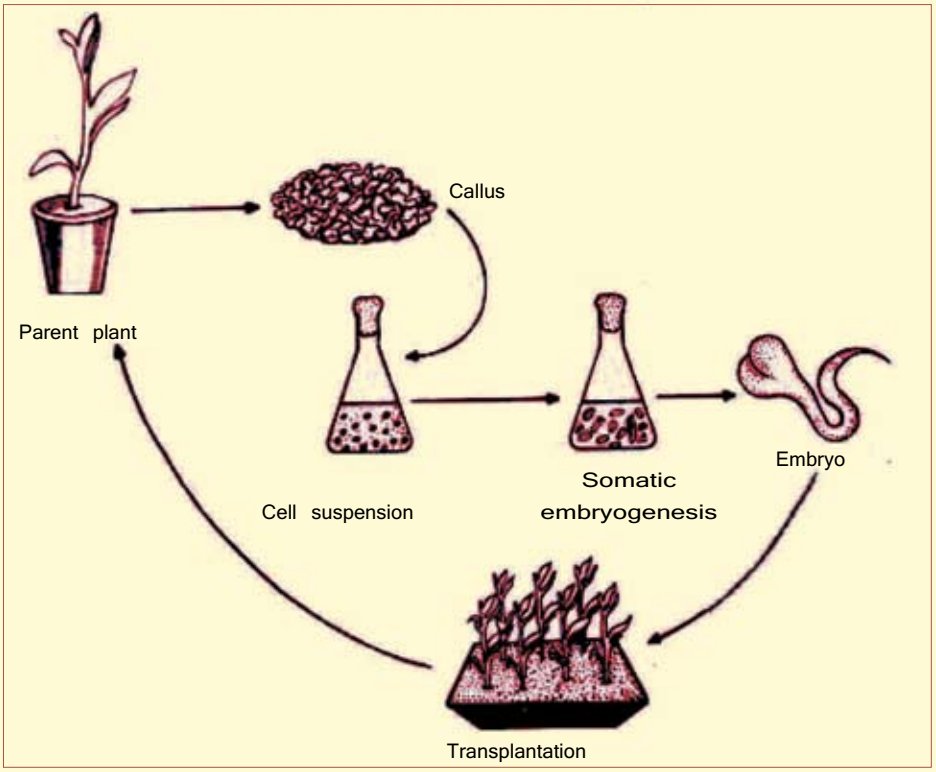
Right, let me tell you about the history of plant tissue culture. It begins with the concept of the "cell theory" developed independently by the German botanist Matthias Schleiden in 1838 and the German physiologist Theodor Schwann in 1839. These two scientists said that the cell is a "functional unit". The first plantlet formation in vitro was reported as early as the 1940s. In 1946, Ernest Bell reported it in plants such as Tropaeolum and Lupinus. In 1965, the French botanist George Morel tried to obtain a virus-free orchid plant and discovered, that a millimetre-long shoot could be developed into complete plantlets by micropropagation. This was the beginning of plant tissue culture.

Kunto:


Kunto

The plant tissue culture techniques must be an old one then.

Tissue culture stages



Sterile culture hood where the cells are isolated and plated for culture

Dr. Mardea:

Yes, Kunto, it is.
You know, three criteria should be satisfied for the tissue culture techniques to be successful: first, the parts of a plant which have totipotent cells should be used; second, this plant material must be clean, disease-free and not contaminated; and third, this material must be grown in an aseptic or a sterile environment in a sterilized nutrient medium.


Dr Mardea

Dalila:

And what are the culture cells grown in?


Dalila

Dr. Mardea:

Tissue culture can be carried out in any test tube, a small container or a dish that contains the culture medium. The culture medium is a special solution that scientists prepare, that contains all the necessary ingredients for the plant to grow, including food.


Dr Mardea

Dafina:

Is the culture medium essential for the cells to grow?


Dafina

Dr. Mardea:

Yes, Dhakiya. While the germplasm is going through the culture phase, it can also be cleaned of viruses and possible diseases. After this, it has to be stored in a place where it will retain its viability. Cryopreservation ensures long-term and safe storage of plant species. A large collection of germplasm can be stored in liquid nitrogen using the cryopreservation technique.


Dr Mardea

Efia:

Yes, we talked about cryopreservation in our biology class, but I have forgotten now what it means.


Efia

Dr. Mardea:

Cryopreservation is used for storing samples at ultra-low temperatures, like 196°C, often in liquid nitrogen. For several species, such as potato, apple, banana or cassava, procedures have been developed which allow this technique to be routinely applied for conservation. Besides this, cryopreservation is also used for the long-term storage of seeds.


Dr Mardea

Photo: Deo Resurreccion

Cryopreservation of germplasm

Cryopresrvation of plant parts

Efia:

How does tissue culture help in this technique? Is there any connection between tissue culture and cryopreservation?

Dr. Mardea:

Yes, there is a direct link between these two techniques. Cryopreservation is used for long-term storage of PGR. When we wish to reuse the stored PGR we have to use the tissue culture method to propagate it.

Dhakiya:

How about cassava tissue culture? Is a lot of cassava germplasm analysed and stored using cryopreservation?

Dr. Mardea:

Yes, quite a lot of work has been done on cassava germplasm conservation using the cryopreservation technique. I will explain to you how cassava PGR can be collected and stored for future use and how it is being cultured in order to increase the number of plants available for farm use.

Efia:

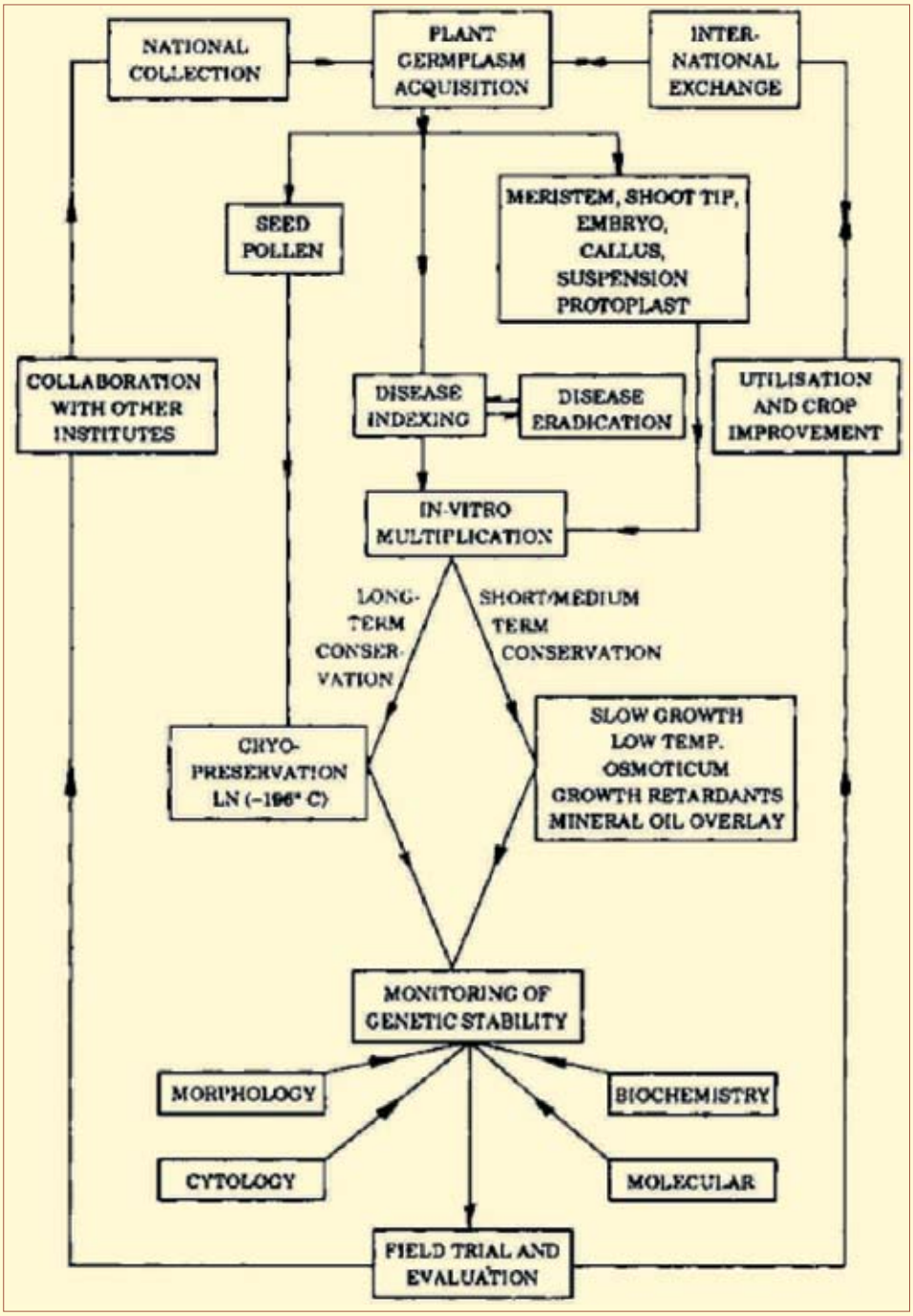
Is the process of culturing and storing cassava PGR for future use called cassava PGR conservation?

Dr. Mardea:

Yes. I am glad that you already know about conservation. Now, I want you to keep in mind, that there is more than one biotechnology tool used for conserving cassava genetic diversity.

There are tools used: for collection and assessment of wild and cultivated cassava varieties; as diagnostic methods for clean germplasm transfer; for long-term conservation of genetic resources; and for tissue culture, particularly for germplasm conservation, exchange and micropropagation.

So, you see, tissue culture is a biotechnology tool that can be used in many ways in the process of conserving cassava varieties.



Schematic representation of in-vitro conservation and cryopreservation of germplasm in the National Plant Tissue Culture Repository, NBPGR

Source: Plant Genetic Resources Conservation: Recent Approaches - K.P.S. Chandel and Ruchira Pandey Plant Genetic Resources Conservation and Management Concepts and Approaches Published by the International Board for Plant Genetic Resources, Regional Office for South and

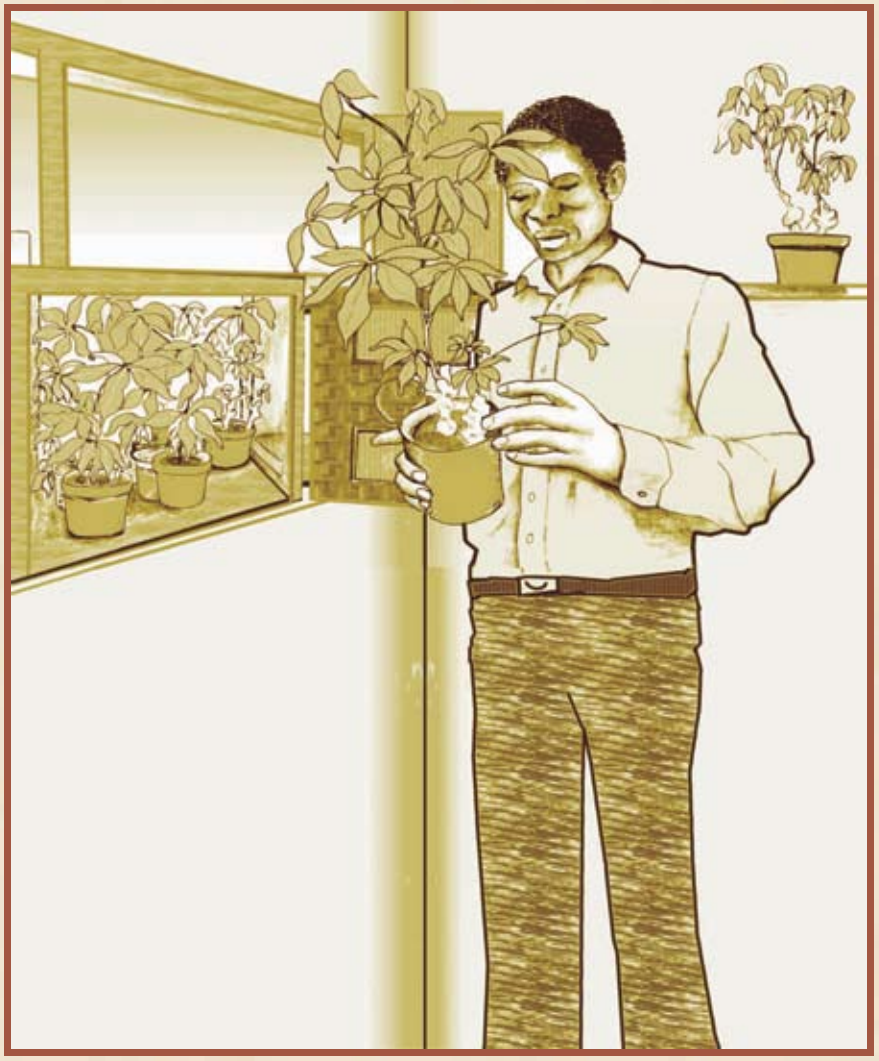
Teacher:

Thank you, Dr. Mardea for the valuable information you presented to us today.

Well, class, it is Friday and it is time for your lunch break. After lunch, come back here to spend as much time as possible looking at these pictures. Off you go now and have a nice weekend.

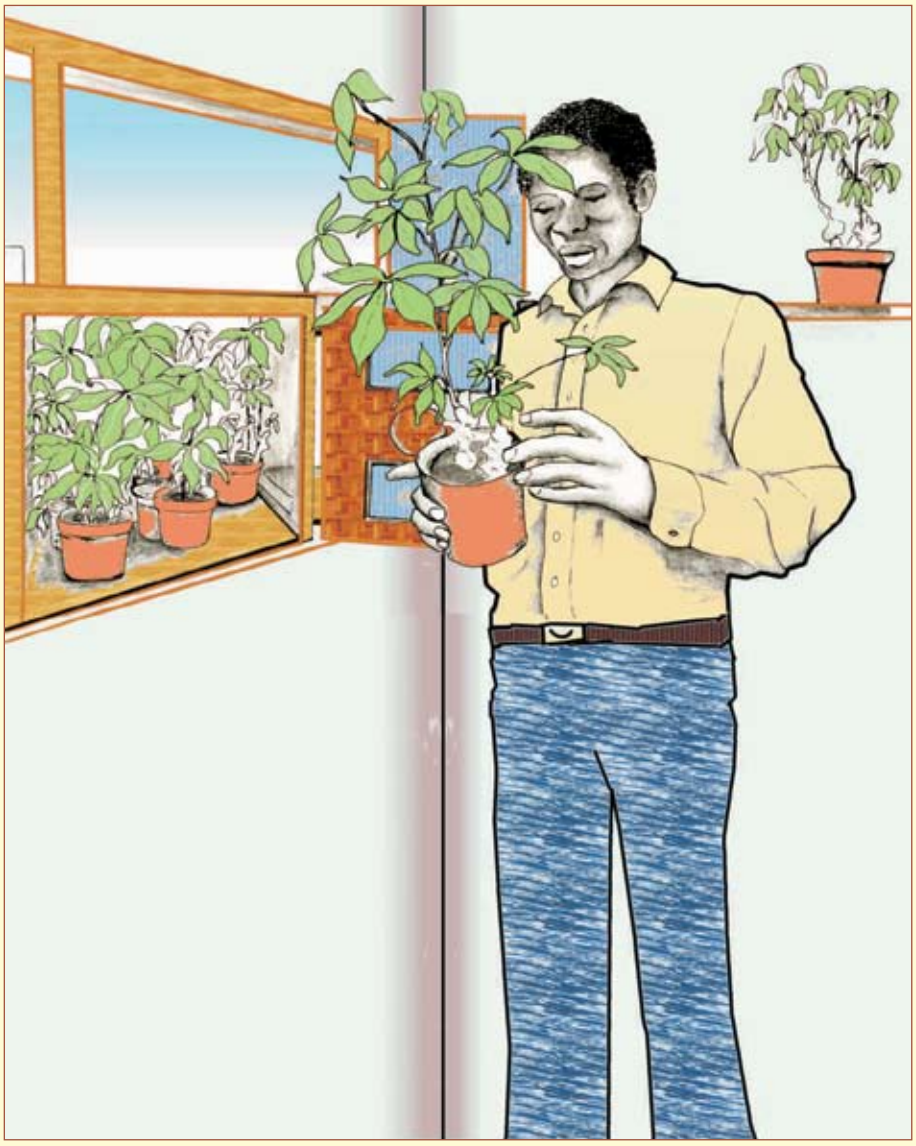
(***The students leave the classroom to go to lunch and on the way they are discussing PGR. They continue to talk about it during lunch. They rush back afterwards to check what is on the wall in their classroom. To their surprise, there are many pictures showing all kinds of equipment used in tissue culture and cryopreservation, as well as a nice schematic diagram showing the steps involved in tissue culture. There is also another schematic diagram showing the steps used in freezing and thawing samples for cryopreservation. The students spend some time looking at the pictures until the bell rings for the afternoon classes. As everything seems so interesting, at the end of the class they go back again to*** **discuss** ***the pictures on the wall.***)

Scene Nine



Friday morning

Scene Nine:	Friday morning
Place:	Classroom
Characters:	Students, teacher and Dr Mardea
Situation:	Dr Mardea talks with the students about tissue culture and micropropagation



Fully grown cassava plants obtained using micropropagation

Micropropagation (1)under the hood, (2) in the culture room & (3) outdoor

Scene Nine: Friday morning

Place: Classroom

Characters: Students, teacher and Dr Mardea

Situation: Dr Mardea talks with the students about tissue culture and micropropagation

Teacher:

Good morning. On Wednesday Dr.Mardea told us all about tissue culture and how cryopreservation is essential in order to use tissue culture techniques to conserve genetic resources. Does anyone have any questions regarding tissue culture techniques?

Thandiwe:

(***Asks with a big question mark on his face***)
I have a question. If we culture plant cells in the culture room and take them immediately to plant in the field, we will not need cryopreservation technique. So why is cryopreservation considered as an essential technique for propagating plants and conserving germplasm?

Teacher:

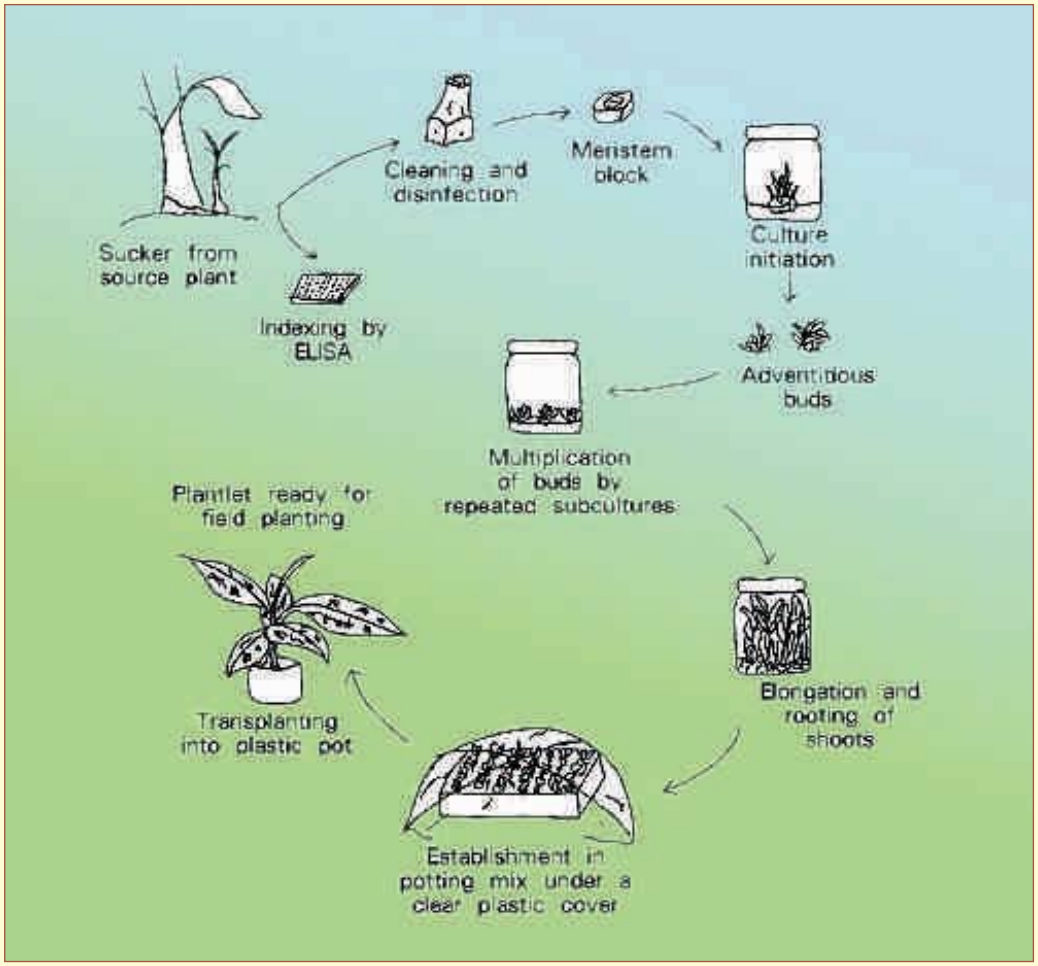
Well Thandiwe, do you remember why we need to conserve germplasm and if we can take it for granted that the plants will always be available in the lab and in the field?

Runako:

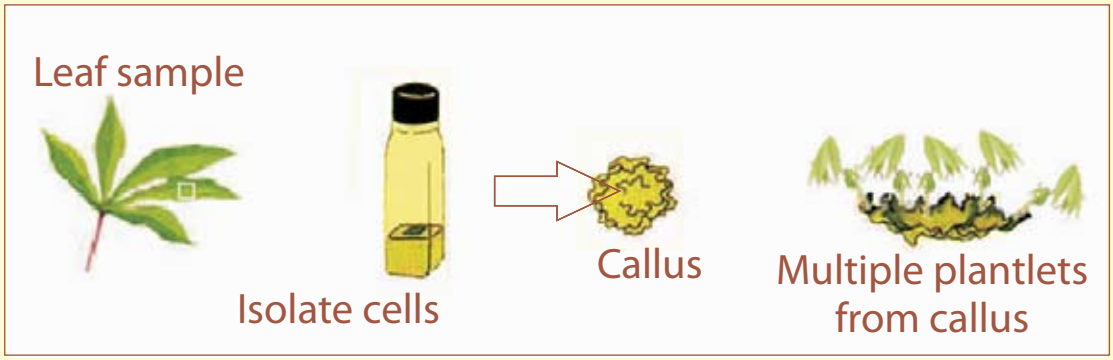
Thandiwe, if we want to save the germplasm for a long time and keep it safe from disasters we have to save it somewhere where it will not be spoiled and that has to be the deep freezing cryochamber ***(with a bright smile).***

Teacher:

Well done Runako. Thandiwe, your thinking is not all that wrong but you must remember that conservation is the critical process that will give us safety and security. You must think along these lines about how and why these tools are useful. The tools on their own will not be useful if they are not applied for a higher, safer and secure cause, for the well-being of humanity. Very well then, if there are no more questions, today we will learn more about plant tissue culture and micropropagation from Dr Mardea.



Steps in plant propagation



Steps in micropropagation

Dr Mardea:

Hello students. Are you ready? Shall we talk about tissue culture technique and how micropropagation is essential to use tissue culture as a biotechnology tool for conserving genetic resources?
You know a lot about plant tissue culture. Let me tell you about another biotechnology tool that is very closely related to tissue culture. It is called "micropropagation".


Dr Mardea

Dene:

Propagation at a very small level?


Dene

Dr Mardea:

No Propagation at microlevel. Micropropagation is also initiated in a culture room under sterile conditions. You see how close these two techniques are?


Dr Mardea

Masara:

If they are so similar, why do they have two such different names as tissue culture and micropropagation?


Masara

Dr Mardea:

Even though these two biotechnology tools are closely related, there is a subtle difference between them in the way we use them. The common term between them is propagation. We propagate cells in the tissue culture room. We propagate small growing plantlets using the micropropagation technique.


Dr Mardea

Adesimbo:

So there is a common technical factor but the samples used are different?


Adesimbo

Dr Mardea:

Not really. The commonality, as I said before, is the concept of "propagation". Tissue culture, is the process whereby a small piece of living plant tissue called explant, is isolated from a plant and grown aseptically on a nutrient medium under controlled conditions. Micropropagation is the method whereby large numbers of plants, are produced from small pieces of the stock plant, or parent plant in


Dr Mardea

Types of plant propagation

Sexual propagation

Seeds and spores are used for reproduction

Asexual propagation

Seeds produced without fertilization. The embryo arises from an unfertilized egg or embryo and is formed from the surrounding embryo sac tissue. Plants are genetically identical from one generation to the next.

Vegetative propagation

A type of asexual reproduction without production of seeds or spores. Both **a natural process** as well as one utilized by horticulturists to obtain quantities of economically valuable plants - uses vegetative plant parts or roots, stems and leaves

Micropropagation

Used by people to multiply plants. Used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative propagation

Establishment

Micropropagation begins with the collection of a sterile explant(s). This small portion of plant tissue, which may be as small as a cell, is placed on a growth medium containing sucrose as an energy source and one or more plant growth regulators like plant hormones. Usually the medium is thickened with Agar to create a gel which supports the explant during growth. The plant tissue now begins to grow and differentiate into new tissues.

Pretransplant

The plantlets/shoots are treated to produce root growth and "hardening" (preparation of the plants for a natural growth environment). This is performed *in vitro*, or in a sterile "test tube" environment.This stage (pretransplant) is not always performed.

Transfer from Culture

In the final stage of plant micropropagation, the plantlets are removed from the plant media and transferred to soil or potting compost for continued growth by conventional methods. This stage is often combined with the "Pretransplant" stage.

Multiplication

Following the successful growth of plant tissue, the establishment stage may be repeated, by taking tissue samples from the plantlets produced in the first stage. Through repeated cycles of this process, a single cell sample may be magnified to hundreds or thousands of plants.

relatively short periods of time using tissue culture techniques.


Adesimbo

Adesimbo:

Oh! I understand. Tissue culture is one of the techniques used in micropropagation. Am I right Dr. Mardea?


Dr Mardea

Dr Mardea:

Yes! You are correct. But can anyone tell me what other steps are used in the practice of micropropagation?

(There is a silence and then Papukayi says.)


Pepukayi

Pepukayi:

No! What are the steps involved in micropropagation?

Dr Mardea:

Micropropagation involves 4 steps. A stock plant is a parent plant. Micropropagation is usually used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative propagation


Dr Mardea

I have one large picture, which I will leave with you, that shows all the steps used in micropropagation. This will be useful for the presentation that you are supposed to be working on. Each of these carries a description underneath that you should read in order to understand what the steps are.

Teacher:


Teacher

Thank you Dr Mardea for the valuable information you presented to us today.

Well, class, it is Friday and it is time for your lunch break. Off you go now and have a nice weekend.

(The students leave the classroom to go to lunch and on the way they talk about micropropagation.)