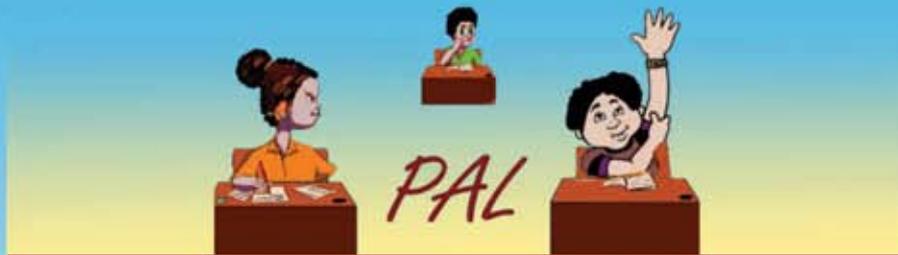


# Participate and Learn



## *Biotechnology Tools for Conservation and Use of Plants* A school play for senior students



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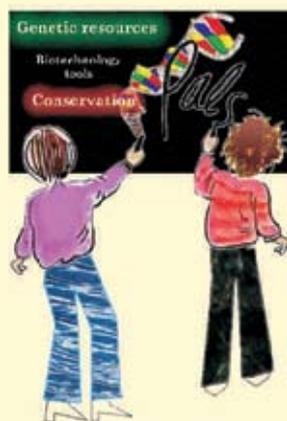




## ***Acknowledgments***

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***PAL: story line, script and graphic design***  
***by***  
***Roopmathy Speedy***



***Character illustration***  
***by***  
***Ivy Steele & Roopmathy Speedy***

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## Preface

With the droughts and other natural disasters in the African continent, which adversely affect agricultural production, people often struggle to survive. Droughts have caused hunger problems in Africa and malnutrition is widespread, even during times when growing conditions are good. Experts argue that ploughing up yet more wilderness, cutting down forests or increasing the area of land under agriculture is no longer a viable option to solve food production problems. To conserve the present ecosystems, increased food production must be limited to the cropland currently in use. Therefore, new ways to increase food production from existing resources need to be applied.

An extension of traditional plant breeding, plant biotechnology, uses genetic knowledge and scientific techniques to add specific traits to crops. These traits benefit farmers, including those in developing countries where crop losses due to weeds, pests and diseases are high and conventional tools to overcome these problems are unavailable or unaffordable. It is now argued that biotechnology is by default our best and, maybe, only way to increase production to meet future food needs.

With the increasing importance of biotechnology, especially in developing countries, educating young students, who will become the decision makers of the future, is a key factor in spreading knowledge about use of biotechnology.

The Food and Agriculture Organization of the United Nations (FAO) is the leader of international efforts to defeat hunger. Achieving food security for all is at the heart of FAO's efforts - to make sure people have regular access to enough high-quality food to lead active, healthy lives.

Through this book, and in line with its mandate to provide information to raise the levels of nutrition, improve agricultural productivity, better the lives of rural populations and contribute to the growth of the world economy, FAO is helping young generations to understand the concept of conservation and how knowledge of biotechnology can help conserve genetic resources. In addition, this book invests in the introduction of methods of conservation to youth at an early stage of their education. This is in line with the International Treaty on Plant Genetic Resources for Food and Agriculture whose objectives are the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising out of their use for sustainable agriculture and food security.

This book is an elementary introduction of a complex subject to encourage students to learn further about biotechnology for the future. The knowledge in this book is presented in the form of a play. This knowledge is intended to help students in their decision making process during their higher education.

It is hoped that this book will be both entertaining and educational.

**Roopmathy Speedy**  
Consultant

**Elcio P. Guimarães**  
Senior Officer  
Cereals/crops Breeding  
Crops & grassland Service (AGPC)  
FAO

**Kakoli Ghosh**  
Agriculture Officer  
Seed and Plant  
Genetic Resources Service (AGPS)  
FAO

## *Biotechnology Tools for Conservation and Use of Plants*

### *About the book*

This book, with script, graphics and photographs, is written as a “play” for high school students aged 15 to 16 years. It is intended to introduce students to the term “biotechnology” and to inform them about the role of biotechnology tools in the conservation and use of plant genetic resources (PGR). The play encourages active participation of students by adding a touch of entertainment to scientific content. Besides the term “biotechnology”, the play also introduces the concept of “conservation” and the logic behind it. In particular, it is expected to educate students in understanding the fundamentals associated with biotechnology and to explain how biotechnology can contribute to the process of conserving plant genetic resources. The cassava plant, which is a major staple food in Africa, is used as an example, whenever possible. The first few examples of biotechnology tools discussed in the book are the plant tissue culture technique, micropropagation, cryopreservation and the techniques associated with marker-assisted selection.



*I hope, this plant will be healthy*

By the end of this play, students are expected to have gained enough knowledge to confidently discuss “biotechnology” and “conservation” with their teachers and friends. This play is also intended to create interest in young minds to encourage them to learn more about applying biotechnology tools towards conserving existing natural resources.

## How to use this book

### Information for the teacher & the students

There are many characters in this play. It is recommended that the teacher plays the part of "biology teacher" as well as that of "Dr. Mardea". All other characters should be assigned to students.

There are limited numbers of "student" characters. Therefore, students in the class may have to share the parts of the "student" characters. Each student may play a character in one scene and give the opportunity to play the same character in the following scene to another student. This means that a specific character will be played by more than one student before the completion of the play.

Depending on the class size undergoing this exercise, the number of students reading each "student" character needs be previously assigned by the teacher. This will allow every student in the class to take part in this exercise.

In order to provide a smooth transition between the students reading the same character, participants should read their lines at home and try to understand the content before they read their part in the class room. If they do not understand the content then they should contact their teacher and request clarification before their reading session. It is important that the students have a clear knowledge of what they read when they play their role.

Once a student has read/played a character in a particular scene, he/she should listen and follow the same character throughout the play. At the end of the play, the students who read the same character should get together and discuss the role of that character in the play and the interest of that character pertaining to "PGR", "conservation" and "biotechnology tools".

After the completion of the play, each student in the class will be expected, individually or in groups, to demonstrate what he/she has understood regarding "PGR", "conservation" and "biotechnology tools" in any acceptable format such as a poster, drawing, essay, painting, poetry, slide show, presentation or even another play script.





## Classmates from school:



"Masara" means "joy"



Dalila means "gentle"



"Dene" means "water lily"



"Adesimbo" means "noble birth"



"Mandisa" means "sweet"



"Hakika" means "truth"



"Hadiya" means "gift"



"Runako" means "handsome"



"Gathii" means "wanderer"



"Sabola" means "hot"



Pepukayi" means "wake up"



"Safwani" means "sincere"



## Teachers:

Biology teacher

Visiting scientist Dr. Mardea



*Characters in this play (each name has a meaning):*

## A family:



← Father "Abeeku" means "born on Wednesday",

Mother "Adowa" means "Born on Tuesday",



← Son "Ekow" means "born on Thursday",

Daughter "Efia" means "Born on Friday",



← Grandpa "Atu" means "born on Saturday",

Grandma "Ama" means "born on Saturday"



← Uncle "Sisi" means "born on Sunday";

## Classmates from school:



"Dhakiya" means "Intelligent"



"Dafina" means "precious"

"Gamba" means "warrior"



"Kunto" means "third child"

"Thandiwe" means "beloved"



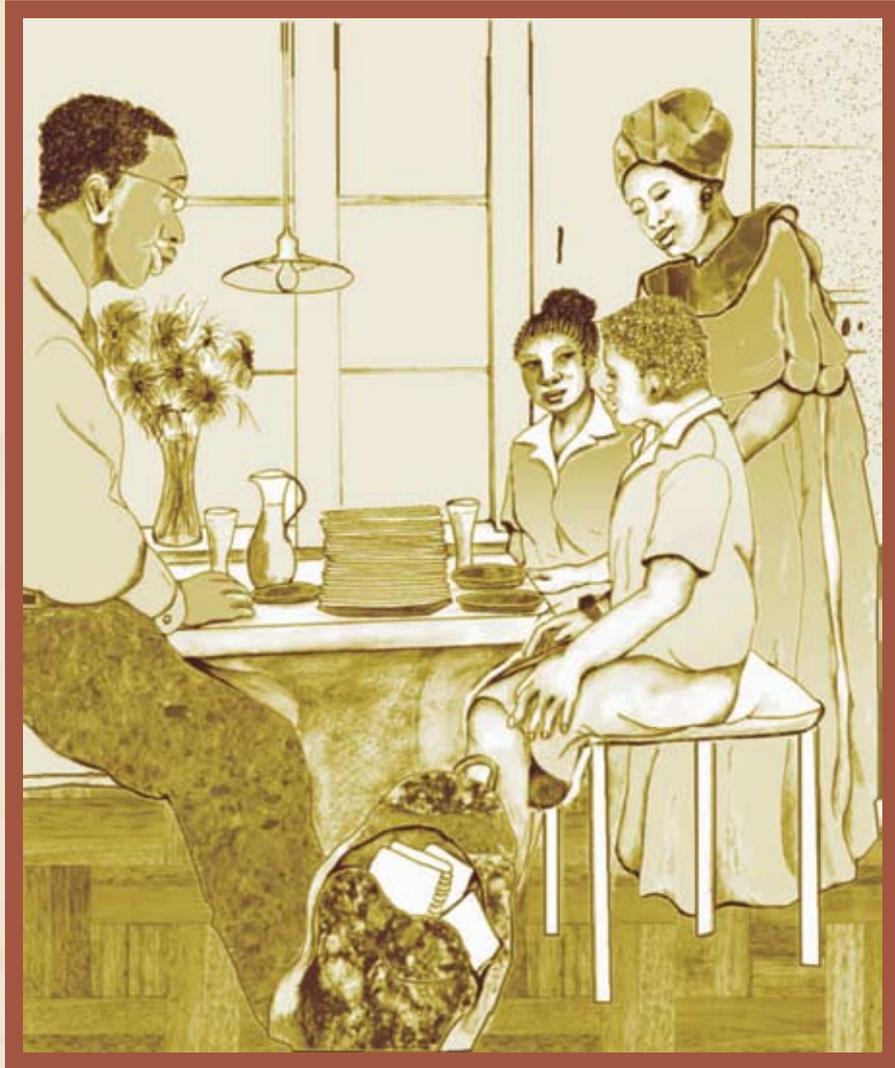


*In this play, Efia, Ekow and their classmates discuss the role of biotechnology in conservation and use of plants, with their Biology teacher and Dr. Mardea.*

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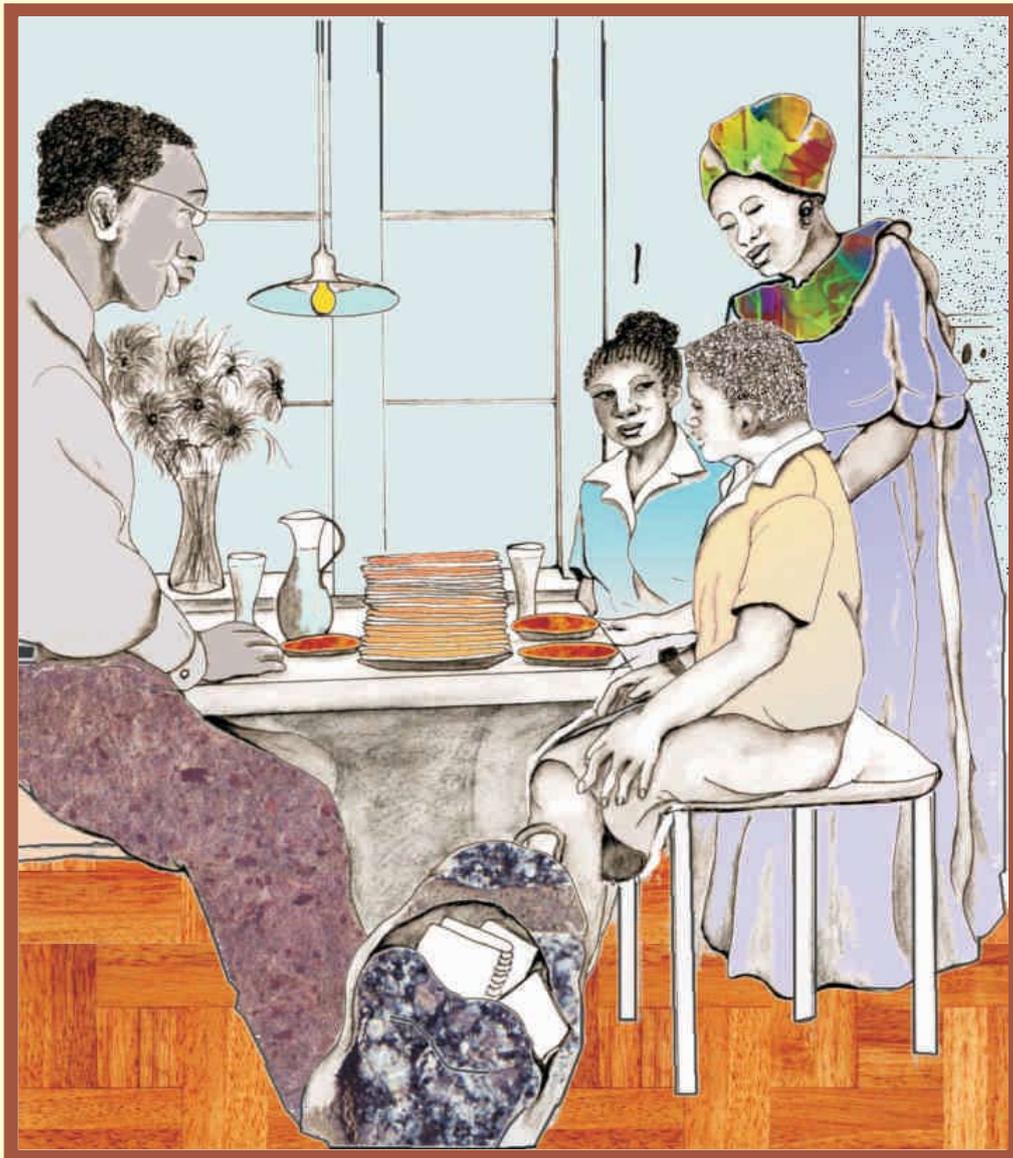


# Scene One



## *Friday morning*

<i>Scene One:</i>	Friday morning
<i>Place:</i>	Home (dining room)
<i>Characters:</i>	Father, Mother, Son (Ekow) and Daughter (Efia)
<i>Situation:</i>	Breakfast time
<i>Breakfast:</i>	Beju (cassava pancake)



*Ekow and Efia are having breakfast at home.*



*Wow! All these are made from cassava?*

Scene One:  
Friday morning

Place:  
Home (dining room)

Characters:  
Father, Mother, Son (Ekow)  
and Daughter (Efia)

Situation:  
Breakfast time

Breakfast:  
Beju (cassava pancake)

**Efia:**

Mum! How did you make these pancakes? They are delicious!



**Efia**

**Mother:**

Well, you need cassava powder, sugar and grated coconut. You mix the sugar and grated coconut with cassava powder and then add the milk and mix it again into a thick paste. You pour this on to a greased hot pan and cook the pancake until it is golden brown. Et voilà!



**Mother**

**Ekow:**

We have had cassava before, but I thought that we ate it as a root. Isn't cassava a root? Don't we have this plant in the garden behind the house?



**Ekow**

**Mother:**

Yes. Cassava powder is made from cassava roots. We have a few cassava plants in our backyard, but your Uncle Sasi has a cassava field. Maybe you should ask him about it.



**Mother**

**Ekow:**

OK, Mum. Can we go to Uncle Sasi's place now?  
(**Ekow is giggling because he thinks they can escape the morning class.**)



**Ekow**

**Father:**

Well! Well! Well! I know the trick you are trying to pull.  
Maybe you can go to your Uncle Sasi's in the evening after you finish your homework.  
Now it is time to go to school.



**Father**

**Ekow:**

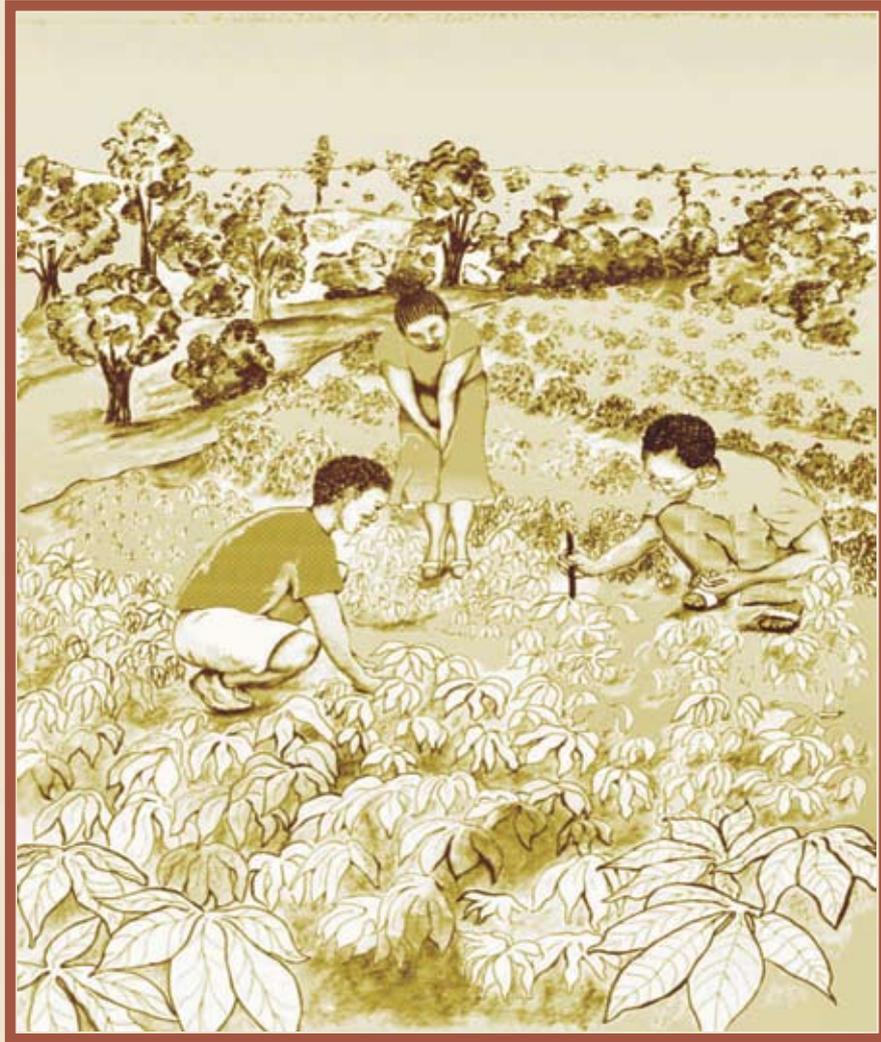
OK, Dad.  
(**Ekow and Efia grab their school bags and leave for school.**)



**Ekow**



# Scene Two



## *Saturday evening*

*Scene Two:*

Saturday evening

*Place:*

Uncle Sasi's cassava field

*Characters:*

Uncle Sasi, Ekow and Efia

*Situation:*

Walking through the cassava field



*Uncle Sasi's cassava field*



*Healthy leaves*

*CMD infected leaves*

*Healthy & diseased cassava leaves*

Scene Two:

Saturday evening

Place:

Uncle Sasi's cassava field

Characters:

Uncle Sasi, Ekow and Efia

Situation:

Walking through the cassava field

**Uncle Sasi:**

Hello kids! How was school today? Do you have any special news?

**Ekow:**

Well, Uncle Sasi, no news from school but we have learned some new information about cassava from Mum. She also said that you have a huge cassava field and that you know a lot about cassava. Do you? Is it easy for you to grow it in your field? Do you make cassava powder?

**Uncle Sasi:**

Yes, I do know quite a bit about cassava, but your biology teacher is the best person to tell you all about growing it.

One of the things I can tell you is that all these plants in my field are disease-free and healthy. There is a special word that describes this condition; it is axenic.

**Efia:**

What exactly does axenic mean? It sounds like a poisonous plant!  
(Laughs)

**Uncle Sasi:**

The word axenic means that the plants are free of disease. I wanted to be sure that my plants will grow to be healthy plants, so I got the plantlets from a plant tissue culture distribution centre.

**Ekow:**

Culture? Do you mean cassava can be grown without soil? Even in a lab?

**Uncle Sasi:**

Yes, a cassava plant can be grown in a lab. The plant tissue culture method is used to produce several plants of the same type in a short time, starting with only a single plant. The plants that are produced this way are also called clones. Cloned plants are identical copies of the original plant. But you should discuss this at school with your teacher who can tell you more about this technique.



Uncle Sasi



Ekow



Uncle Sasi



Efia



Uncle Sasi



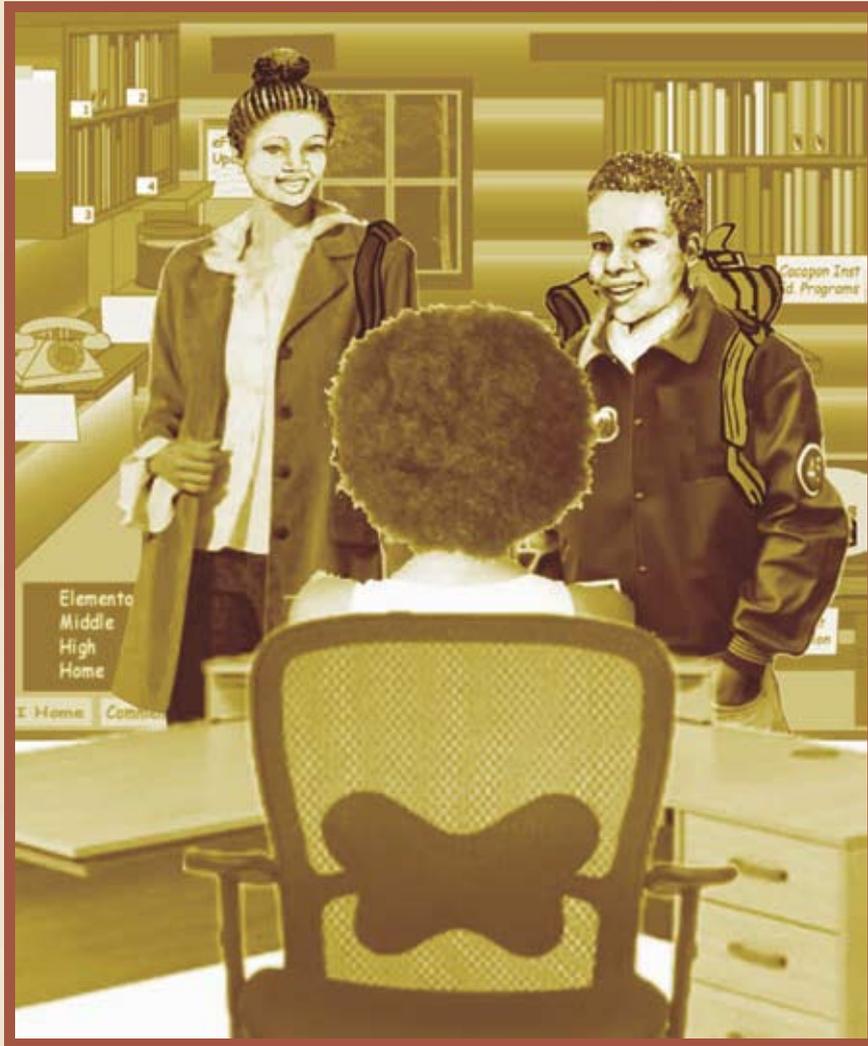
Ekow



Uncle Sasi



# Scene Three



## *Monday morning*

*Scene Three:*

Monday morning

*Place:*

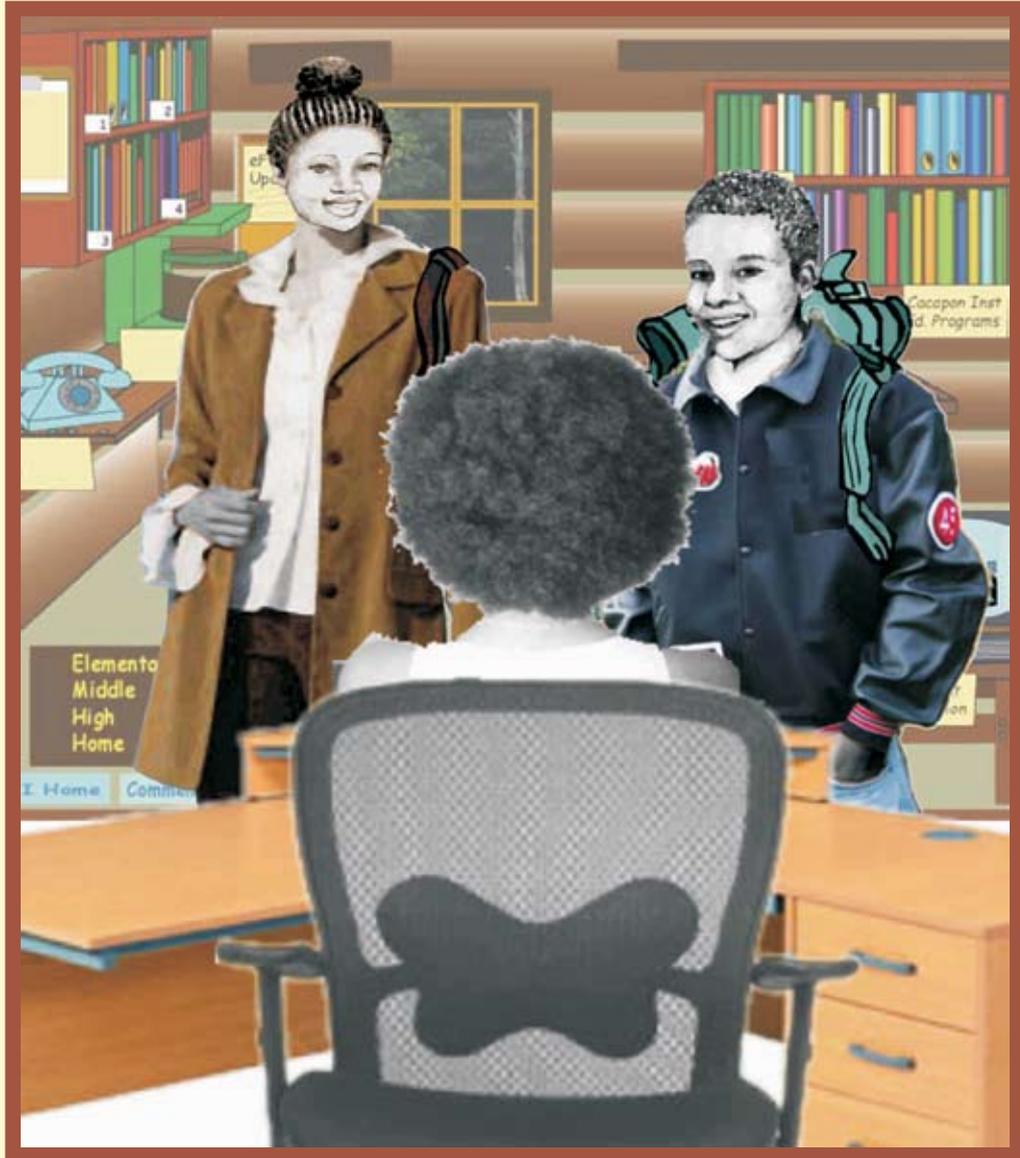
Teacher's room

*Characters:*

Ekow, Efia and the biology teacher

*Situation:*

Talking to the biology teacher



*Efia and Ekow meet with their biology teacher*



*Scenes from plant tissue culture room*

Scene Three:

Monday morning

Place:

Teacher's room

Characters:

Ekow, Efia and the biology teacher

Situation:

Talking to the biology teacher

**(Ekow and Efia are standing and talking near the entrance of the biology teacher's room.)**

**Ekow:**

We must ask our biology teacher about cassava tissue culture today.



**Efia:**

Yes, we have a biology class this afternoon. Maybe we can go and talk to our biology teacher now and ask her to tell us about plant tissue culture during our biology class.



**(They knock on the door of the biology teacher's room and enter.)**

**Teacher:**

Good morning Efia. Good morning Ekow. How can I help you?



**Efia:**

My brother and I visited our Uncle Sasi's cassava field on Saturday. Uncle Sasi told us that he got small cassava plants from a plant tissue culture distribution centre. When we asked him about plant tissue culture he said that you could tell us about it. Could you please tell us more about this technique during our biology class?



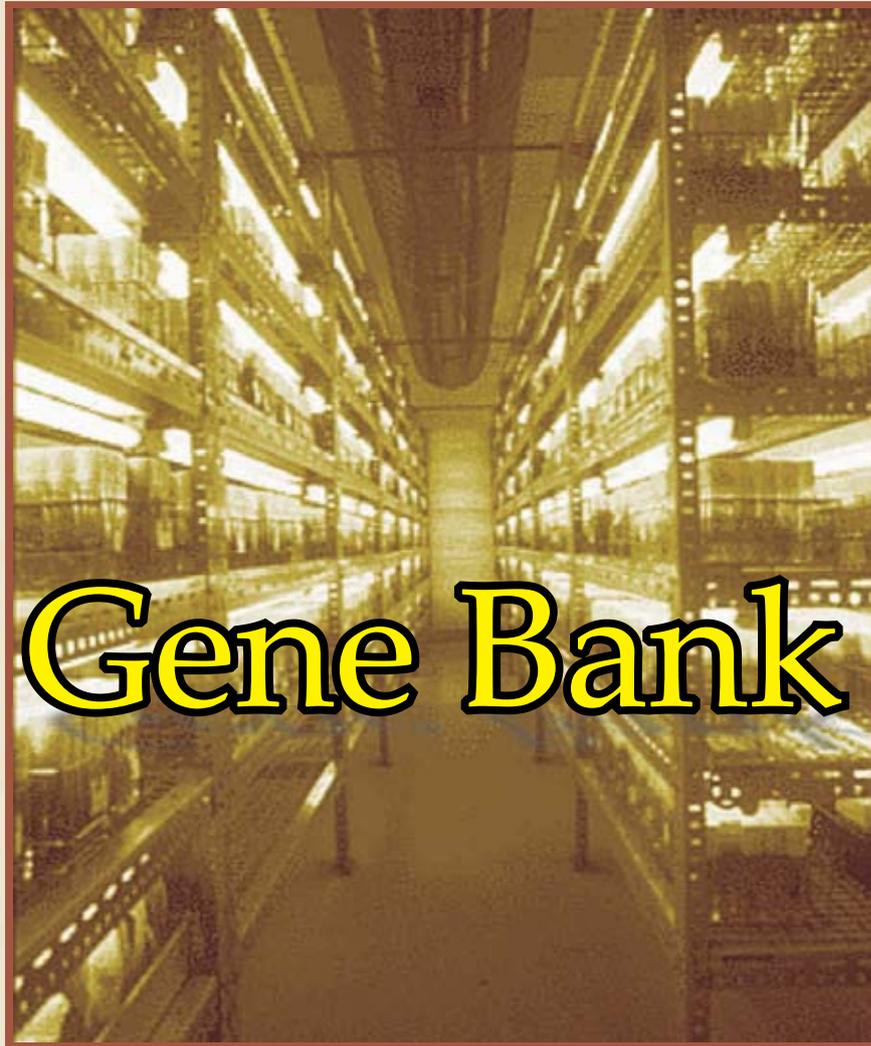
**Teacher:**

Actually, I was going to talk to your class about plant varieties. You have now given me a new idea. I will tell you more about tissue culture during the class this afternoon. See you in the classroom.





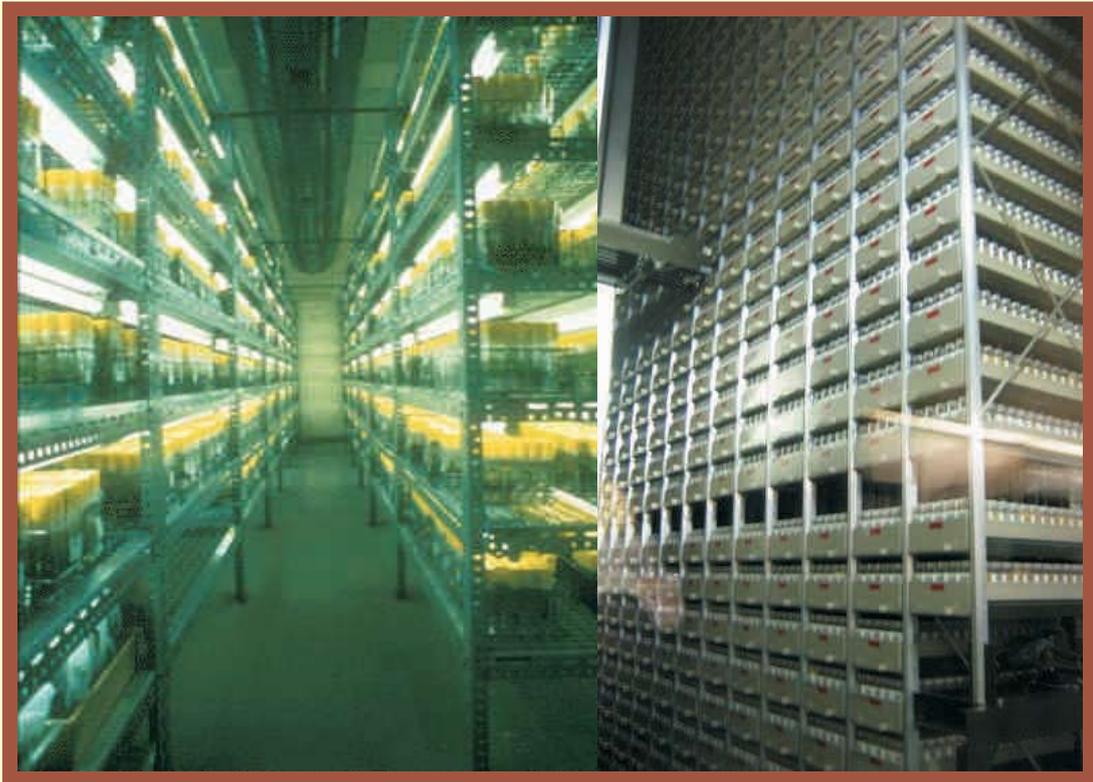
# Scene Four



## Gene Bank

### *Monday afternoon*

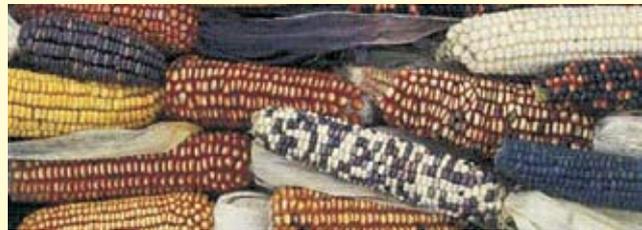
<i>Scene Four:</i>	Monday afternoon
<i>Place:</i>	Classroom
<i>Characters:</i>	Students & biology teacher
<i>Situation:</i>	Discussion on plant genetic resources (PGR) and conservation



*Gene banks*



*Sorghum varieties*



*Maize varieties*



*Seeds in gene bank*

*The seeds conserved and stored may be those of food crops, or those of a rare species, all saved to protect biodiversity.*

*Scene Four:*

Monday afternoon

*Place:*

Classroom

*Characters:*

Students & biology teacher

*Situation:*

Discussion on plant genetic resources (PGR) and conservation

**Teacher:**

Today in our biology class we will learn about how we can save, store and use plants that give us our food. I want you to follow every new term and concept we talk about very carefully. I will ask you to prepare a presentation on the topics when we have finished discussing this subject. The presentation can be a poster, a painting, a poem, a slide show, a skit, or any other idea that you can come up with. You have to be creative.

Now, can anyone tell me anything about the terms I have written on the board?

**Gamba:**

Conservation means "to save."

**Teacher:**

Good! Does anyone know why we might want to save something?

**Kunto:**

Yes, we should save things so that they are available for use when we need them in the future.

**Teacher:**

Good. Can we save plants for future use? And if so, which parts of a plant can we save and for how long?

**Gamba:**

Well, we can save vegetables, fruits and leaves in the refrigerator for some time.

**Kunto:**

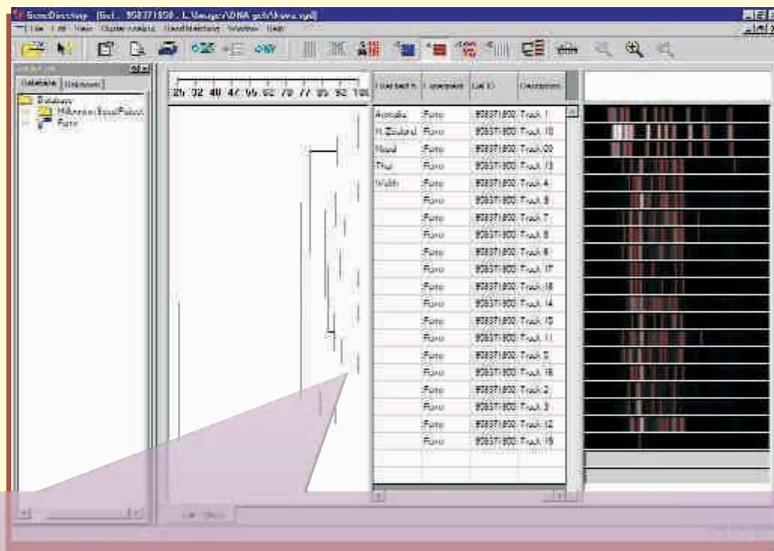
We can also save plant seeds and use them to grow new plants the





*Regenerating seeds for the gene bank is not a simple process. With cross-pollinated crops valuable genetic traits can easily be lost forever.*

*When distributed, as much of the available genetic diversity as possible is included in samples.*

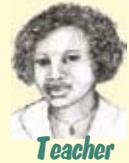


*Gene directory gel data storage and analysis software is used as a tool, to assist rapid and precise identification of seeds to preserve some of the world's rarest plant species.*

following year. But they have to be kept in a cold, dry place.

**Teacher:**

That's correct. And how do you think seeds save the huge plants inside them selves and make sure that they will grow when they are sown in the soil?



**Thandiwe:**

Seeds carry, in their genes, all the information necessary for a new plant to grow. When the seed is left under ideal conditions with water, it can grow into a plant.



**Teacher:**

Yes. All the information needed to grow a full plant is stored in the seed. The structure within the seed that stores this information is called the plant's genome. Genes are inherited, so you can say that the "genetic" make-up of the plant is conserved inside the seed.



Can anyone tell me how seeds in large quantities are stored for future seeding in large fields and how these seeds can be saved from natural disaster?

**Ekow:**

Under natural conditions, seeds are protected from the sun and heat because they have a protective cover, and sometimes they are covered by the soil.



**Teacher:**

You are right, Ekow. This is true for the seeds that fall from the trees and grow again in the following season. However, how about the seeds that farmers store for future use? Natural disaster is when there is unexpected heavy rain, flood, extremely hot sun, fire or sometimes even the destruction of seeds by animals. Do you know how the seeds can be saved from this damage and conserved for future use?



**Gamba:**

Does the word "conserved" also mean "stored"?



**Teacher:**

Yes Gamba. However, the word conserved means stored for a long time.

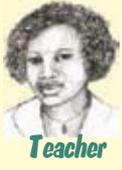




Ekow

**Ekow:**

I know. Seeds are stored in seed banks under ideal conditions to protect them from natural disasters. But I am not sure for how long they can store seeds in a seed bank.



Teacher

**Teacher:**

Correct, Ekow. Seeds are stored in the dark at ideal temperatures in seed banks, where the seeds are kind of sleeping until the farmers use them for seeding. However, there are also places called plant gene banks where plant genetic resources can be stored for a very long time. Can you tell me what is stored in a plant gene bank?



Efia

**Efia:**

Yes! My dad told me that a plant gene bank is a safe repository for crop varieties. It is a very important place because it is one of the storage places for plant genetic resources.



Teacher

**Teacher:**

Very good, Efia. You have mentioned a few important words to remember. Can you tell the class what you mean by "repository" and "plant genetic resources"?



Thandiwe

**Thandiwe:**

Efia, may I answer this question please? A repository is a place where something can be stored safely. The plant's genetic resources are the genetic material that the plants carry in their cells. These can be reproduced into new plants. Am I right?



Efia

**Efia:**

Does your dad also work in a plant genetic resources center?  
(*Thandiwe winks and both Ekow and Thandiwe laugh.*)



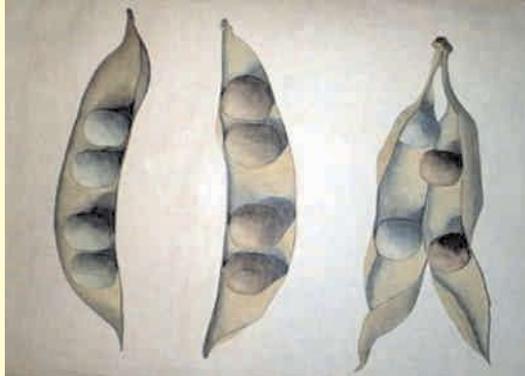
Teacher

**Teacher:**

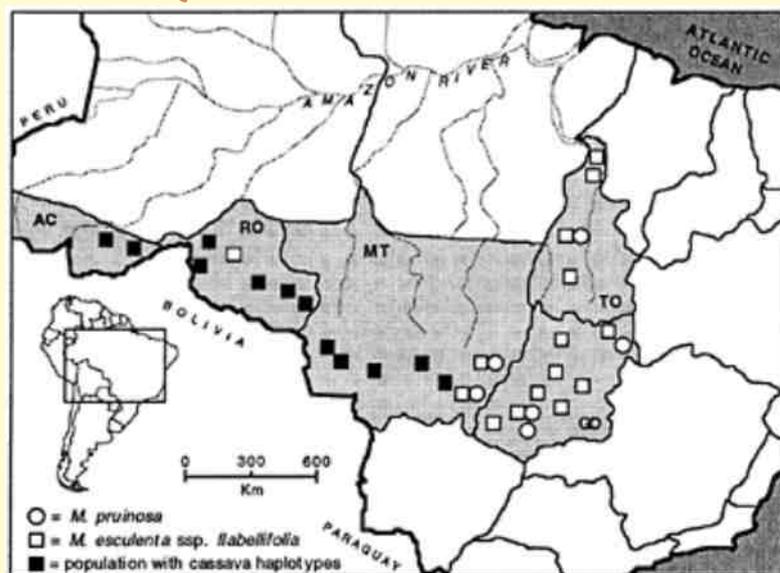
Yes, a plant genetic resource, includes any genetic material of a plant that has value and can be reproduced. It is the basic raw material for research and an important source for food production. Plant seeds are one of these resources.

Can you tell me about any other genetic resource of a plant? Can a new plant be grown from a plant in any other way?

## ***Genetic variation within a species (size and shape differences)***



## ***Genetic diversity of cassava in South America***



***The locations of populations of *M. esculenta* subspecies *flabellifolia* (squares) and *M. pruinosa* (circles). The shaded squares indicate populations containing one or more haplotypes found in domesticated cassava accessions.***

*Original source: Olsen and Schaal (1999).*

*Source: The National Academe Press/Openbook (2000)  
Title: Variation and Evolution in Plants and Microorganisms  
By MICHAEL T. CLEGG AND MARY L. DURBIN*



Efia

**Efia:**

Yes, it is possible to grow some plants by cutting their stems into small pieces and planting the pieces in the soil. But I am not sure whether short branches of plants can be stored in the gene bank.



Teacher

**Teacher:**

Well, a piece of branch cannot be stored forever in a gene bank. However, do you know what this type of propagation is called, Efia?



Efia

**Efia:**

Mmmmm ....., yes, I remember. It is called asexual reproduction or vegetative reproduction.



Teacher

**Teacher:**

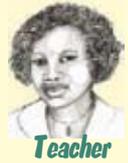
Correct. We will talk about vegetative reproduction or "propagation" a little later. Now we know that the genetic resource of a plant is any part of the plant that has the ability to reproduce that plant as a whole new plant. Is it clear now?



Runako

**Runako:**

Is it true that plant genetic resources can also be called PGR?



Teacher

**Teacher:**

Yes, PGR is the abbreviation of plant genetic resources. Shall we use the abbreviation PGR from now on?

**(The students nod their heads in agreement.)**



Teacher

**Teacher:**

PGR has a broad meaning because it includes the genetic material contained in all plant varieties found on earth.



Runako

**Runako:**

Do you mean that PGR includes the genetic information of all plants from all over the world?



Teacher

**Teacher:**

Yes. For example, the PGR of a particular species includes not only the genetic information of that species but also that of its relatives and traditional and modern varieties. All these together make the genetic diversity of a plant species.

**Efia:**

What is genetic diversity? Is this the same as the gene pool of that species?



**Efia**

**Teacher:**

Genetic diversity means the genetic variation within a population of a species. The genetic diversity of a species is a crucial indicator of how that species copes over time within its environment. The genetic diversity constitutes the gene pool of a species.



**Teacher**

**Runako:**

So, a gene pool is made up of all the genes in a population of a particular species and its relatives.



**Runako**

**Teacher:**

Very good! So, let me see. Who can give us a brief note on PGR; Ekow?



**Teacher**

**Ekow:**

I understand that plant genetic resources in short, is called PGR and that PGR means the collection of plant "genetic" material from various plants and their relatives all over the world. This collection of PGR contributes to plant genetic diversity. The genetic diversity of a species is a crucial indicator of how that species will survive any change in the environment.



**Ekow**

**Teacher:**

Very good, Ekow.



**Teacher**

**Kunto:**

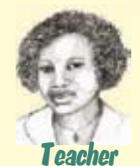
So, genetic diversity means a variety of gene material that belongs to only one species?



**Kunto**

**Teacher:**

No. There is genetic variability among the populations and the individuals of the same species. Genetic diversity of any species is the diversity of genes within that species and all its relatives, including the wild and domesticated relatives and all new improved varieties. The extent of diversity in a plant species depends upon the number of individual plants, where they grow and how far apart the individual populations of plants are separated from each other.

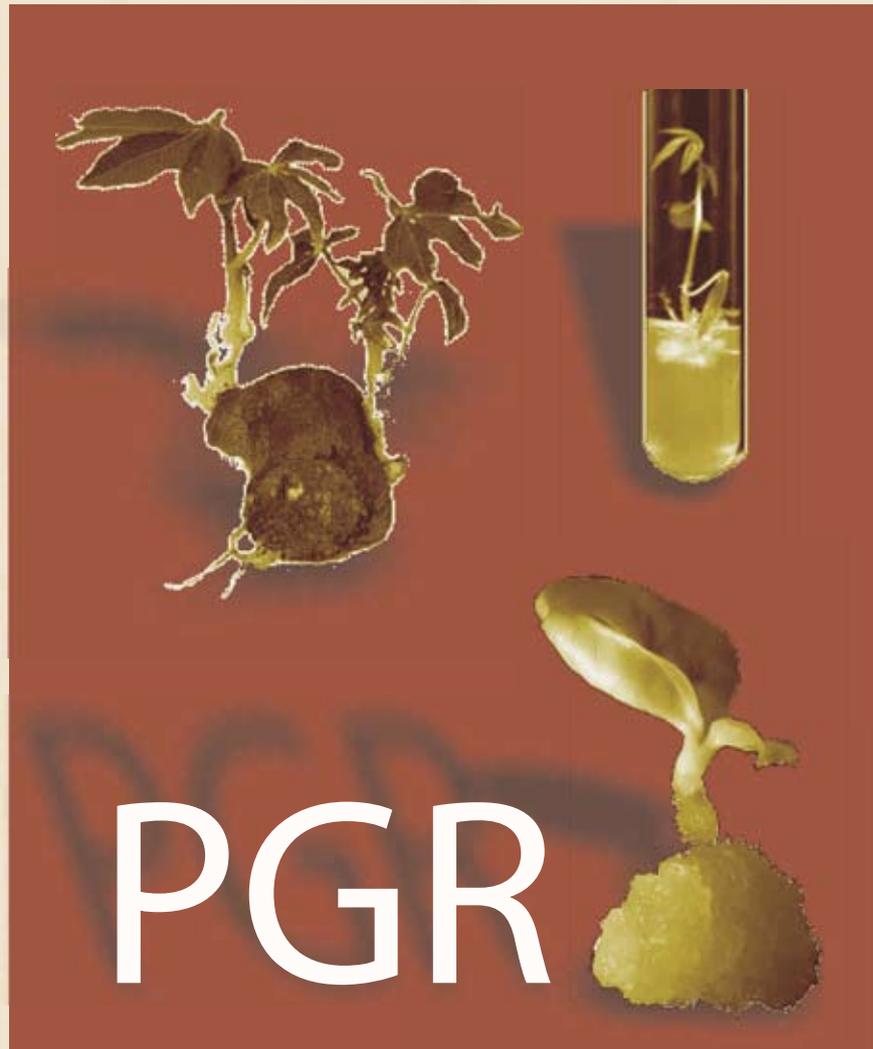


**Teacher**

**(The school bell rings and everybody gets ready to go to the next class.)**



# Scene Five



## *Wednesday morning*

*Scene Five:*

Wednesday morning

*Place:*

Classroom

*Characters:*

Students & biology teacher

*Situation:*

A discussion on the importance of PGR



***Cassava plant  
growing in a  
test tube***



***Cassava plant  
growing from  
callus***



***Cassava plant***



***Cassava root growing***



***Cassava seeds***



***Cassava root***



***Cassava  
shoot  
growing  
root***

Source: Floridata.com

Scene Five:  
Wednesday morning

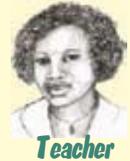
Place:  
Classroom

Characters:  
Students & biology teacher

Situation:  
A discussion on the  
importance of PGR

**Teacher:**

Good morning class. Shall we continue our conversation on PGR conservation and use?



**Efia:**

Yes, but first I have a different question; is the genetic variety of plants useful in any way?



**Teacher:**

Your question is not so unrelated to PGR conservation and use as you may think, Efia. Genetic variety is essential for a species and its populations to be able to adapt to changing environmental conditions and is therefore a prerequisite for their survival.



**Ekow:**

Just like asking questions is a prerequisite for being inquisitive, Efia. (Giggles.)



**Teacher:**

Ekow! Pay attention, please. Let us take the example of the cassava you were talking about. By the way, do you know the botanical name for it?



**Ekow:**

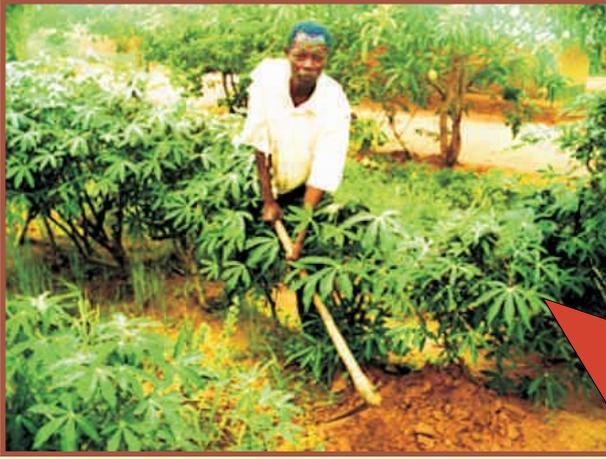
No.



**Teacher:**

It is called *Manihot esculenta* Crantz. Now, think of all the different types of cassava you see in gardens or markets; thin and long, thick and short, with a dark brown skin or a light colour skin and so on. All these varieties contribute to cassava genetic diversity.

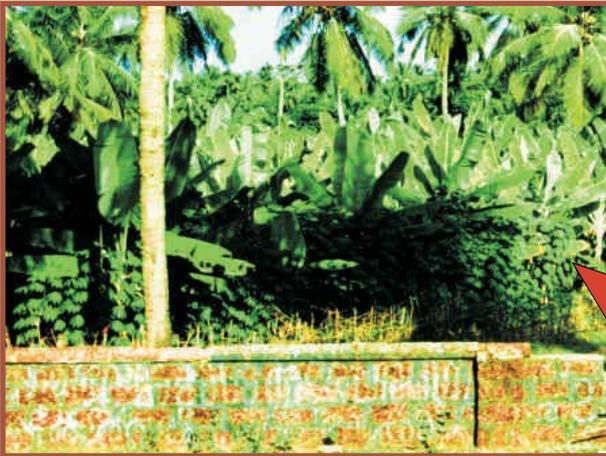




*Cassava field in Zambia*



*Cassava field in Venezuela*



*Cassava field in India*



*Cassava field in Brazil*



**Gathi:**

I know we have many varieties of cassava in our country.



**Teacher:**

Gathi, cassava does not only grow here in our country. Different varieties of cassava grow in many parts of the world. For example, cassava grows in Brazil, Colombia, India and many other places. Some cassava varieties grow under wild conditions. Some are domesticated and are grown as a crop. Some cassava plants are transformed in the lab to develop new or improved characteristics. All the genes belonging to this species contribute to the cassava gene pool and therefore to the cassava genetic resources.



**Mandisa:**

If PGR is just the genetic material of all the plant species we have in the world, why should we conserve it? After all, these plants are present everywhere and, as far as cassava plants are concerned, we have lots of them.



**Teacher:**

Let me tell you about a real life experience to explain why we need to conserve PGR.



In 1983, a scientist from Brazil led an expedition to collect some of the wild relatives of cassava from Mexico. He was looking for a cassava wild relative called *Manihotoides pausiflora*. He discovered that this variety was in danger of becoming extinct. This means that once we lose it we will never be able to grow it again.

**Hadiya:**

Is this how a variety becomes extinct?



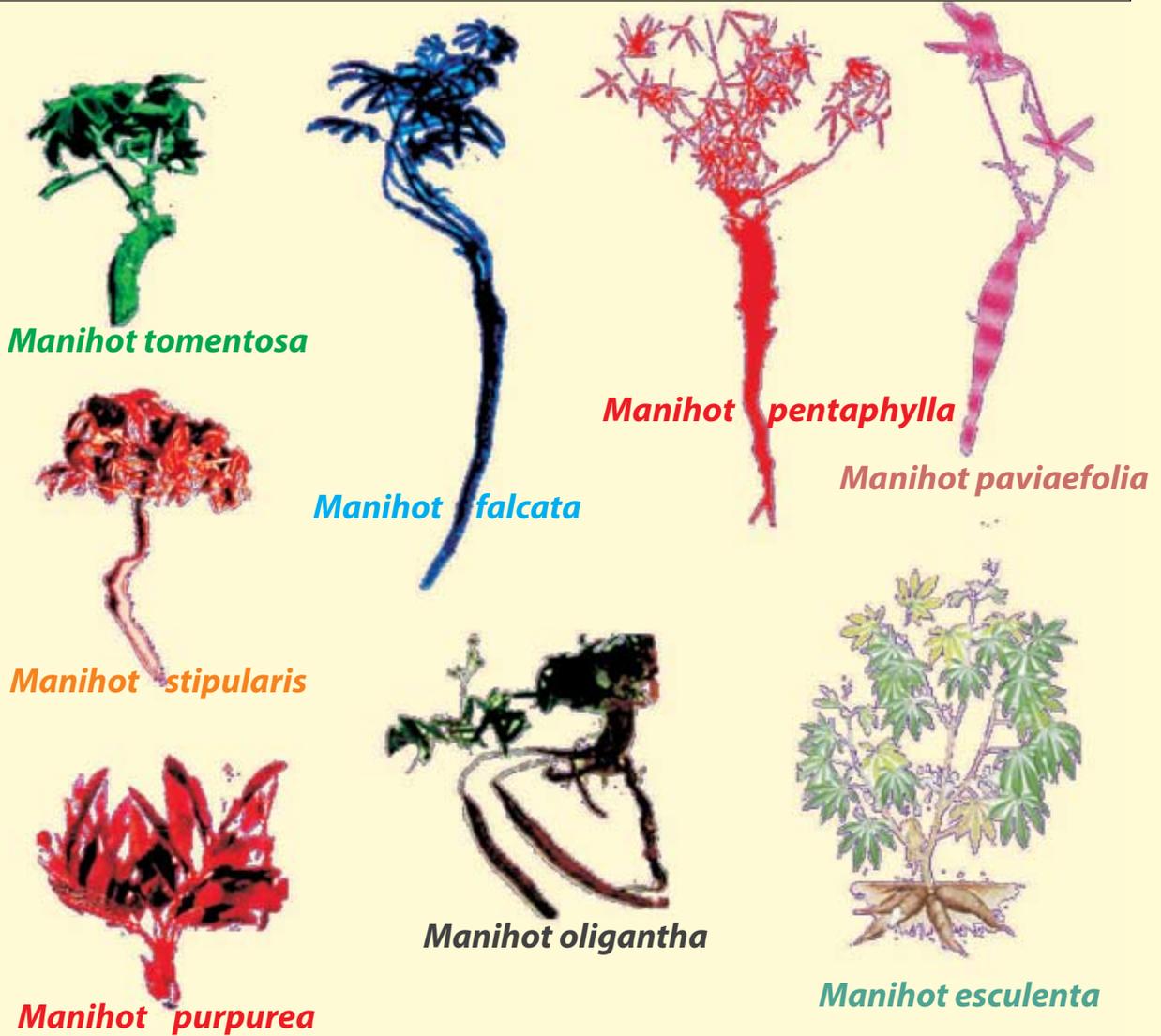
**Teacher:**

Yes, Hadiya, that is correct. However, we must be very careful before we declare a species extinct. This is because all species try to adapt to any bad conditions and will try to survive in different parts of the world until it is impossible for them to survive at all. Therefore, we must carefully search for the variety and verify its complete absence before condemning a species to extinction. On the other hand, you are right in saying that a species is lost to us if we can not find it when we need it. This is why we have to conserve it in a place where we can find it again. Therefore, a gene bank is a good place for storage.



# Genetic diversity in Cassava

Photo source: Geneconserve/By Nagib M. A. Nassar



## Variation in cassava leaf structure and appearance

Photo source: FAO



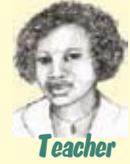
**Ekow:**

Tell us about the scientist, please. Why was he looking for wild cassava?



**Teacher:**

The scientist was interested in finding a specific species of cassava because this variety has three-lobed pubescent leaves which make it tolerant to both drought and insects. These primitive characteristics make it a strong candidate as a reputed ancestor of the genus *Manihot*. It can adapt to very dry habitats.



**Efia:**

Excuse me teacher, what does "pubescent" mean?



**Teacher:**

Pubescent means that the leaves are covered with fine, hair-like structures. These leaves can retain water better than those that do not have these structures.



Now, to get back to our story, the scientist had heard that this plant had last been seen in the period from 1921 to 1960 in a place called Tehuacan in Mexico. He went to visit the site and found that a road had been constructed near the area and all these plants had almost disappeared. He knew that many varieties of crops such as cassava were disappearing as a result of pests and diseases, natural catastrophes and rapid socio-economic changes, such as changes in diet or urban migration. Yet, this endangered diversity is vitally important. Each variety has particular characteristics, requirements, advantages and disadvantages. We need the genetic information from a particular plant variety in order to crossbreed this plant with another variety. For example, a cassava plant with characteristics such as disease resistance can be crossbred with a variety of a cassava plant that has drought-resistant characteristics.

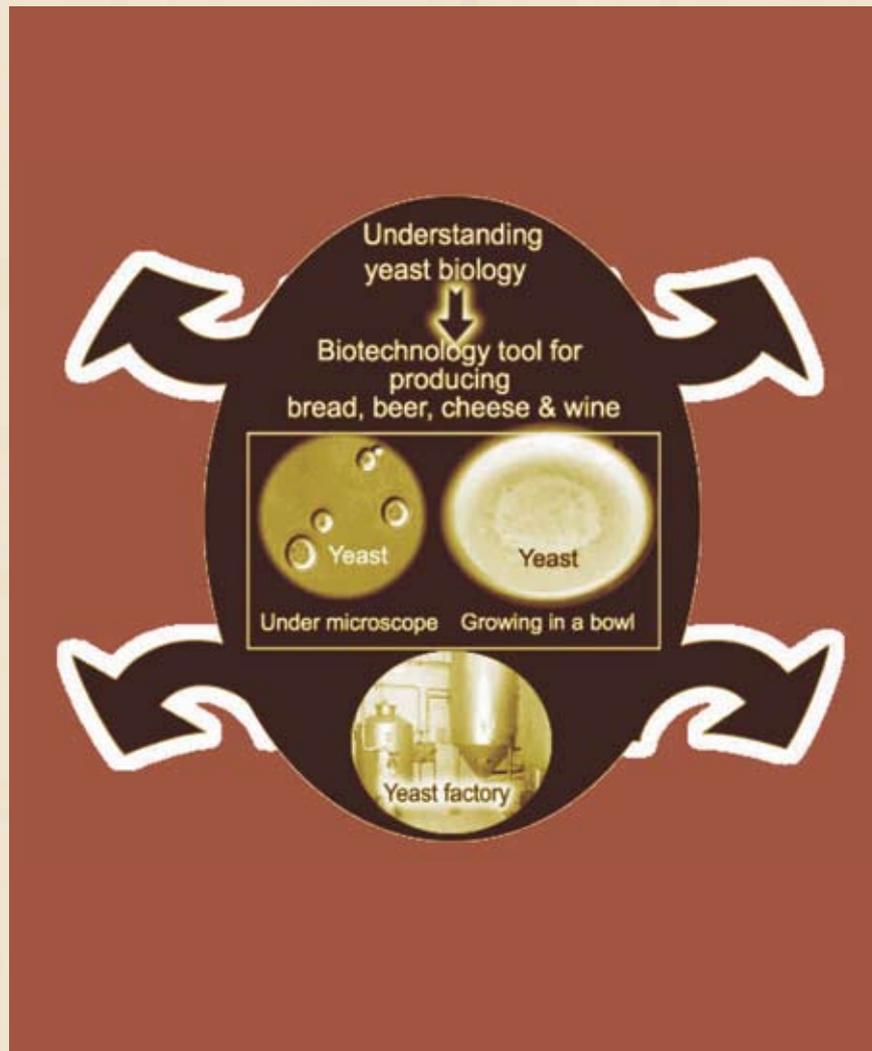
That is why it is important to know the genetic information of all available cassava varieties and to try to conserve their genetic resources.



Now, it is time for the next class. I will see you all again on Friday. In our next class we will talk about how the knowledge of biotechnology helps in PGR conservation.



# Scene Six



## *Friday morning*

*Scene Six:*

Friday morning

*Place:*

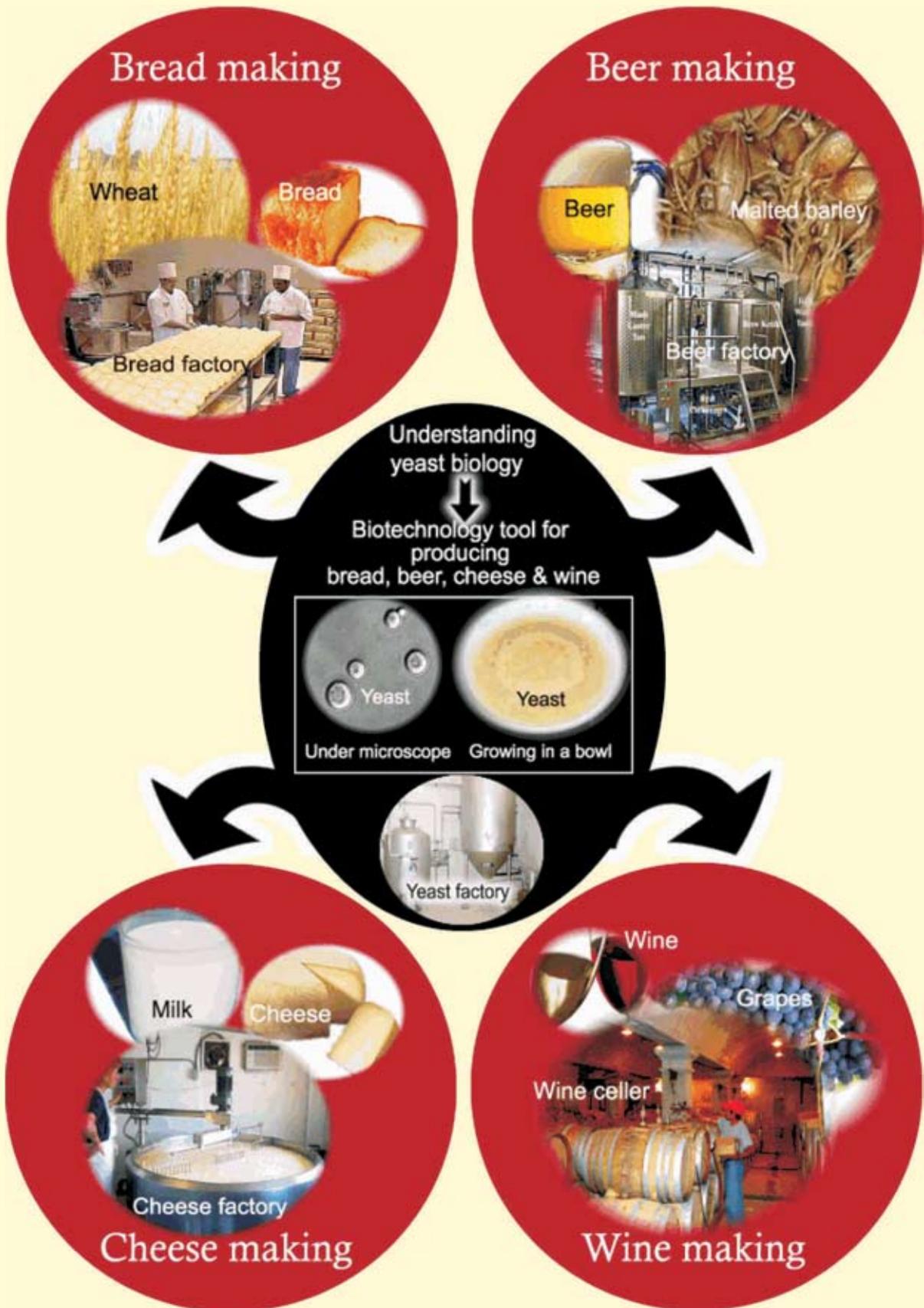
Classroom

*Characters:*

Students & biology teacher

*Situation:*

A discussion on biotechnology tools



## *Simple biotechnology tools*

*Scene Six:*  
Friday morning

*Place:*  
Classroom

*Characters:*  
Students & biology teacher

*Situation:*  
A discussion on biotechnology tools

**Teacher:**

Good morning students. During our last biology class, we discussed "PGR", "conservation" and "gene banks". Today we will discuss "biotechnology". Who can tell me something about biotechnology?



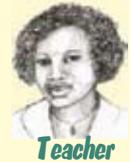
**Efia:**

Biotechnology uses biological material in order to make, modify or improve plants, animals or micro-organisms for specific uses.



**Teacher:**

Very good! So can you tell me what biotechnology tools are?



**Mandisa:**

Biotechnology tools are all the techniques that use biological material to make, modify or improve plants, animals or micro-organisms.



**Dhakiya:**

So, when we use live yeast to produce something, are we using a biotechnology tool?

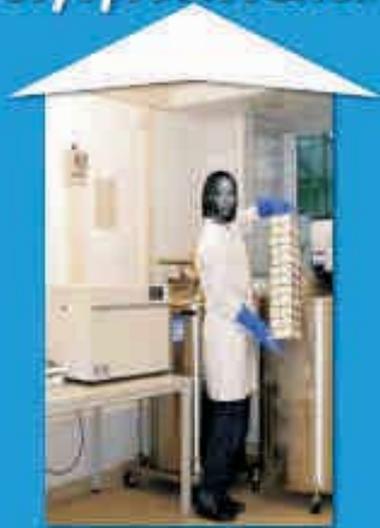


**Teacher:**

Yes, the processes of making beer, bread, wine and cheese using yeast and bacteria are good examples of biotechnology tools. For example, the knowledge of how to use yeast to produce soft bread came from an understanding of how and when yeast reacts. So this knowledge about yeast biology is used as a biotechnology tool. The term "biotechnology" was coined only in 1917 when plant and animal breeding techniques were developed on a large scale.



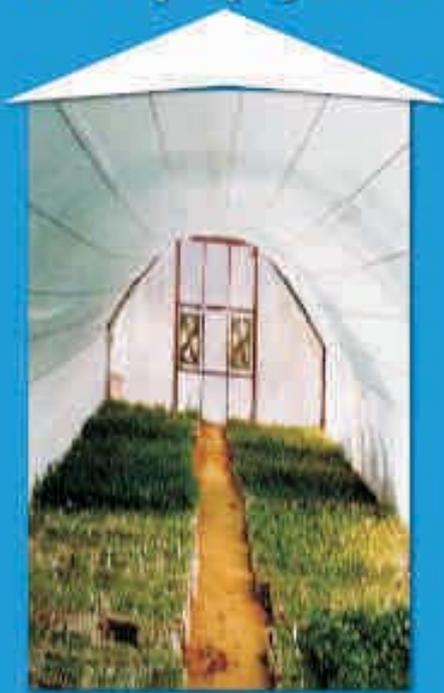
## Cryopreservation



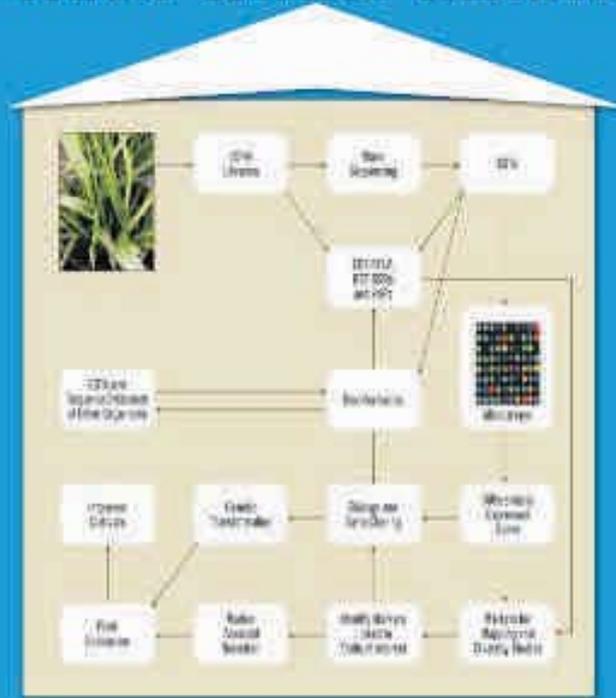
## Tissue culture



## Micropropagation



## MAS Marker assisted selection



Examples of **Biotechnology tools** that can be used for conservation and use of plants

**Biotechnology tools are those techniques that use biological material such as plant and animal cells.**

**Gathi:**

Are you going to tell us how biotechnology tools can now be used for conservation of PGR?



**Teacher:**

Yes, that is the focus of our discussion today. The simple biotechnology tools are "cryopreservation", "tissue culture" and "micropropagation".



**Dhakiya:**

I have heard that biotechnology tools were used to develop a cassava plant that is virus-resistant.



**Teacher:**

Yes, that is right, Dhakiya. What do you know about virus-resistant cassava?



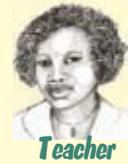
**Dhakiya:**

My dad was talking with his friends about it. He said that we can now buy small cassava plants that will not be killed by a virus that normally kills them when they grow into bigger plants.



**Teacher:**

Do you know the name of the virus, Dhakiya?



**Dhakiya:**

No, I don't know the name of the virus.



**Teacher:**

It is called "cassava mosaic virus". The disease is called cassava mosaic disease or CMD. It is caused by a white fly that carries the virus within it.



**Pepukayi:**

Was this insect-resistant cassava variety made using biotechnology tools?

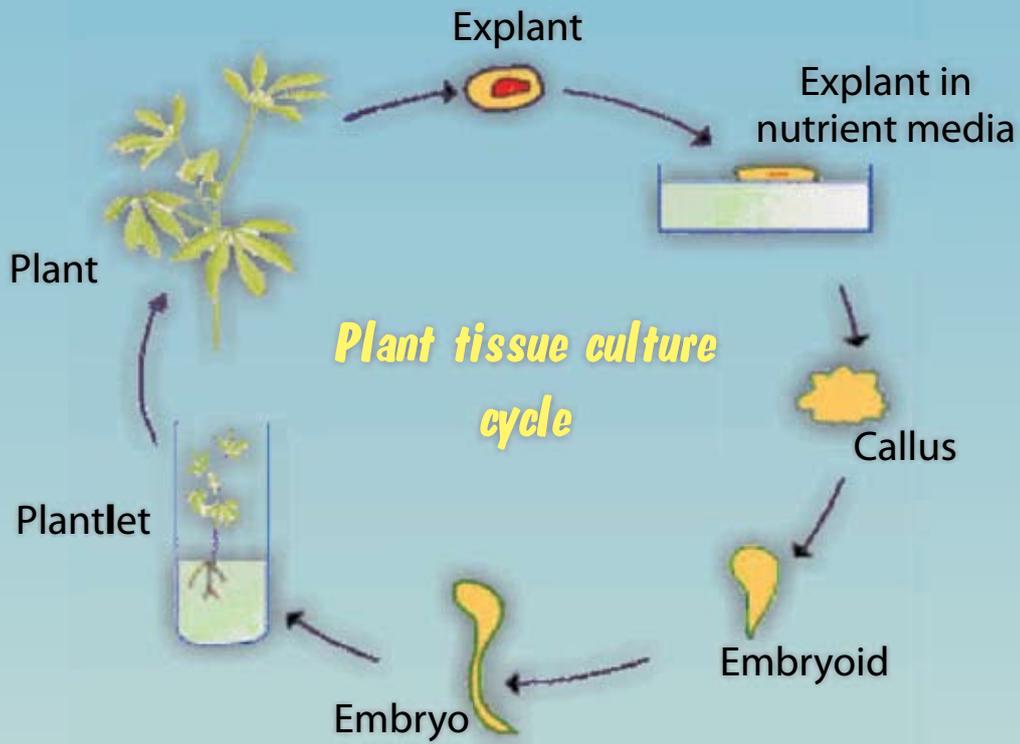


**Teacher:**

Yes. The biotechnology tools used in growing and propagating these varieties are called plant tissue culture and micropropagation.



# Plant tissue culture



**Cassava culture**  
Source: GD Stone



**36** *Cassava plantlet growing from callus*



*Cassava plantlet growing in test tube*

**Efia:**

Are these tools also useful for conserving these varieties?

**Teacher:**

Yes. The tissue culture technique is very useful for conserving the richness of plant diversity. The technique helps in preparing and storing disease-free plants and plant material. At the same time plant genetic resources are the raw materials for any application of biotechnology.

**Efia:**

If we want to reuse something we must have a way to save and store it. If we want PGR for future use then we must conserve them in some way.

**Dafina:**

So, what type of plant tissue is used for tissue culture?

**Teacher:**

Tissue culture utilizes plant cells that are capable of generating a whole new plant; in other words, these cells are totipotent. The totipotent single cell or group of cells can be encouraged, in the right nutrient medium, to grow into a whole new plant.

**Dhakiya:**

What is so special about tissue culture in comparison with the normal way of growing a plant?

**Teacher:**

Tissue culture can produce many identical copies of a plant from a single cell of that plant, so this speeds up the process of making a new variety available to everyone. It is done in a laboratory under sterile conditions. Don't you think this biotechnology tool is amazing, Dhakiya?

**Dhakiya:**

Yes, it is very interesting. Tissue culture increases the number of plants, but how can this technique help in conserving PGR?



**Efia**



**Teacher**



**Efia**



**Dafina**



**Teacher**



**Dhakiya**



**Teacher**



**Dhakiya**

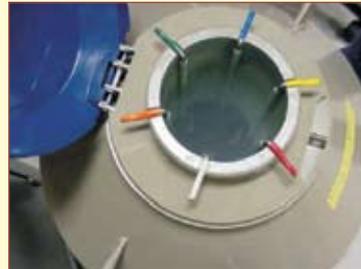
# Cryopreservation



*Collecting liquid nitrogen from a big container to a small flask*



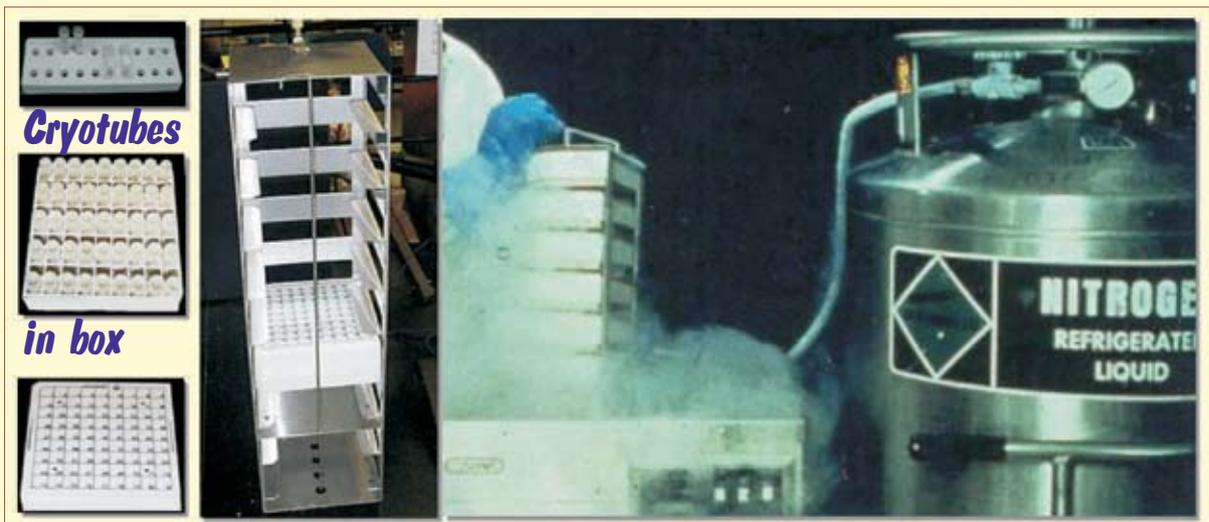
*Storing samples in a smaller container*



*Open container*



*Closed container*



*Cryotubes*

*in box*

*box*

*Rack*

*Rack with boxes in Cryo-tank*

*Equipment for cryopreservation*

**Teacher:**

Tissue culture technique is not only used to multiply the number of cells but also to store the plants for use over a long period of time in the future. This technique has a huge potential to contribute towards conservation.



**Hakika:**

Do farmers protect their PGR using biotechnology tools?



**Teacher:**

Not the farmers themselves, but for their benefit, many national and international organizations do. In many ways, saving, storing and reusing PGR requires a knowledge of biology and biotechnology.



**Kunto:**

I know what "biotechnology" means but I still do not understand what the term "biotechnology tool" means?



**Dhakiya:**

Kunto, that is very easy. Biotechnology tools are the techniques used to increase, improve and conserve biological materials such as plants. We need to know biology, the appropriate methods and the kinds of equipment needed for these techniques, so as to actually use the biotechnology tools in the laboratories.



**Teacher:**

Can anyone give me some examples of techniques that are commonly used to conserve biological materials?



**Dalila:**

How about cryopreservation? It is a quick deep-freezing method for biological materials.



**Ekow:**

Is cryopreservation a biotechnology tool for the conservation of PGR?



**Teacher:**

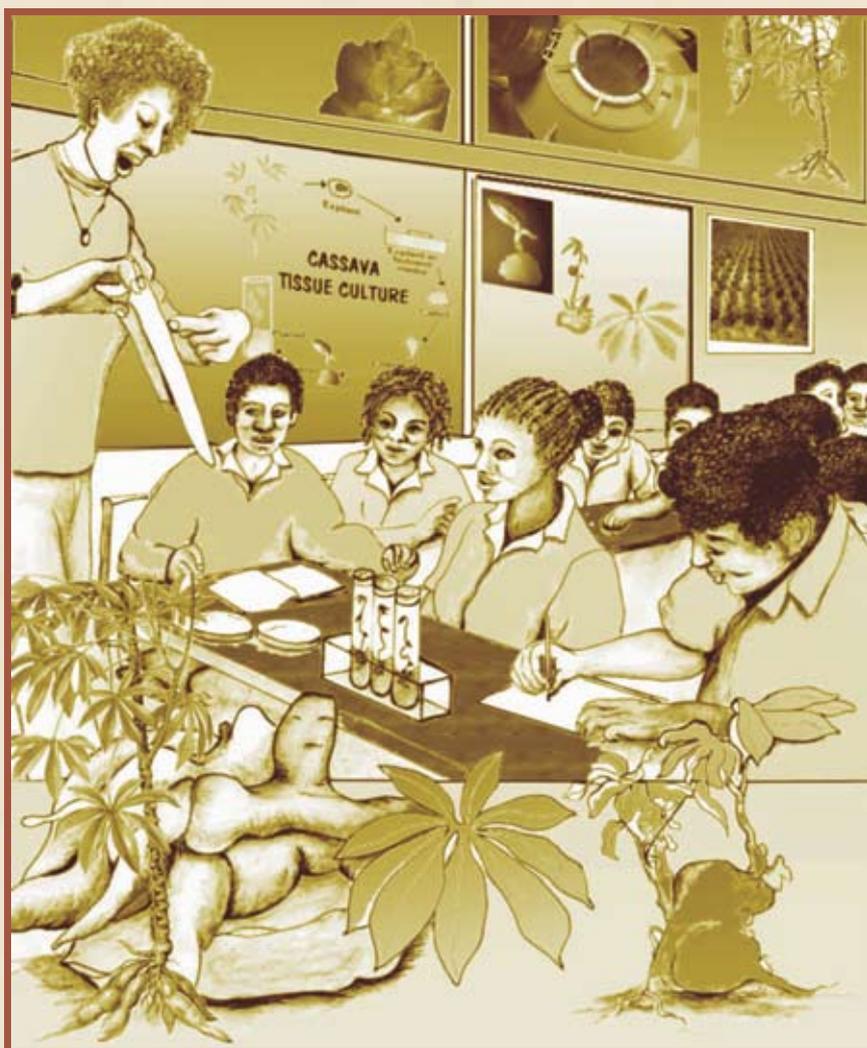
Well, to find out the answer to your question, you will have to wait until the next biology class. We have a guest speaker called Dr Mardea who will tell us about it tomorrow. She is a biotechnologist who knows all about cryopreservation and tissue culture.



That is all for today. I will see you in the next class with Dr Mardea.



# Scene Seven



## *Monday afternoon*

*Scene Seven:*

Monday afternoon

*Place:*

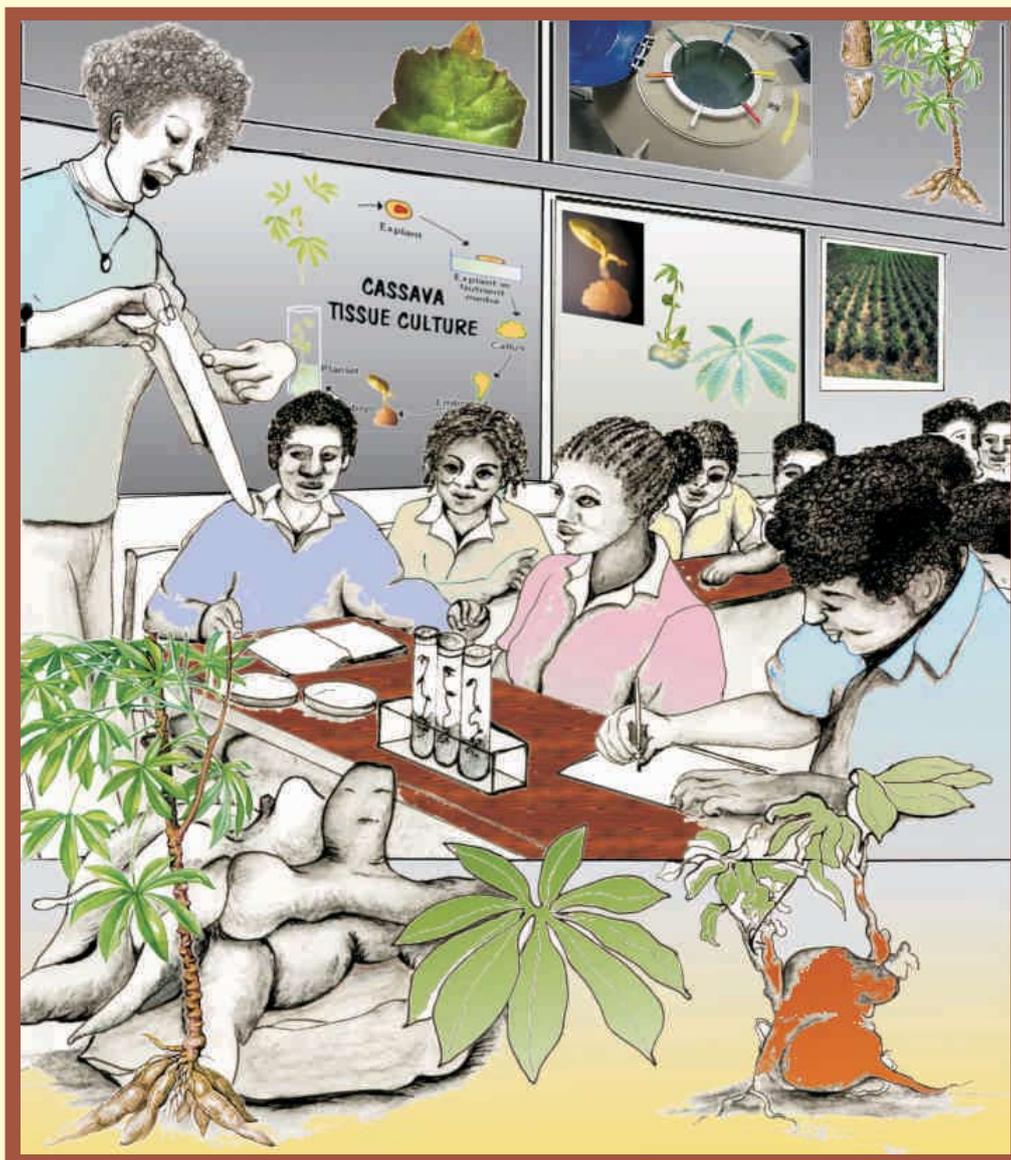
Classroom

*Characters:*

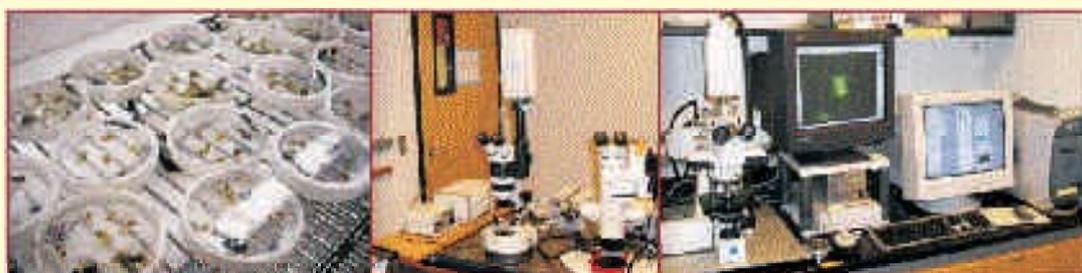
Students, teacher and  
Dr Mardea

*Situation:*

Dr Mardea visits: discussion  
about the link between  
biotechnology tools and PGR  
conservation



*Dr. Mardea is explaining plant tissue culture to students.*



*Culture grown in petri dishes*

*Plant tissue culture room with microscopes*

*Scene Seven:*

Monday afternoon

*Place:*

Classroom

*Characters:*

Students, teacher and  
Dr Mardea

*Situation:*

Dr Mardea visits: discussion  
about the link between  
biotechnology tools and PGR  
conservation

**Teacher:**

Good morning everyone. This is Dr Mardea. She will talk to you about plant tissue culture and explain why it is one of the biotechnology tools used for conserving PGR. Listen carefully. If you have questions write them down immediately so that you can ask her when it is time to ask questions.



**Dr Mardea:**

Hello everyone. Are you all ready to listen to some fascinating information about biotechnology?



**Students:**

Yes, Dr Mardea.

**Dr Mardea:**

Right. Everything I will talk about is related to one of our favourite and most important tuber crops here in Africa, called cassava. Do you know cassava?



**Students:**

Yes, we do; our teacher has already told us a lot about it.

**Dr Mardea:**

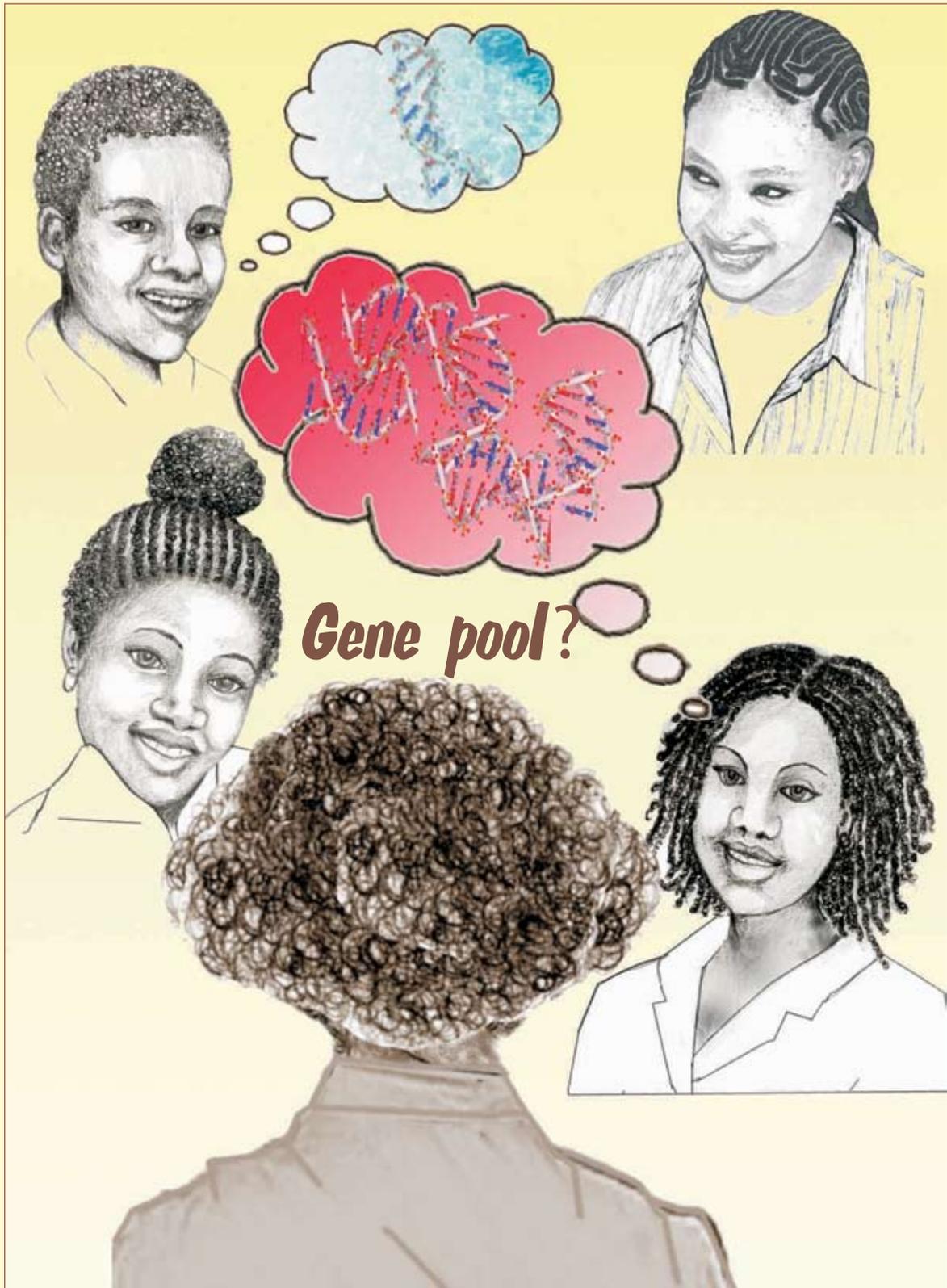
Do you also know what scientists do to improve, conserve and reuse cassava using tissue culture?



**Ekow:**

Is this what you are going to tell us about today?





***A gene pool is the sum of all the genetic information carried by all members of a population of a (plant) species.***

**Dr Mardea:**

Yes, today I will tell you about cassava conservation. The plant tissue culture technique is useful for many different purposes but, most of all, it is useful for conserving clean, disease-free and healthy genetic resources in a safe manner, so that they can be used in the future when they are needed.

**Ekow:**

We already know this because our teacher discussed it in our last class.

**Efia:**

Ekow, please be patient and listen.

**Hakika:**

**(With a smile)** I think we should quiz Ekow after class as he knows everything.

**Ekow:**

Yes, that would be great.

**Dr Mardea:**

You seem to be very bright students. I am glad that you are knowledgeable in this field. The plant tissue culture technique involves the utilization of biological materials and therefore it is a biotechnology tool. Before we learn the details of tissue culture, I think we must discuss the term germplasm and PGR and what PGR conservation means. Who can tell me what germplasm means?

**Dafina:**

Germplasm is the genetic material that carries the inherited characteristics of an organism.

**Dr Mardea:**

Very good. Can anyone tell me what a gene pool is?

**Hakika:**

The gene pool is the sum of all the genetic information carried by all members of a population of a plant species.

**Ekow:**

Hakika, do you realize that there is no diving in the deep end of the gene pool? **(Everybody has a good laugh. Efia makes faces to let Ekow know that it is not the joking time)**



Dr Mardea



Ekow



Efia



Hakika



Ekow



Dr Mardea



Dafina



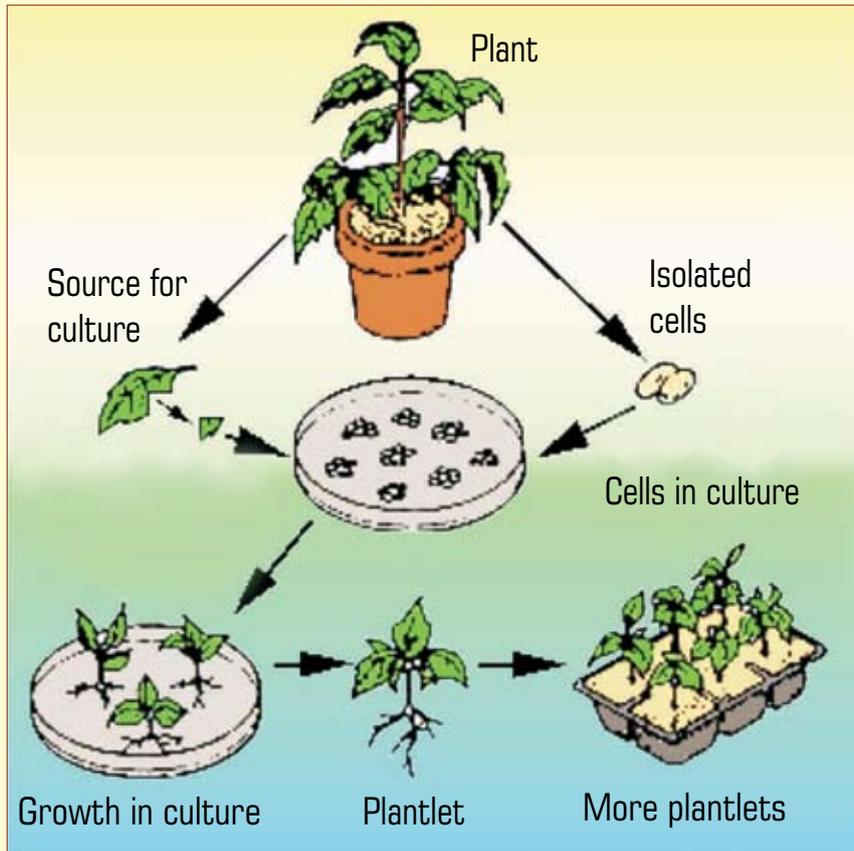
Dr Mardea



Hakika



Ekow



### ***Steps in Micropropagation***



### ***Simple mathematics...micropropagation***

*Micropropagation is a very efficient tool for propagation of a genetic material. This is especially so in the case of the “elite plants” which cannot be multiplied through seeds or even through horticulture. Somatic embryogenesis, especially with cell suspensions, offers a powerful tool for multiplication.*



***In the labs***

***In a green house***

**Dr Mardea:**

Very good Hakika. I am impressed. Can anyone tell me what PGR means in this context?

**Dene:**

The genetic resource of a particular crop type is any genetic material of the original crop that has the potential value to create a new, or even an improved, crop.

**Ekow:**

Dr Mardea, our biology teacher told us, that we must protect the germplasm diversity of any crop from being lost, so as to make it available for future use. Does this mean, we have to store, I mean conserve, all PGR?

**Dr Mardea:**

Yes, it is true. Now, in order to save and store the germplasm and the genetic variety, the germplasm has to be stored in a place where it can be distributed for further use. This has to be somewhere outside the plant body itself. This type of conservation is called in vitro conservation.

**Sabola:**

Dr Mardea, if we have to conserve the germplasm outside the plant, it has to be in a form that can be stored in a container. Then later on it has to be taken out and grown to increase the number of plants. How does the tissue culture technique help in this?

**Dr Mardea:**

The tissue culture technique is essential for the entire process of germplasm conservation, maintenance, distribution and micropropagation. We shall see this later on.

**Efia:**

I know that "micro" means small, but what is micropropagation?

**Dr Mardea:**

Micropropagation involves growing very small plant parts in a test tube or other container with a nutrient medium under sterile conditions. You can simply say that micropropagation is plant tissue culture for regenerating a whole plant from the cells, tissues or organs of that plant.

There are two important concepts that we must know about plant cells to understand micropropagation; these are "totipotency" and "plasticity".



**Dr Mardea**



**Dene**



**Ekow**



**Dr Mardea**



**Sabola**



**Dr Mardea**



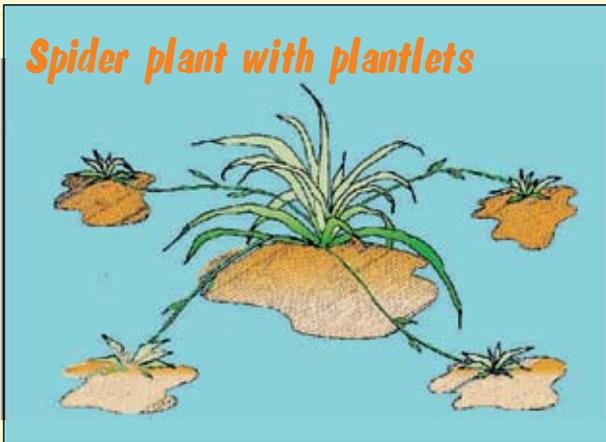
**Efia**



**Dr Mardea**

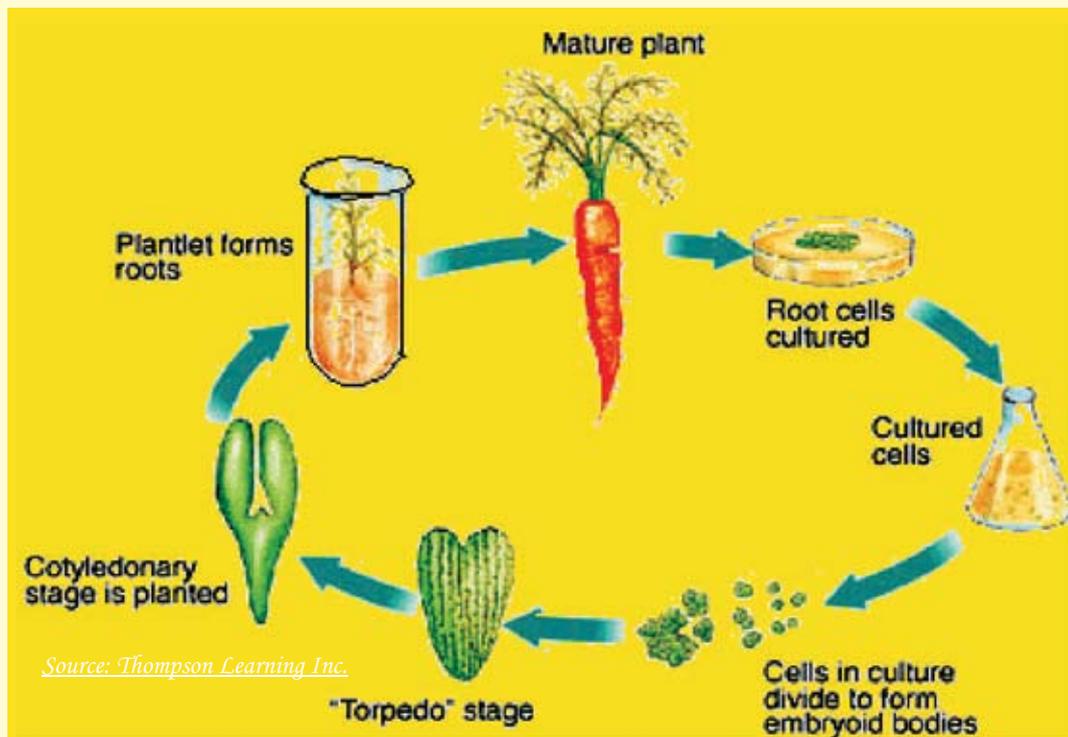
## Link between "Totipotency" and "PGR"

*Spider plant with plantlets*



*A plant reproduces itself vegetatively using its totipotent cells.*

**Totipotency: the ability of a cell to multiply into a whole organism.**



*Source: Thompson Learning Inc.*

*A carrot root is cut into discs and cultured in a liquid nutrient medium. Individual cells are divided to form clumps of undifferentiated cells known as embryoid bodies. Transferring the embryonic tissue to a solid nutrient medium stimulates the tissues to form small plants, called plantlets, which then develop into mature plants.*

**Dhakiya:**

Totipotency is familiar to us because our teacher talked about it. Plasticity sounds like the cells would be flexible. Am I correct, Dr Mardea?



**Dr Mardea:**

You are almost right. First, can you explain what "totipotency" means?



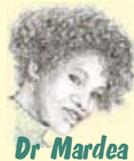
**Dhakiya:**

"Totipotency" of the cell or tissue means, that the starting tissue has the potential to generate all other cells and tissues necessary to grow a whole new plant. In other words, the starting cell or tissue has the ability to generate not just its own type of cell but any type of cell found in that particular plant.



**Dr Mardea:**

Very Good. "Cellular totipotency" is a very old concept. The term was coined in 1902. Your comment about plasticity is also correct. Plasticity of a cell or tissue means that it is able to adjust to the changes in the environmental conditions.



**Ekow:**

Dr. Mardea, please wait, I am very confused. I think we are talking about two different things here. One is that plant tissue culture is a biotechnology tool. It can be used to grow a whole new plant from a small part of a plant in sterile conditions with proper nutrition using cells that are totipotent. But how is this subject connected to our previous discussion about PGR and the need to conserve PGR in the form of germplasm so that we can use it when we need it. Tissue culture is a biotechnology tool, but how is it used for germplasm conservation?



**Dr Mardea:**

A very good question. This means that you have understood everything up to now.



**Ekow:**

**(With a big smile)** But I still don't understand the direct link between plant tissue culture and plant germplasm conservation.



**Dr Mardea:**

OK. Now, tell me, what is plant germplasm?

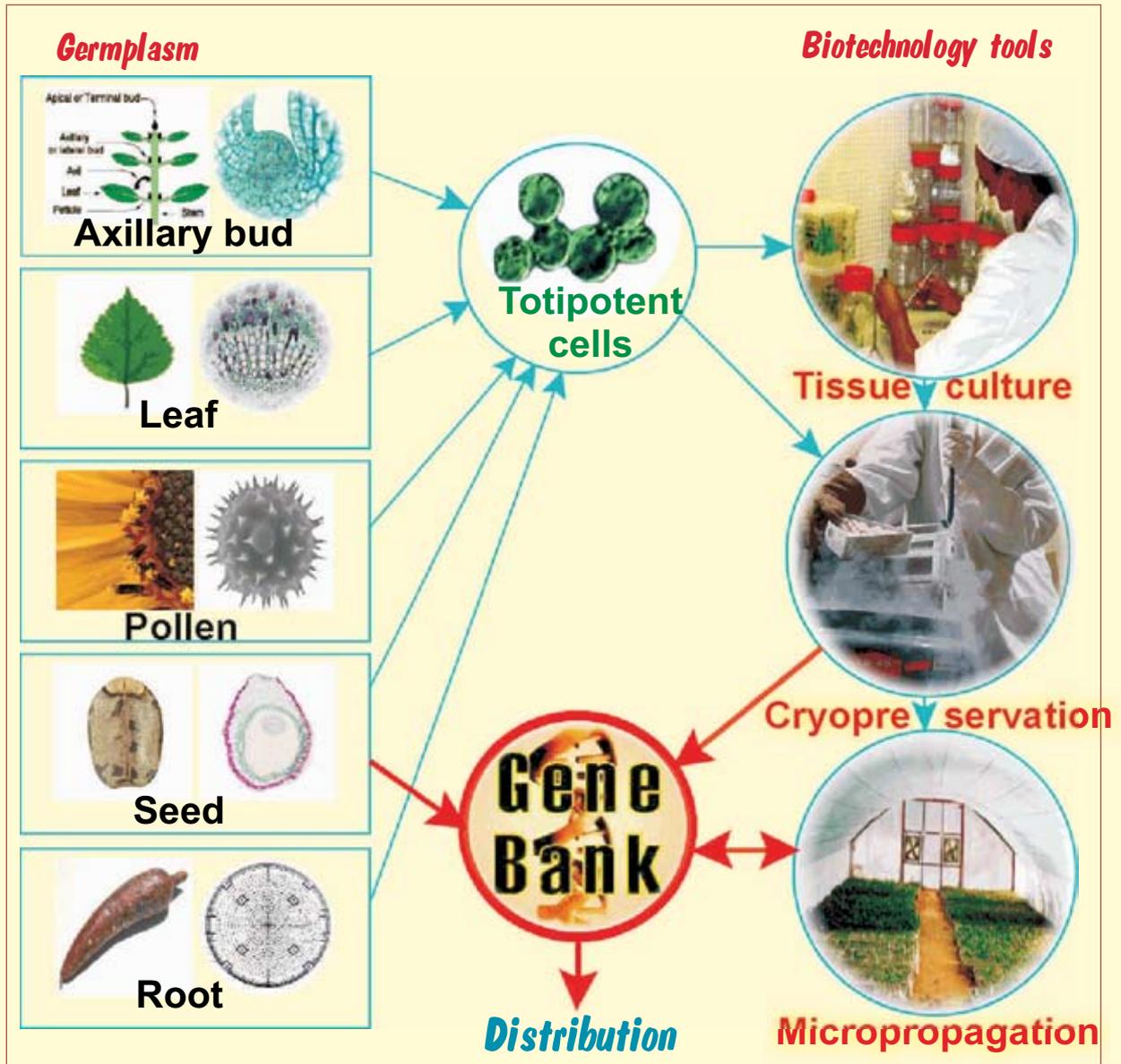


**Ekow:**

Plant germplasm is the genetic material of the plant that can give rise to a new plant.



## Link between biotechnology tools and PGR conservation



*Plant germplasm is the living tissue from which new plants can be grown. Germplasm is usually seed, or another plant part - a stem, a leaf, or pollen or even just a few cells that can be cultured into a whole plant.*

*Use of biotechnology tools, when appropriate, will stop the decline of agricultural biodiversity and will enhance the wealth of genetic resources for future sustainable use.*

**Dr. Mardea:**

And what is that genetic material?

**Safwani:**

It is the seed of a plant that contains the whole genome of that plant.

**Dr. Mardea:**

So, if a plant does not have any seeds or cannot produce good seeds, would you then say that it does not have any germplasm to produce a new identical plant?

**Ekow:**

No, that is not true. We know that cassava plants can be propagated by the asexual method or through tissue culture. It is not necessary to have cassava seeds to grow a cassava plant.

**Dr. Mardea:**

Right. Plant germplasm is the living tissue from which new plants can be grown. Plant germplasm is not only the plant seed but also other plant parts, such as the stem, leaf or pollen. For example, a few cells from a plant can be cultured into a whole new plant. Plant germplasm contains all the genetic information for the plant's hereditary make-up.

Now do you understand why the tissue culture technique is used for germplasm conservation, exchange and micropropagation?

**Ekow:**

Not really.

**Dr. Mardea:**

If you think logically, you will understand Ekow.

If we want to conserve or propagate a plant, tissue culture techniques and sterile conditions are essential, to get a clean sample of the germplasm from the appropriate part of that plant.

If the germplasm is to be transferred from place to place and is to be used after a long period of time, it has to be stored in a suitable way using cryopreservation. All these activities are steps in germplasm conservation, exchange and reuse, to save and provide healthy and disease-free resources which then can produce healthy disease-free plants. Therefore, a knowledge of biotechnology tools is essential for the conservation and use of plants.

**Ekow:**

Now I understand. **(Smiles with relief)**

**Dr. Mardea:**

Wonderful! I am glad that you understand.

**(Bell rings and the class is dismissed and the students leave.)**



**Dr Mardea**



**Safwani**



**Dr Mardea**



**Ekow**



**Dr Mardea**



**Dr Mardea**



**Ekow**



**Ekow**



**Dr Mardea**



**Dr Mardea**



**Ekow**



**Ekow**



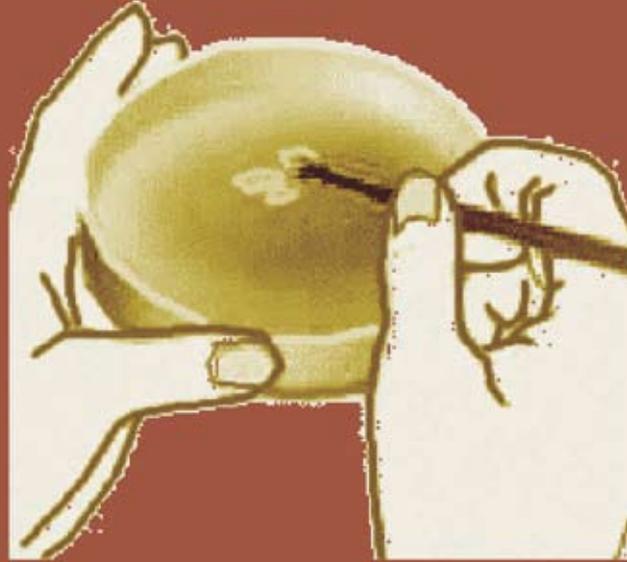
**Dr Mardea**



**Dr Mardea**



# Scene Eight



## *Tissue culture*

# *Wednesday morning*

*Scene Eight:*

Wednesday morning

*Place:*

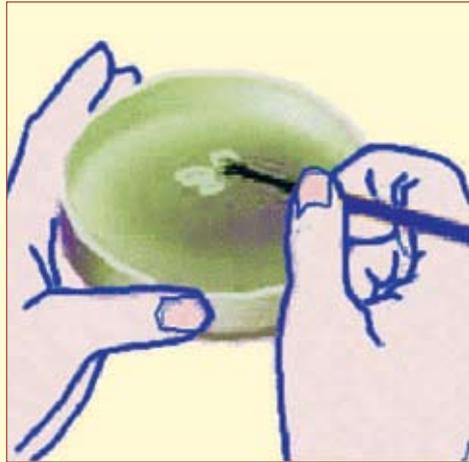
Classroom

*Characters:*

Students, teacher and  
Dr Mardea

*Situation:*

Dr Mardea talks with the  
students about tissue culture  
and cryopreservation

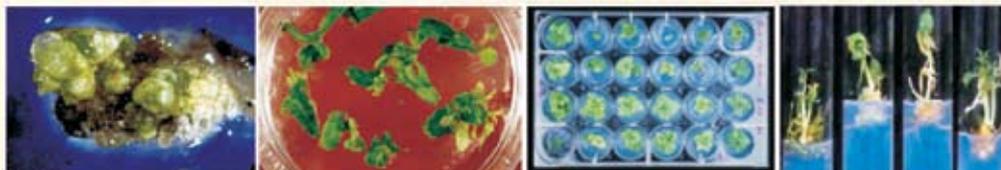


*A petri dish used for plating cells in a nutrient medium in the form of gel*



Photo source: G.D. Stone

*Cassava plants growing from callus*



*Different stages of cell culture*

Scene Eight:

Wednesday morning

Place:

Classroom

Characters:

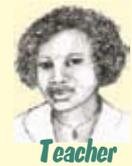
Students, teacher and  
Dr Mardea

Situation:

Dr Mardea talks with the  
students about tissue culture  
and cryopreservation

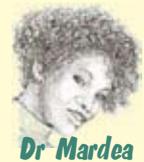
**Teacher:**

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:**

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:**

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



**Dhakiya:**

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



**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.

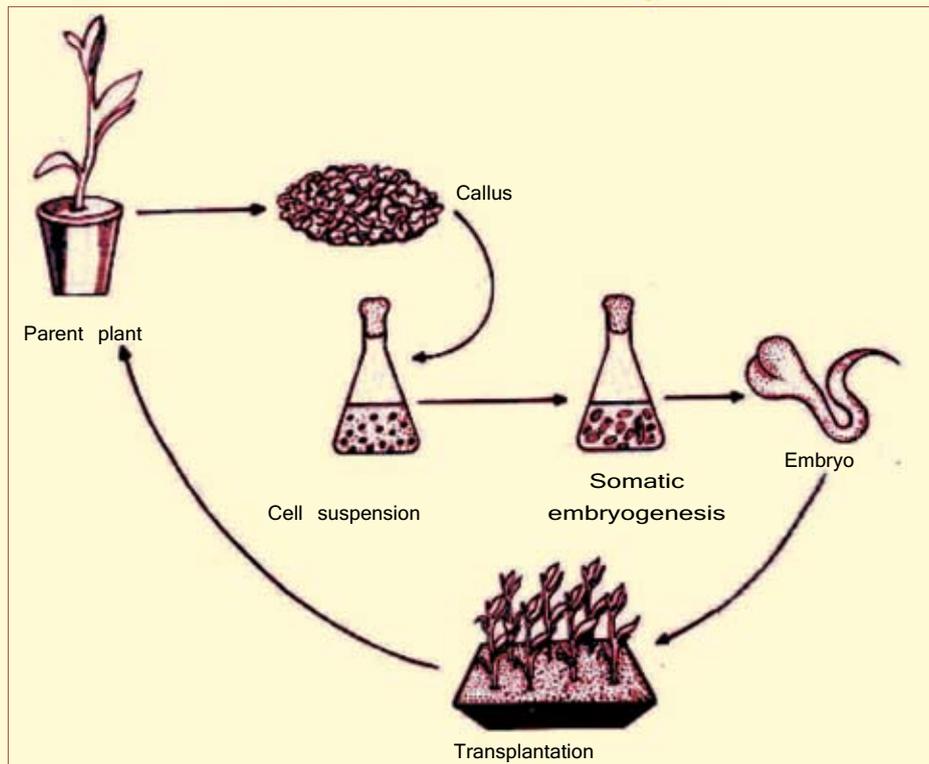


**Kunto:**

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  $196^{\circ}\text{C}$ , 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*



### *Cryopreservation 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?



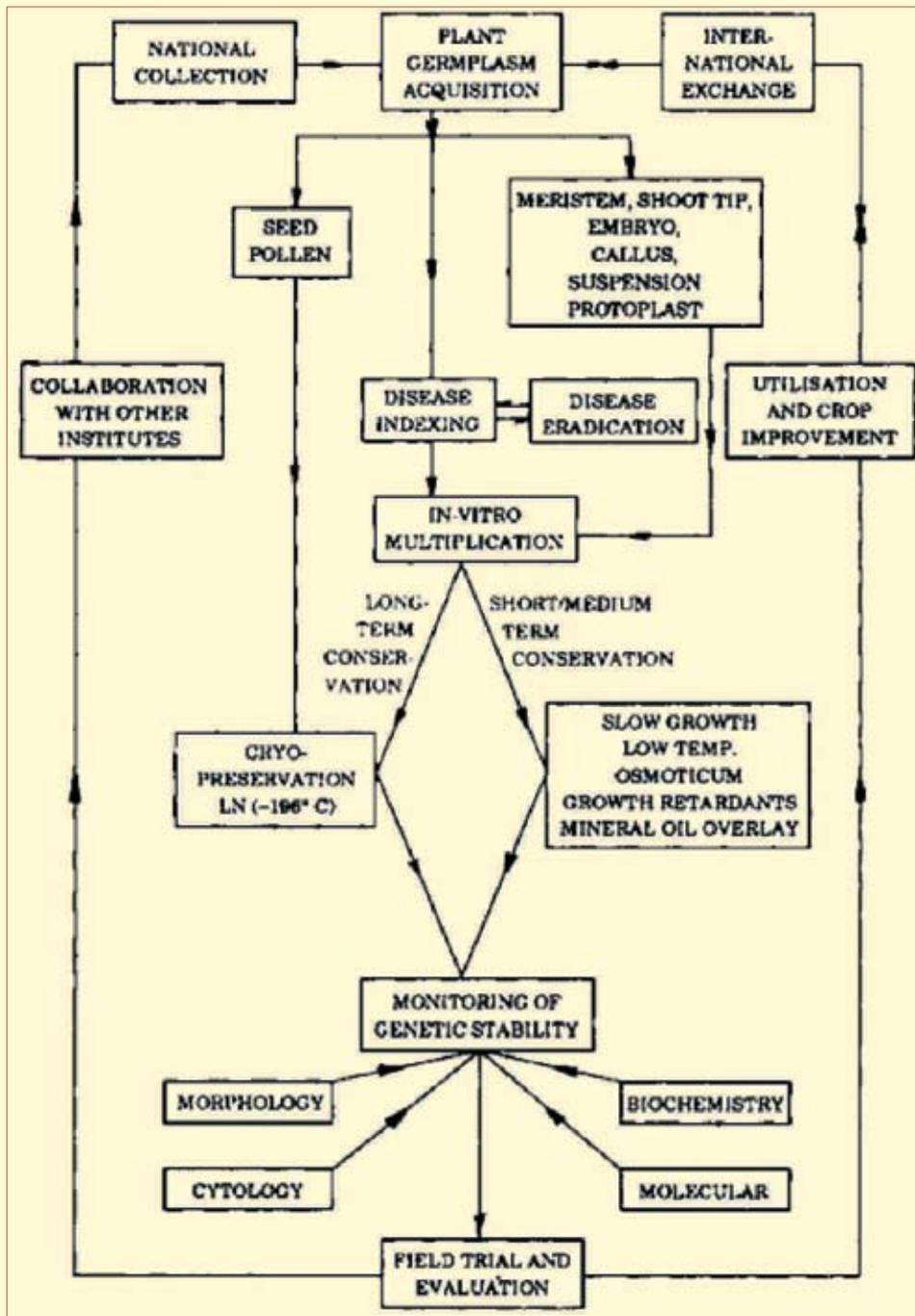
**Dr. Mardea:**

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*

<i>Scene Nine:</i>	Friday morning
<i>Place:</i>	Classroom
<i>Characters:</i>	Students, teacher and Dr Mardea
<i>Situation:</i>	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?

**Teacher****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?

**Thandiwe****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?

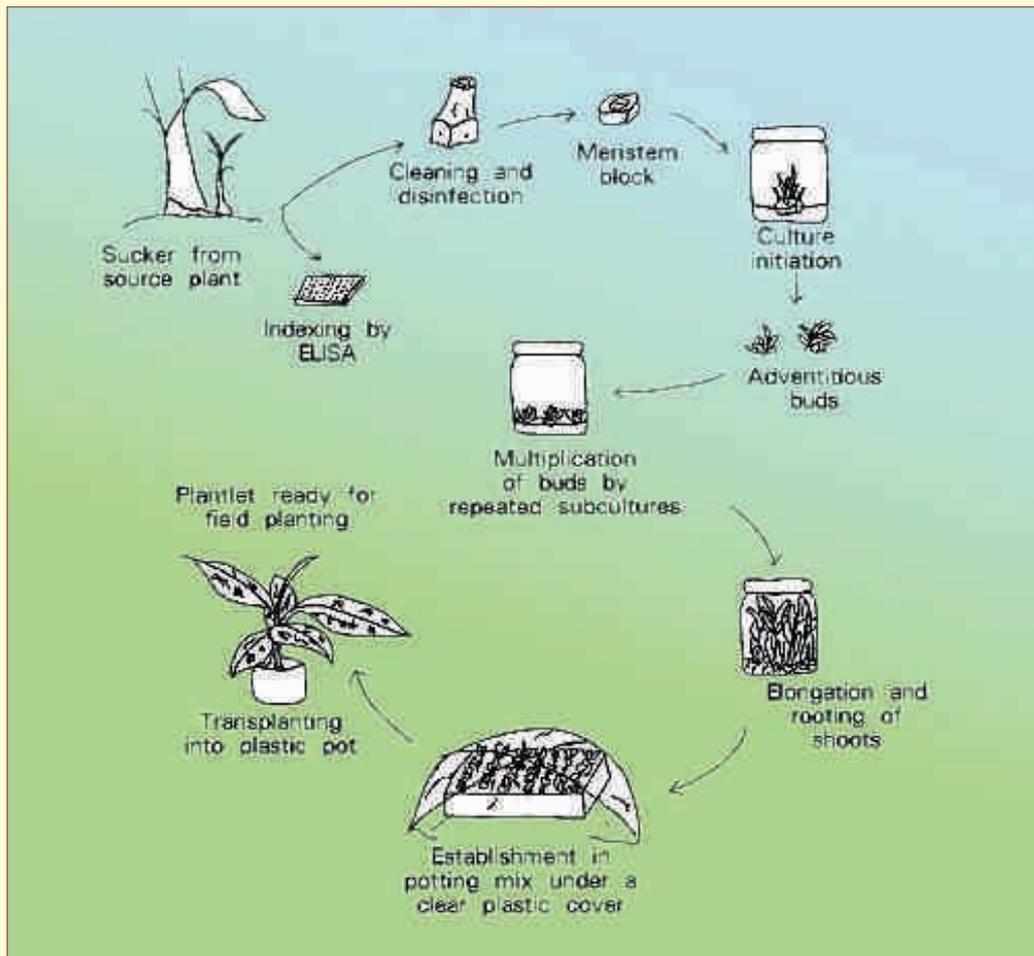
**Teacher****Runako:**

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)**.

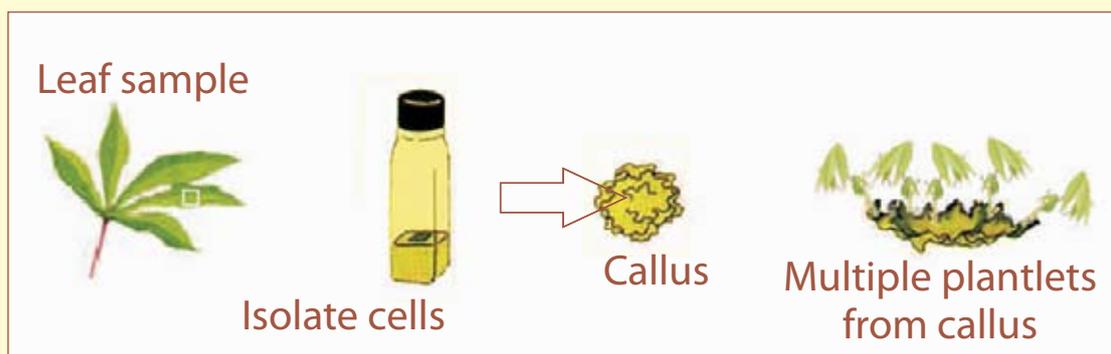
**Runako****Teacher:**

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.

**Teacher**



### *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

### 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.

### 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.

### 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.

### 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.

relatively short periods of time using tissue culture techniques.

**Adesimbo:**

Oh! I understand. Tissue culture is one of the techniques used in micropropagation. Am I right 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:**

No! What are the steps involved in micropropagation?



**Dr Mardea:**

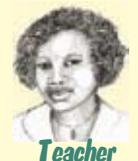
Micropropagation involves 4 steps. A stock plant is a parent plant. Micropropagation is usually used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative propagation



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:**

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

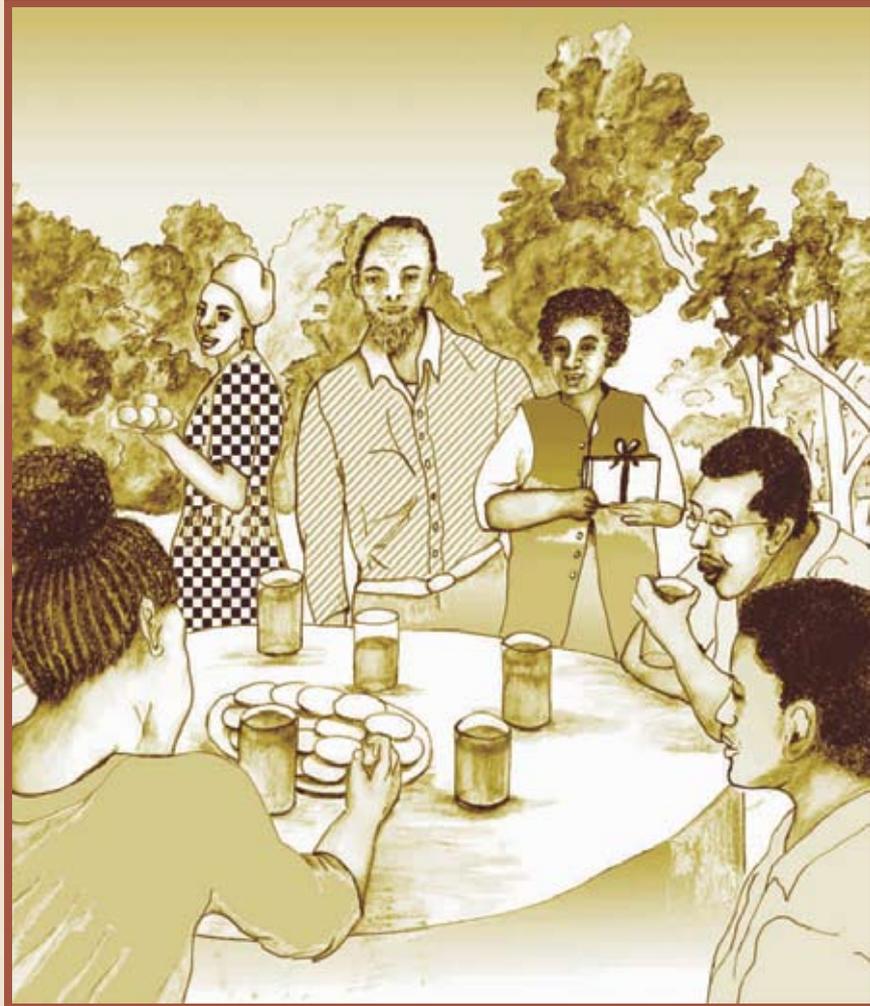


Well, class, it is Friday and it is time for your lunch break. Off you go now and have a nice weekend.

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

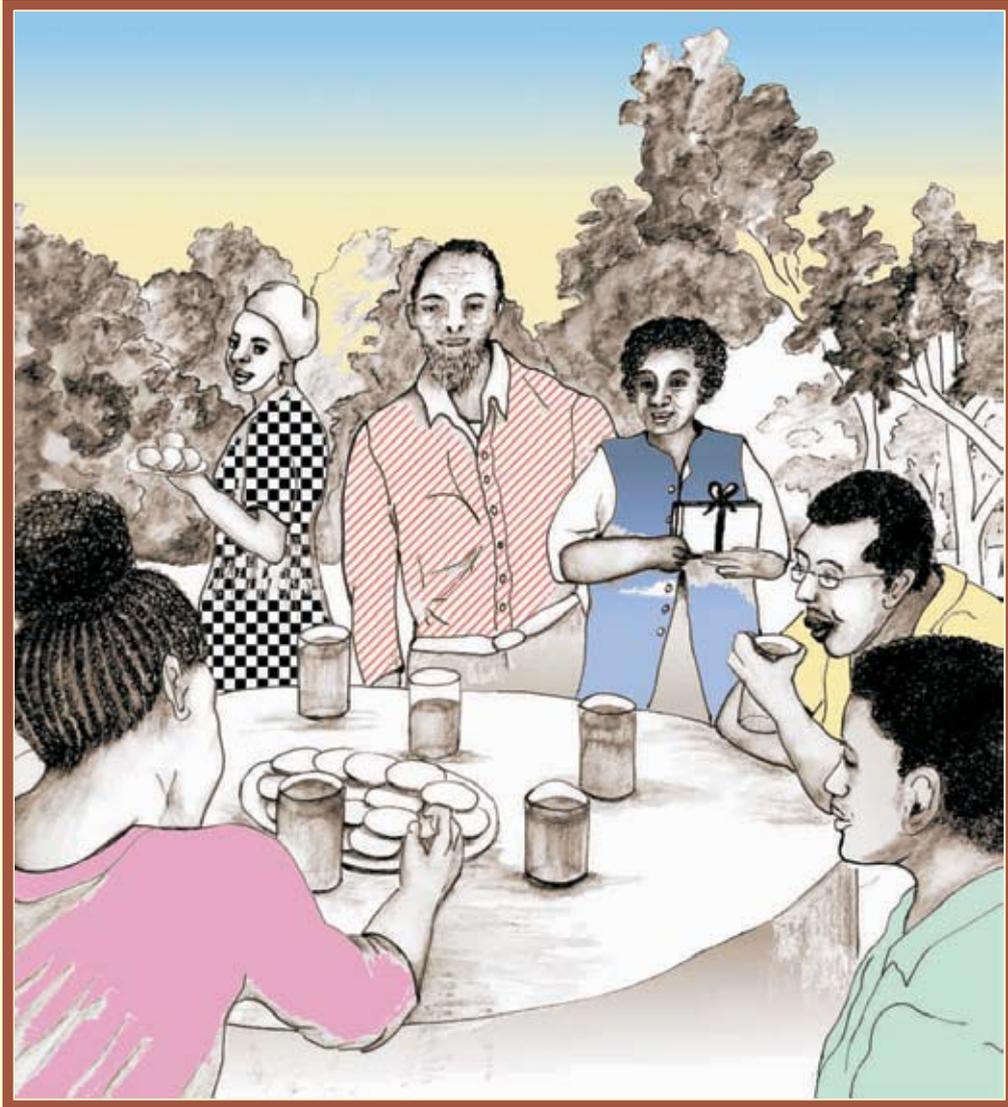


# Scene Ten



## Friday evening

<i>Scene Ten:</i>	Friday evening
<i>Place:</i>	Home
<i>Characters:</i>	Efia, Ekow, their parents and grandparents
<i>Situation:</i>	The family is sitting in the garden



*Efia, Ekow, their parents and grandparents in the garden at home*  
**Cassava dishes**



Scene Ten:  
Friday evening

Place:  
Home

Characters:  
Efia, Ekow, their parents and grandparents

Situation:  
The family is sitting in the garden

**(Ekow and Efia are sitting in the garden with their parents telling them all about tissue culture. Their father works for a PGR centre and he is impressed by their knowledge of tissue culture.)**

**Ekow:**

Dad, do you culture a lot of varieties of plants at work?

**Father:**

Son, the place where I work is called a PGR centre. We do not culture plants, but we collect and conserve the germplasm of PGR for the local area. We characterize, evaluate and document PGR under conservation. We encourage the utilization of the conserved resources by appropriate users, such as plant breeders, researchers and farmers. We serve as a central agency for the introduction and exchange of PGR. We also carry out research on any problems that may arise from any of the above objectives.

**Efia:**

This sounds complicated. Our teacher explained it in a simple way. Anyhow, maybe you can take us there one day.

**Father:**

Yes, when you have understood the seriousness of conserving plant genetic resources I will take you and explain what we do.

**(There is some noise near the front door. Grandma and Grandpa are walking in. Grandma has a big package in her hands. The kids run to the door to greet them.)**

**Ekow and Efia:**

Hello Grandma! Grandpa!

**Ekow:**

What did you bring us Grandma?

**Grandma:**



Ekow



Father



Efia



Father



Ekow

## Diseases affecting cassava

### BACTERIAL

Bacterial blight  
Bacterial leaf spot  
Bacterial stem gall  
Bacterial stem rot  
Bacterial wilt

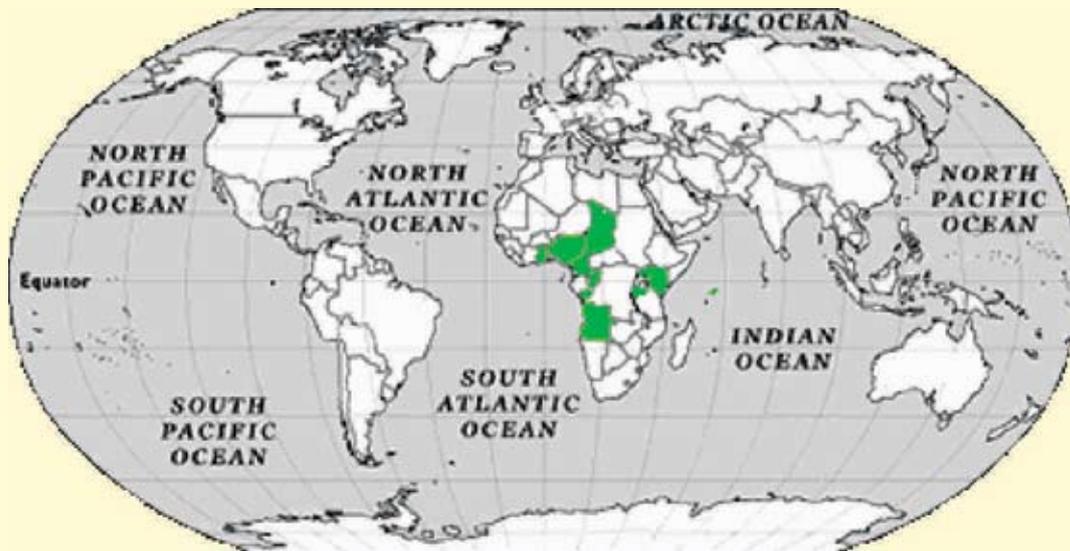
### VIRUS

African cassava mosaic  
Cassava common mosaic  
Cassava frog skin  
Cassava green mottle  
Cassava green mottle virus  
Cassava symptomless infections  
Cassava vein mosaic  
Indian cassava mosaic  
Witches' broom

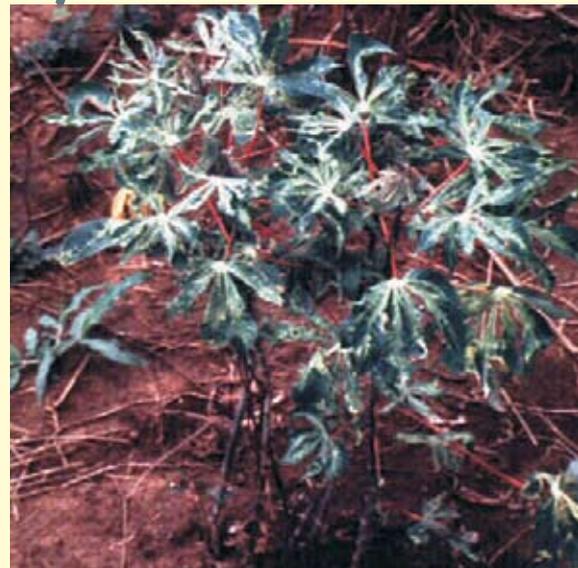
### FUNGAL

Armillaria root rot  
Black root and stem rot  
Blight leaf spot  
Cassava ash  
Concentric ring leaf spot  
Rust

### African cassava mosaic distribution in Africa



### *M. Esculenta* affected by African cassava mosaic



Source: <http://gemini.biosci.arizona.edu/viruses/acmv/index.htm>

I brought you a cassava sweet. Your Grandpa liked it so much, I thought I should bring some for you too.

**Efia:**

There you go, another recipe for the good old cassava!

**Grandma:**

Well, Efia, you are right, you can do so many things with cassava roots. These days there are so many varieties, but I still miss some of those cassava roots we used to get. I think they have all been lost as we couldn't keep them alive when we had problems with some cassava diseases.

**Father:**

Mother, we have not lost the varieties. We just don't grow them all at all times. Most of them are stored in gene banks where you can get them if you want to grow them.

Efia, did you learn about gene banks in your biology class?

**Efia:**

Yes, Dad. Our biology teacher mentioned them briefly to us when she talked about plant genetic resources and conservation.

**Mother:**

In that case, are you going to ask her to talk about them in more detail in your next class?

**Efia:**

Yes, Mum, we will. I am sure she will be very happy to tell us all about them.

**Mother:**

Then you can tell your grandparents all about gene banks the next time they visit us.

**Father:**

Come on, it is time for dinner. I can't wait to eat the cassava sweet that you brought mother!

**Grandma:**

OK, But please make sure to leave some for the children. *(Laughs)*





# Scene Eleven



## *Monday morning*

*Scene Eleven:* Monday morning

*Place:* On the way to school

*Characters:* Efia and Ekow

*Situation:* Efia and Ekow are talking while walking to school



The concept of conservation is very important for sustainable security... I think I would like to be a conservation specialist...

Plant engineer?

I'd like to know about genes. I think I will become a geneticist

I would like to be able to change things to make farmers lives better... what can I be?

Extensionist?

Biologist?

**Dene**

**Dalila**

**Hakika**

**Dhakiya**

*Scene Eleven:*

Monday morning

*Place:*

On the way to school

*Characters:*

Efia and Ekow

*Situation:*

Efia and Ekow are talking while walking to school

**Efia:**

Ekow, what would you like to do when you grow up? Do you think you might become a plant specialist?



Efia

**Ekow:**

I like all these fine details about how things work, so I could become an engineer, maybe a plant engineer; someone who understands the mechanisms that make plants grow well, reproduce and give good food. What would you like to learn when you finish school?



Ekow

**Efia:**

I would like to learn more about finding ways to save all the varieties of plants for future use.



Efia

**Ekow:**

You mean you want to become a conservation specialist?



Ekow

**Efia:**

Well, not a conservation specialist without a specific conservation purpose. I would like to conserve things in such a way that what is conserved will become useful in the future.



Efia

**Ekow:**

You mean like Grandma wanting to see the cassava she used a long time ago?



Ekow

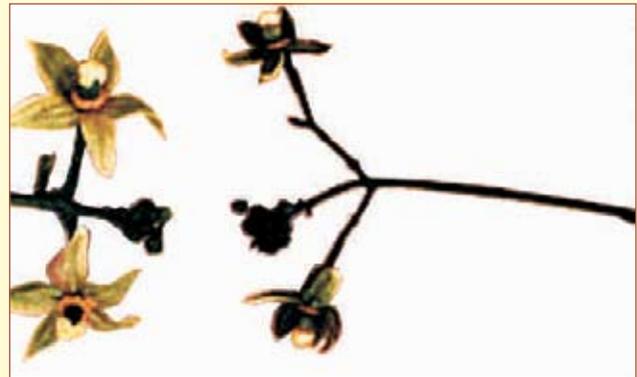
**Efia:**

Ekow, some things are saved for future generations to be able to just look at them. However, old varieties may provide new information in the future. There is always good use for things that are saved. The cassava that Grandma remembers grew well under unfavourable conditions such as drought, but died due to some disease. So, if we find the drought resistant variety in the gene bank and crossbreed it with another cassava variety that is disease resistant, maybe we would be giving a big gift to someone who cultivates cassava in dry-lands.



Efia

## *Cassava flowers*



*Differences in flower discs  
can be used as markers*



*Differences in shapes, sizes and designs of cassava  
fruit can be used as a marker trait*

Review: Gene flow between cassava, *Manihot esculenta* Crantz and wild relatives  
Nagib M.A. Nassar

**Ekow:**

Ah! This is why you are my big sister and I am your little brother. You always think big.

**Efia:**

Well, we may be in the same class, but I am older than you and also bigger than you.

*(Efia puts her right hand on Ekow's head to show him that he is shorter than her and Ekow shirks and moves away from her. Efia compliments him by saying.)*

**Efia:**

Ekow you are the smartest and brightest between the two of us.

**Ekow:**

But that is because you didn't join school as early as I did. Anyway I think you are the best sister ever.

**Efia:**

Thank you, Ekow. Now, we must talk to our biology teacher. Don't forget.

**Ekow:**

OK, let's go.

*(Both walk towards the biology teacher's room.)*

**Teacher:**

Efia, Ekow, good morning. What do you have on your minds?

**Ekow:**

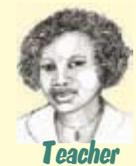
Our grandmother visited us this weekend and brought us a cassava sweet. We told her all about plant tissue culture and how it is possible to have good, healthy cassava roots, but she was saying that the old cassava she had a long time ago is no longer available and that she misses it now.

**Efia:**

My father said that those cassava varieties were not all lost. Some of them are conserved in gene banks. So we would like to know more about gene banks and about saving old cassava varieties. We would also like to know how people recognize and identify the different varieties after storage.

**Teacher:**

It is nice to know that you both talk about science at home and that your parents encourage you to know all about these things. Let me see what the plans are for the biology class this week and then I will announce how and when we can talk about gene banks and other related topics. See you later in the classroom.

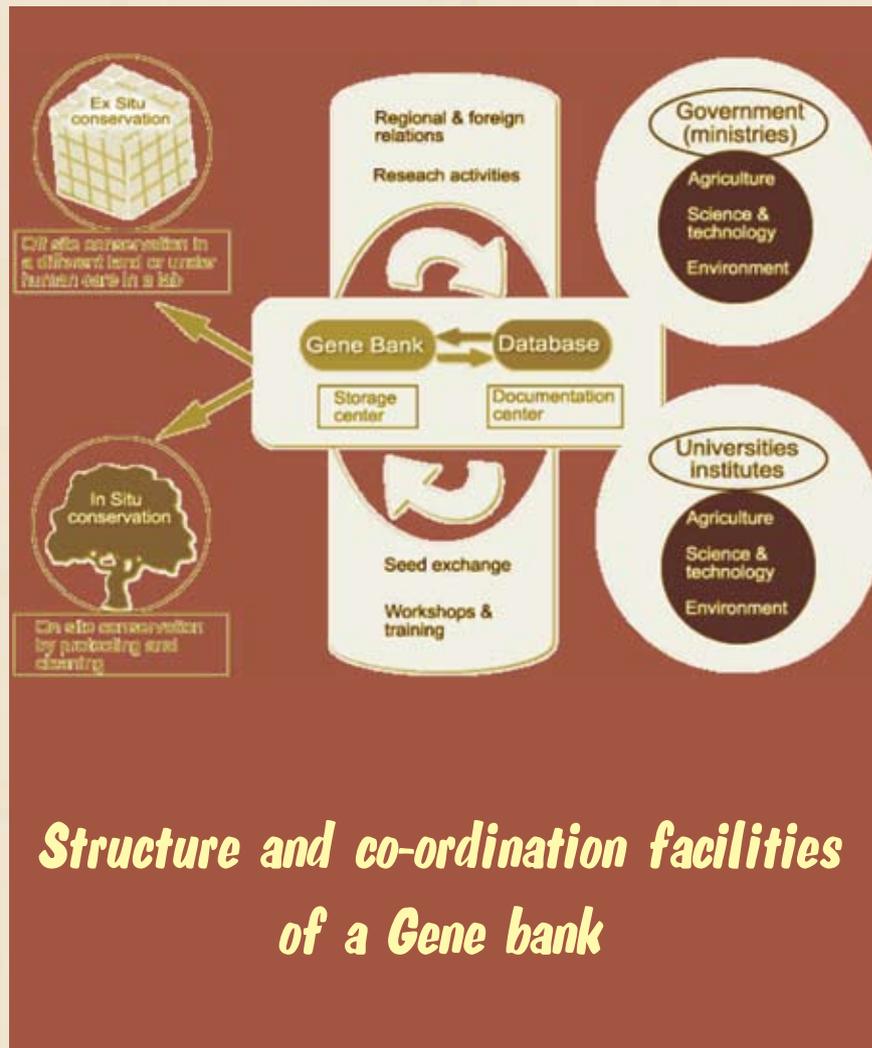
**Efia and Ekow:**

Thank you. See you later.





# Scene Twelve



## Monday afternoon

*Scene Twelve:*

Monday afternoon

*Place:*

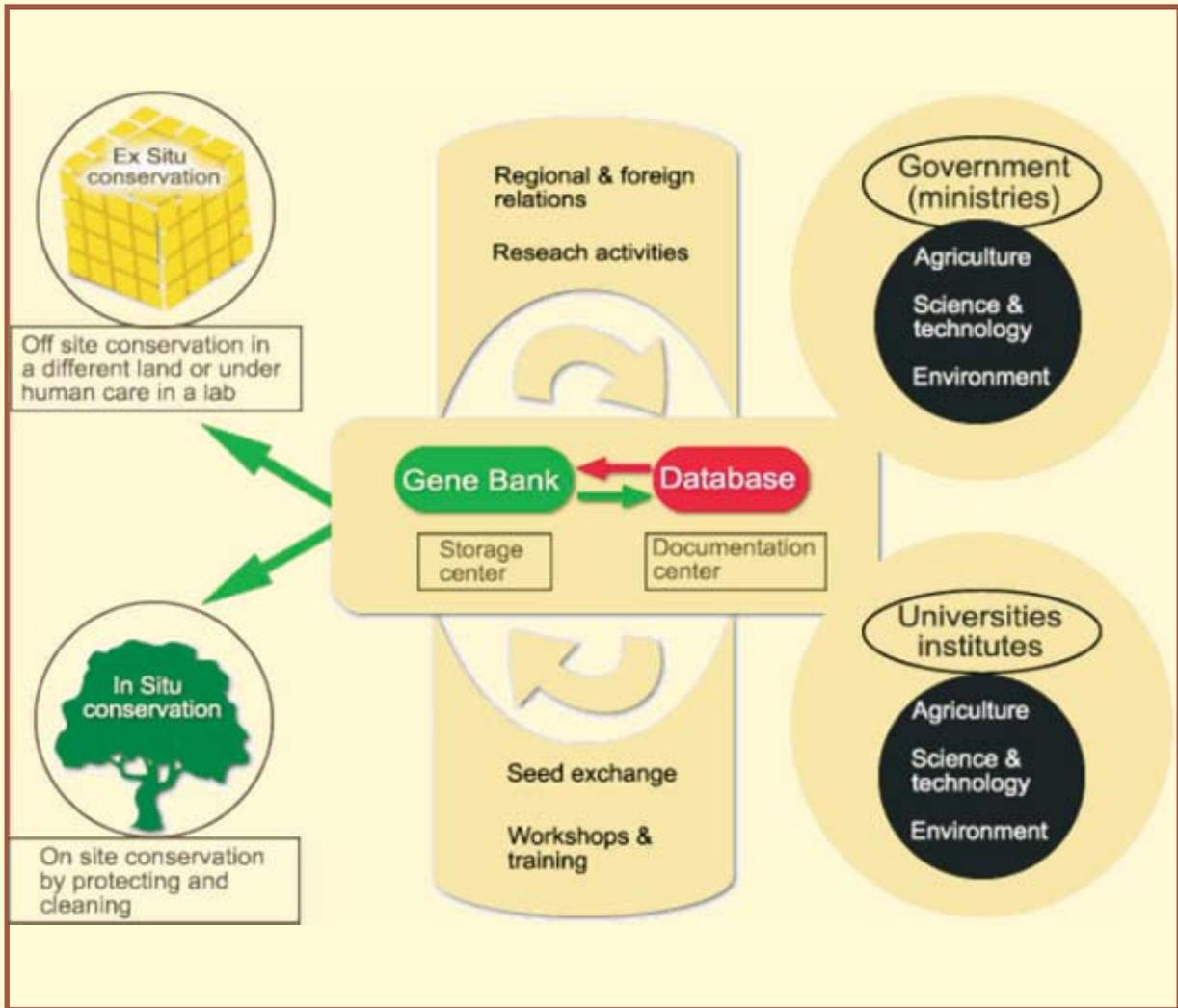
Classroom

*Characters:*

Efia, Ekow, all other students and the biology teacher

*Situation:*

Biology class: discussion about gene banks, DNA and MAS



***Structure and co-ordination facilities of a Gene bank***

The collage shows five activities involved in Gene bank maintenance: **Germplasm selection** (people in a field), **Germplasm diversity** (people at a table), **Gene bank curator** (a person with a clipboard), **Viability test** (a person with a microscope), and **Molecular analysis** (a person with a computer screen).

***Various activities involved in Gene bank maintenance***

Scene Twelve:

Monday afternoon

Place:

Classroom

Characters:

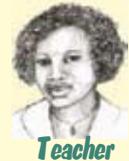
Efia, Ekow, all other students and the biology teacher

Situation:

Biology class: discussion about gene banks, DNA and MAS

**Teacher:**

Good morning everyone. Do you recall where we stopped in our last class?



**Ekow:**

Yes. Last Friday we discussed tissue culture and micropropagation and how they help in PGR conservation. But, I would like to know more about gene banks.



**Teacher:**

Yes. But first, let me ask you a question to see how much you know about gene banks and then I will decide what to talk about in today's class. Can someone tell me what gene banks are?



**Dhakiya:**

A gene bank is a place where genetic information is stored.  
(**Ekow butts in.**)



**Ekow:**

No, it is a facility for *ex situ* conservation, where seeds, tissues or reproductive cells of plants are kept.



**Teacher:**

Ekow, the strict definition for gene bank is "a place where genetic information is stored". *In situ* conservation of plants in the fields are also considered as gene banks when plants are conserved for the purpose of propagation, storage and distribution. Any other definitions?



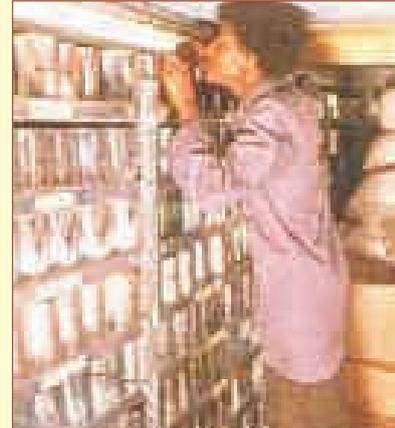
**Efia:**

A gene bank is a place where a collection of germplasm in the form of seeds, pollen, DNA and even whole plantlets is collected and maintained. If you need a specific sample, you can get it from there. That is why it is called a bank.





*Lab workers at a gene bank at one of the Consultative Group of International Agricultural Research centers in Mexico, sorting through seeds and selecting high-protein corn kernels for preservation in cold storage units.*



*This gene bank in Ethiopia stores the seeds of hundreds of wild varieties of crops at sub-zero temperatures for up to 50 years. Every five years the collection is tested for its germination ability.*



*Various ways of storing seeds in gene banks: individual varieties of seeds are packed and labeled (1&4) in bottles, (2,5&6)in packets and (3) in sacks in a room at a cold temperature.*

**Sabola:**

Aren't genes from animals also stored in gene banks.



**Teacher:**

Yes. You are all correct! In simple words, a gene bank is a place where you can go in search of genetic resources. Can anyone tell me about plant gene banks?



**Daila:**

A plant gene bank stores material from plants of interest for future use in agriculture. It also stores information on the species in question and maintains a database on the PGR conserved within a country.



**Pepukayi:**

Are cassava varieties stored in gene banks?

**Teacher:**

Yes, Pepukayi. Many cassava varieties are also stored in gene banks.



**Sabola:**

Do all gene banks have a similar structure inside?



**Teacher:**

Gene banks can be *in vitro* or *in situ* gene banks. Can anyone tell me what *in vitro* means?

**Sabola:**

Yes! We discussed this when we talked about tissue culture. It means the process happens in an artificial environment.



**Teacher:**

Very good. An *in situ* gene bank means conservation of adult plants, in the field or in greenhouses.



**Gamba:**

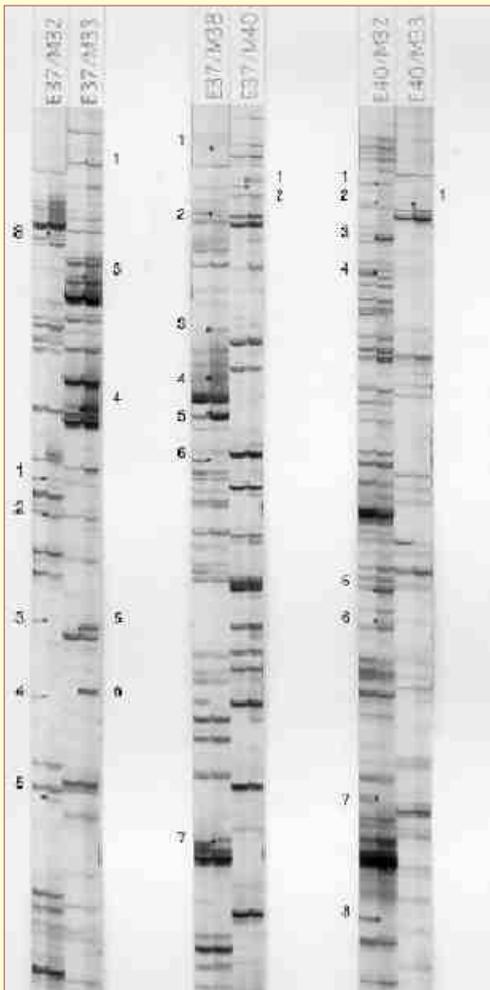
So, *in vitro* gene banks conserve parts of plants, cells and tissues in artificial conditions while *in situ* gene banks conserve complete plants under natural conditions.



**Teacher:**

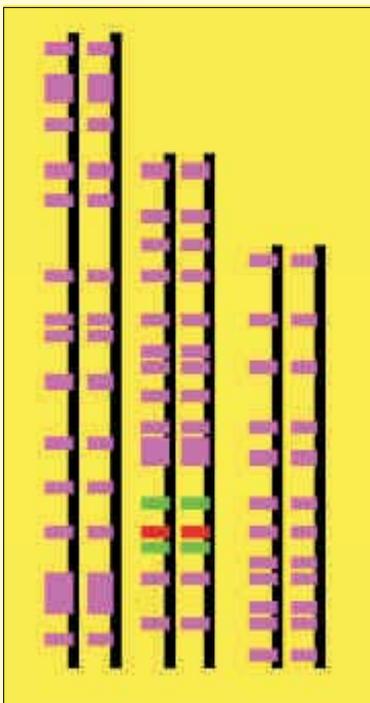
Yes. Originally, gene banks maintained information on the name of the crop variety and on some basic characteristics. But today, new techniques enable us to create gene maps and discover precise information on the roles that specific genes play. As a result, new varieties with desired traits can now be bred much faster. Does this make sense Gamba?





The "DNA fingerprint" on the right shows typical results of Polymerase Chain Reactions (PCR) from plant DNA. In each pair of adjacent lanes in this gel image, DNA from barley cultivars Proctor (left lane) and Nudinka (right lane) are amplified. The results using six different primer sets (indicating the markers) are shown, for a total of 12 lanes. DNA differences (polymorphisms) between Proctor and Nudinka, marked with numbers, appear as a presence or an absence of bands on the gel. For example, the band labeled 1 for a specific (primer) marker is present in cultivar Proctor (left) but absent in cultivar Nudinka (right). This band represents a genetic locus at which the DNA sequence is different between the two cultivars. Between bands 1 and 2 is an unmarked band that is shared in common between Proctor and Nudinka. This band represents a different chromosomal locus that is identical between the two cultivars.

Source: University of Manitoba, Canada



### Reconstituted elite cultivar:

A selection of plants in a given generation of backcross generation is done by scoring dozens of markers using DNA from individual plants. Each band on a gel can be scored as coming from one parent (presence of a band) or the other parent (absence of a band). In the figure (left), the map locations of markers from the elite cultivar are shown in magenta, and those from the wild parent are shown in green. If we screen enough plants, we can find those plants that maximize the number of markers derived from the elite parent, while retaining the important gene of interest. In this map, a marker located very close to that gene is indicated in red. The majority of the time, if we have the marker, we have the gene.

A cultivar is a cultivated plant that has received a name under the International Code of Nomenclature for Cultivated Plants

**Gamba:**

I think so. But what is a gene map?

**Teacher:**

A gene map is a graphic representation of the arrangement of genes or DNA sequences on a chromosome. It is also called a genetic map. New technologies help crop scientists to catalogue the gene bank samples through identifying the traits of each variety and identifying and marking the genes responsible for key traits.

**Dhakiya:**

A trait is a specific characteristic of a plant, right?

**Teacher:**

Yes, Dhakiya. Traditionally farmers select the best plants in their fields and grow them again. This is called selection and it can take years to get a new variety. Now crop scientists can select a variety known to hold a specific trait, "mark" the gene responsible for that trait and cross it with another known variety. The offspring of these plants are then tested to determine if they hold the marked gene. Those that have it are selected for further tests and eventual field planting.

**Efa:**

So the marker gene is the one that marks a specific trait in a plant and that trait can be identified from the plant's genetic map. But, how does a gene map help conserve PGR?

**Teacher:**

A gene map on its own does not conserve PGR, Efa. But when you have a gene map of a plant, the knowledge you get from it, helps you to conserve a plant and to use its traits for breeding. The techniques that are generally used for accurate rapid detection are:

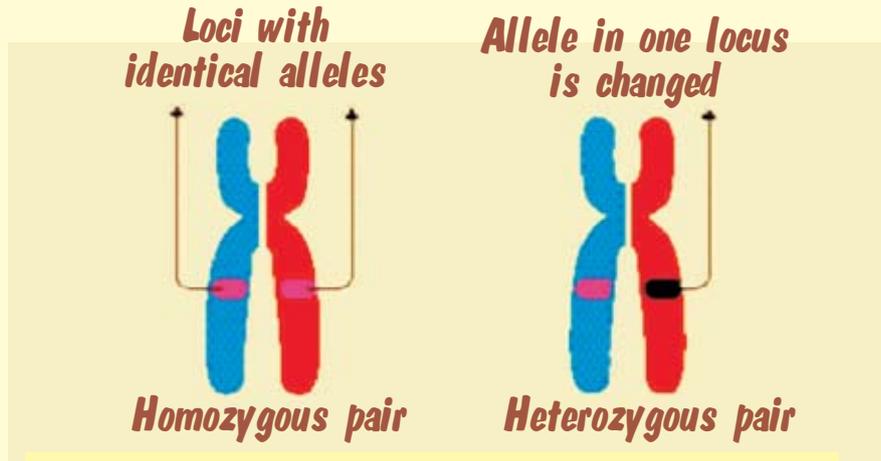
1. **DNA fingerprinting:** to find the degree of relationship between plant populations and also to measure the genetic distance between plant populations.
2. **Gene mapping:** to develop a map of markers using standard reference plant DNA.
3. **Identification of genetic markers:** to provide information on the presence or absence in an individual plant of specific genes, that are associated with plant traits.

**Dhakiya:**

Genetic markers are responsible for identifying the genes that code for specific traits of a plant, right?



## Chromosomes and Loci

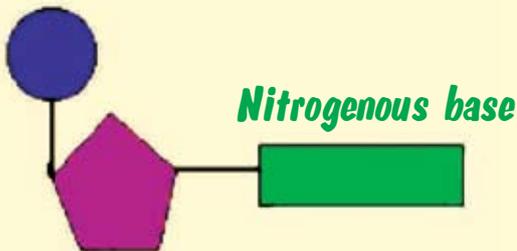


*A drawing of an homologous chromosome under conditions of homozygosity and heterozygosity*

*Homologous chromosomes are those that pair during meiosis, have the same morphology, and contain genes governing the same characteristics.*

## Nucleotide

**Phosphate**



**Pentose sugar**

## Nitrogenous bases

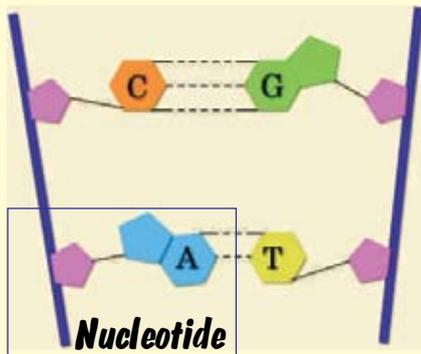
**A** Adenine

**G** Guanine

**C** Cytosine

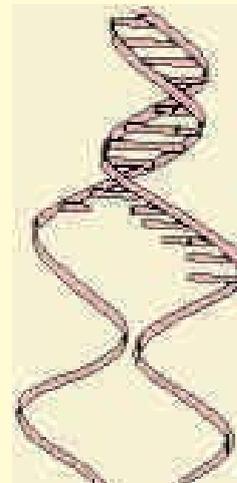
**T** Thymine

*Nucleotides bound by hydrogen bonds to form a double strand*



**Pentose sugar**  
**Phosphate**

*DNA double helix*



**Teacher:**

Yes, Dhakiya. Most such traits are influenced by multiple genes. The identification of DNA markers associated with specific loci will allow their use in marker-assisted selection or MAS.



**Ekow:**

What is a "loci" and how does a marker assist in selection?



**Teacher:**

Ekow, the word "loci" is plural and the singular is locus. Locus is the specific site of a particular gene on its chromosome. Before we discuss MAS, I would like to know more about your knowledge of DNA.



**Ekow:**

But, must we know about DNA to understand MAS?

**Teacher:**

Oh yes, you need to, because knowing DNA structure is essential in order to study anything related to biotechnology tools. The genes in the DNA "code" for various "proteins". Proteins are very important and there are thousands of proteins in a plant encoded by hundreds of genes. Only a small fraction of the DNA sequence carries genes that code for proteins, while the remaining DNA represents non-coding sequences



**Sabola:**

In that case, please tell us more about DNA. I do not remember much about it.



**Teacher:**

Yes, of course. All living things are made up of cells that contain the genetic material called deoxyribonucleic acid or DNA. DNA transmits genetic information. You can say that genes are made up of DNA. The DNA is packaged into chromosomes which are located within the nucleus of all cells. Every cell in the body contains all the chromosomes that collectively make up the genome of that organism.



**Efia:**

Is a plant genome then, a collection of all the chromosomes of that plant cell? Could you tell us a little bit more about DNA structure, please?



**Teacher:**

A single DNA molecule is made up of nucleotides. There are four of them called the adenine, thymine, cytosine and guanine. There is a basic rule regarding the way in which each of these nucleotides pairs with another using hydrogen bonds. Adenine will bind only to thymine with two bonds, and cytosine will bind only to guanine with three bonds. Two strands of the DNA are shaped like a double helix.



# Phenotype

The phenotype of an individual organism is either its total physical appearance or a specific manifestation of a trait, such as size, color, or behavior that varies between individuals.



**Phenotype** is determined to a large extent by genotype, or by the identity of the alleles that an individual carries at one or more positions on the chromosomes. Many phenotypes are determined by multiple genes and influenced by environmental factors. Thus, the identity of one or a few known alleles does not always enable prediction of the phenotype.

# Genotype

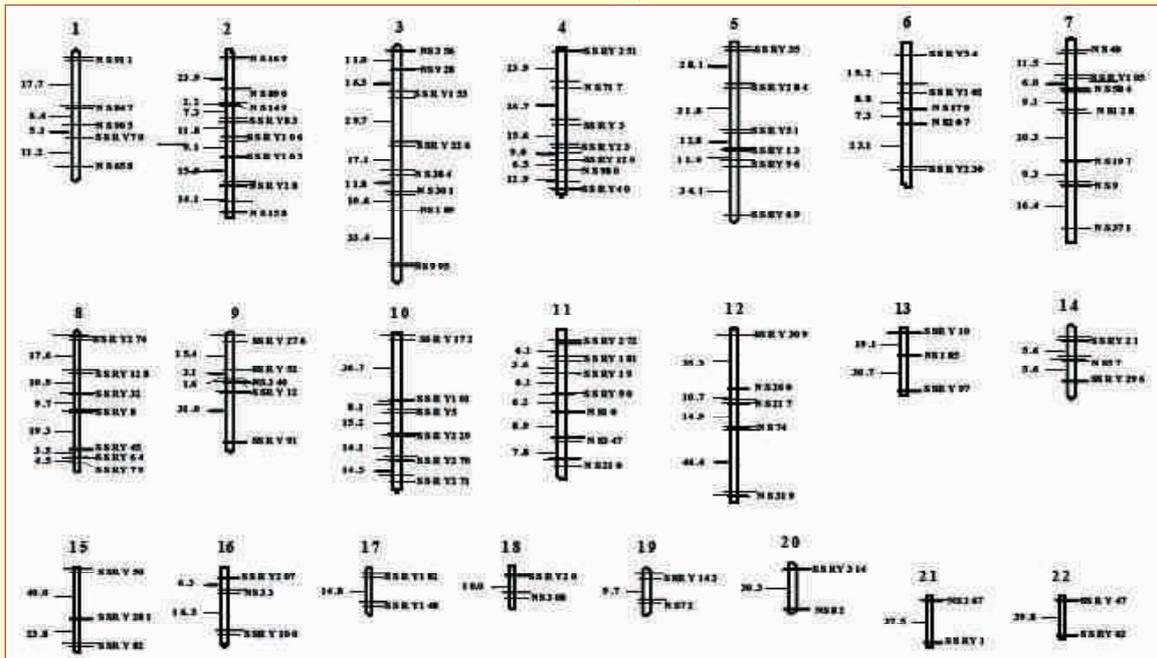


Figure above: A genetic linkage map of cassava based upon a F2 cross and SSR markers. Source: E. Okogbenin, J. Marin & M. Fregene; International Center for Tropical Agriculture, CIAT, Cali, Colombia

The genotype of an organism represents its exact genetic makeup, that is, the particular set of genes it possesses. The term "genotype" refers, then, to the full hereditary information of an organism. A gene map is a graphic representation showing the relative locations of each known gene on a particular chromosome. A gene map can also include DNA sequences on a chromosome. A gene map is used to locate and identify the gene or group of genes that determine a particular inherited trait. The mapping of a set of genotypes with a set of phenotypes is sometimes referred to as the genotype-phenotype map.

**Efa:**

The DNA carries the code for the appearance and the behaviour of the plant?



**Teacher:**

Yes, the genetic material of an organism is organized into sets of chromosomes. In addition to this, every gene has two copies which are called "alleles". The sexually produced new plant receives one allele of each gene from its mother, and one of each from its father. The visible characteristics or phenotype of the plant and the genetic make-up or the 'genotype' of the plant is determined by its DNA.



**Dhakiya:**

So, it is easy to see the phenotype by just looking at the plant but to know the genotype we have to study the DNA of the plant.



**Teacher:**

Yes, Dhakiya. But genotype and phenotype are not always directly correlated. Some genes express only a given phenotype under certain environmental conditions. Therefore, expression of phenotype is a result of interaction between the genes and the environment.



**Ekow:**

Very confusing. Could you explain how a phenotype can be the result of multiple genotypes?



**Dafina:**

Wait. But what about the genotype of a plant? When does the genotype express a specific phenotype due to environmental conditions?



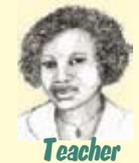
**Ekow:**

Dafina, that is a brilliant question! Teacher, can you give us an example?

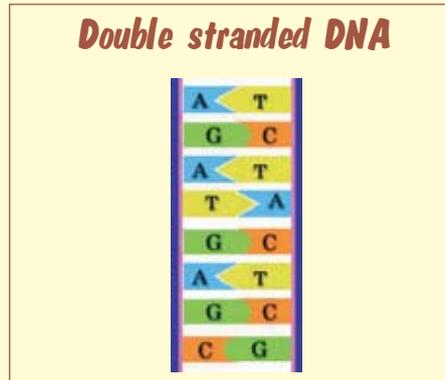
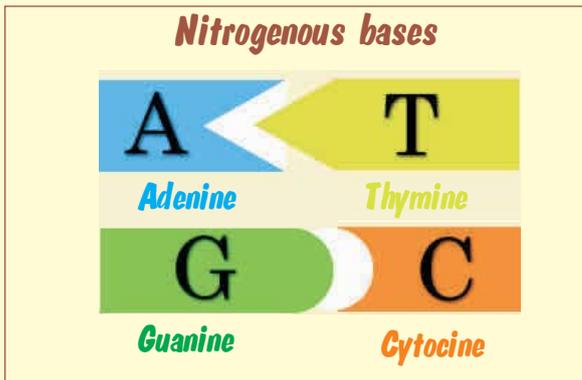


**Teacher:**

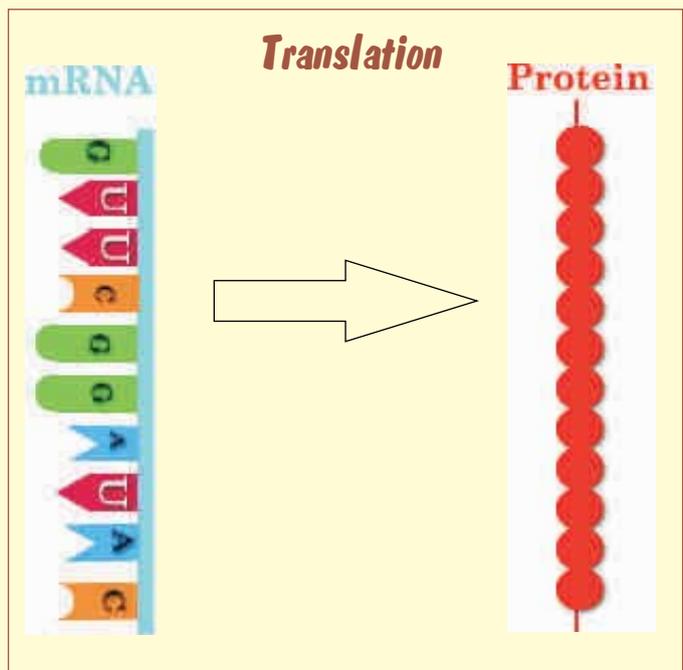
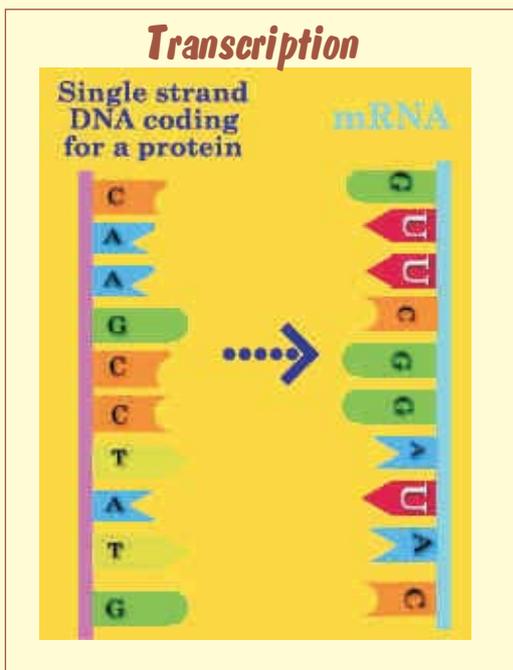
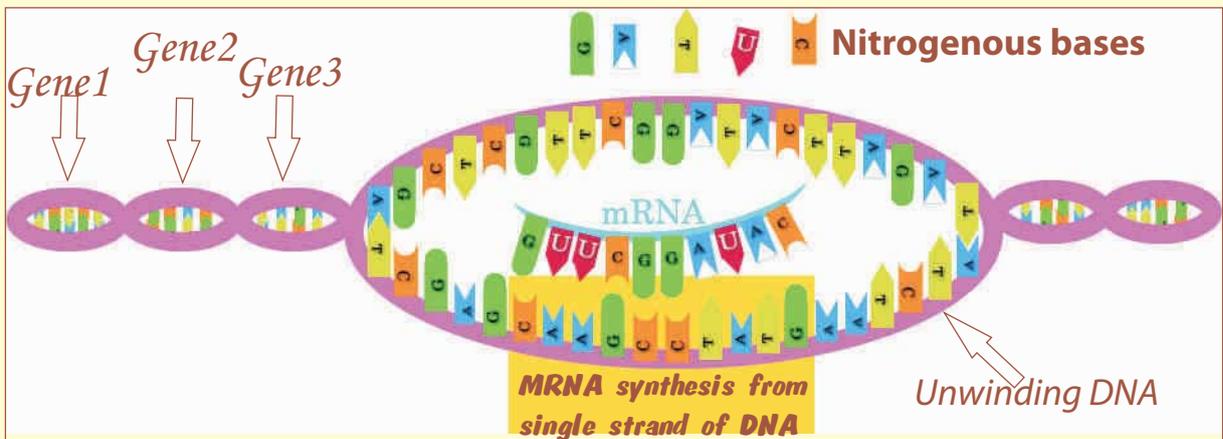
OK, I will give you a very simple example. We can actually do this experiment in the class. We can plant two bean seeds from the same plant in two different pots and let them grow into small plantlets. Then we, place one in a dark room and the other in bright sunlight. Will we see any difference in the appearance of these two plants?



# Gene expression



Message coded in a "gene" in DNA is first re-written (transcribed) into mRNA. In mRNA, Adenine binds with Uracil(U) instead of Thymine. MRNA is a single strand molecule.



**Dhakiya**

Yes. The plant in the dark room will grow taller than the one in bright light and will make less chlorophyll. Wow, so the gene expression is affected by the changed environmental condition and we can see it in the plant with our own eyes!

**Thandiwe:**

In this case, the environment determines the phenotypic pattern of the expression which we can see. This process must involve expression of genes under certain environmental conditions. Can you give us an example?

**Teacher:**

Thandiwe, do you remember that DNA is made up of four different nucleotides that code for proteins and may also even be responsible for regulating protein production? DNA sequences that encode a gene can differ between individual plants. These differences lead to genetic variation.

**Ekow:**

Does this variation also affect the proteins?

**Teacher:**

Yes, Ekow. The difference in the nucleotide sequence can affect the encoded proteins. A change in the DNA nucleotide sequence can also change the production of proteins. These have an effect on the phenotype.

**Dhakiya:**

Does this mean that it is easy to know which gene is responsible for which characteristic of a plant?

**Teacher:**

Well, yes and no. Scientists still do not know the full picture. Genetic variation is phenotypic variance in a population that is due to genetic heterogeneity. The interesting point is that genetic variants can differ from each other by the sequence of a "single" base pair. When there is a single base pair difference, it is called single nucleotide polymorphisms or SNPs, and is pronounced "snips". SNPs are a type of marker used in marker-assisted selection. They are commonly the basis of genotyping tests and are precise.

**Dene:**

Are they called markers because they help identify a trait?



**Dhakiya**



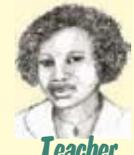
**Thandiwe**



**Teacher**



**Ekow**



**Teacher**



**Dhakiya**



**Teacher**



**Dene**



*Ayalew examines plant tissue regeneration from leaf discs transformed with the plant marker gene. Photo by R. Maxey, The University of Tennessee Institute of Agriculture*

Source: Seed Quest News



*On the DNA double helix, the “marker” (the green coloured area) acts as a red flag to indicate the presence of the gene of interest*

**Quantitative Trait Locus (QTL)** is a polymorphic locus which contains alleles that differentially affect the expression of a continuously distributed phenotypic trait. Usually it is a marker described by statistical association to quantitative variation in the particular phenotypic trait that is thought to be controlled by the cumulative action of alleles at multiple loci.

### **QTL example in cassava breeding**

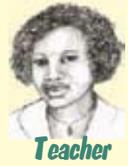
In recognition of the importance of cassava improvement for dry areas and to develop cassava breeding for drought tolerance, molecular markers were studied. This study was funded by the **CGIAR challenge program**

This project planned to develop single nucleotide polymorphism (SNP) markers throughout the genome to identify favorable alleles related to drought tolerance in these mapping populations. In order to achieve this goal, a physical map of the cassava genome was generated that allowed the development of SNP markers that were uniformly distributed around the genome. This facilitated the identification of quantitative trait loci (QTL) associated with drought tolerance in a high-throughput manner. These markers were used for marker-assisted selection of favorable traits.

Quantitative trait loci (QTL) associated with drought tolerance were to be identified by high-throughput genotyping of validated SNPs in two of the mapping populations. Additional SNP markers were to be developed around the “hot spots” identified after QTL mapping to allow marker-assisted selection of desirable QTL alleles for molecular breeding of drought resistance in cassava.

**Teacher:**

Yes! Marker-assisted selection is a biotechnology tool that helps to select the economically important characteristics of trees and crops for breeding. It has the potential to allow rapid, reliable and effective selection. The efficiency and effectiveness of using molecular markers, associated with the selection of useful characteristics, greatly help breeding programs.



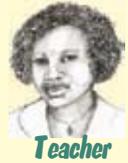
**Ekow:**

What is a marker? Is it a special gene? A part of DNA?



**Teacher:**

Molecular markers are like constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome. They are transmitted from one generation to the next. They can be identified only by DNA tests.



**Ekow:**

So, how does MAS play a role in this matter?



**Teacher:**

The idea behind using marker-assisted selection in this process is that the genes with significant effects can be used specifically for selection of a specific trait. Some traits are controlled by single genes, but many important traits are complex and controlled by a number of genes. These complex traits are usually governed by a number of genes known as Quantitative Trait Loci (QTLs). Looking for the pattern of inheritance at such QTL assists the selection process.



**Dhakiya:**

Why is it called marker-assisted selection and not marker-based selection?



**Teacher:**

The word "assisted" implies that the selection is also influenced by other sources of information. One such source of information is the historical performance record.



**Dhakiya:**

This means that in order to have a good result with MAS we must also consider the historical performance records. Tell us about historical performance records.



**Teacher:**

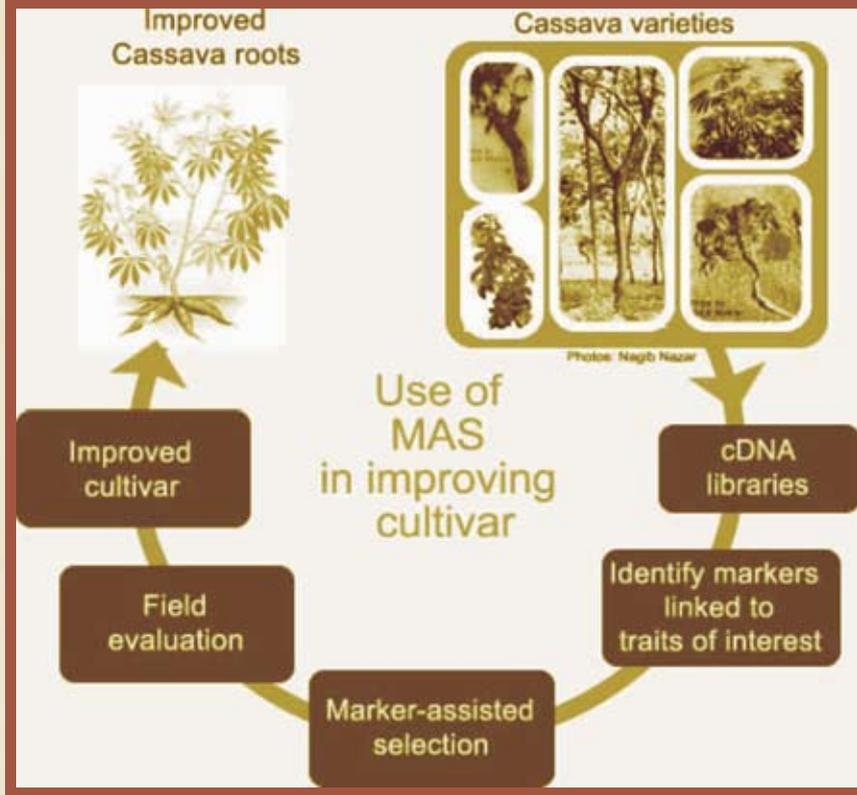
No not now, it is getting late and it is somewhat complicated. You already have a lot to remember. Soon I will explain the meaning of the historical performance records. Enjoy your evening, everyone.





# Scene Thirteen

## *Marker-assisted selection and conservation*



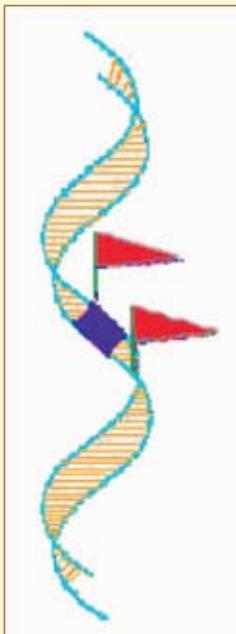
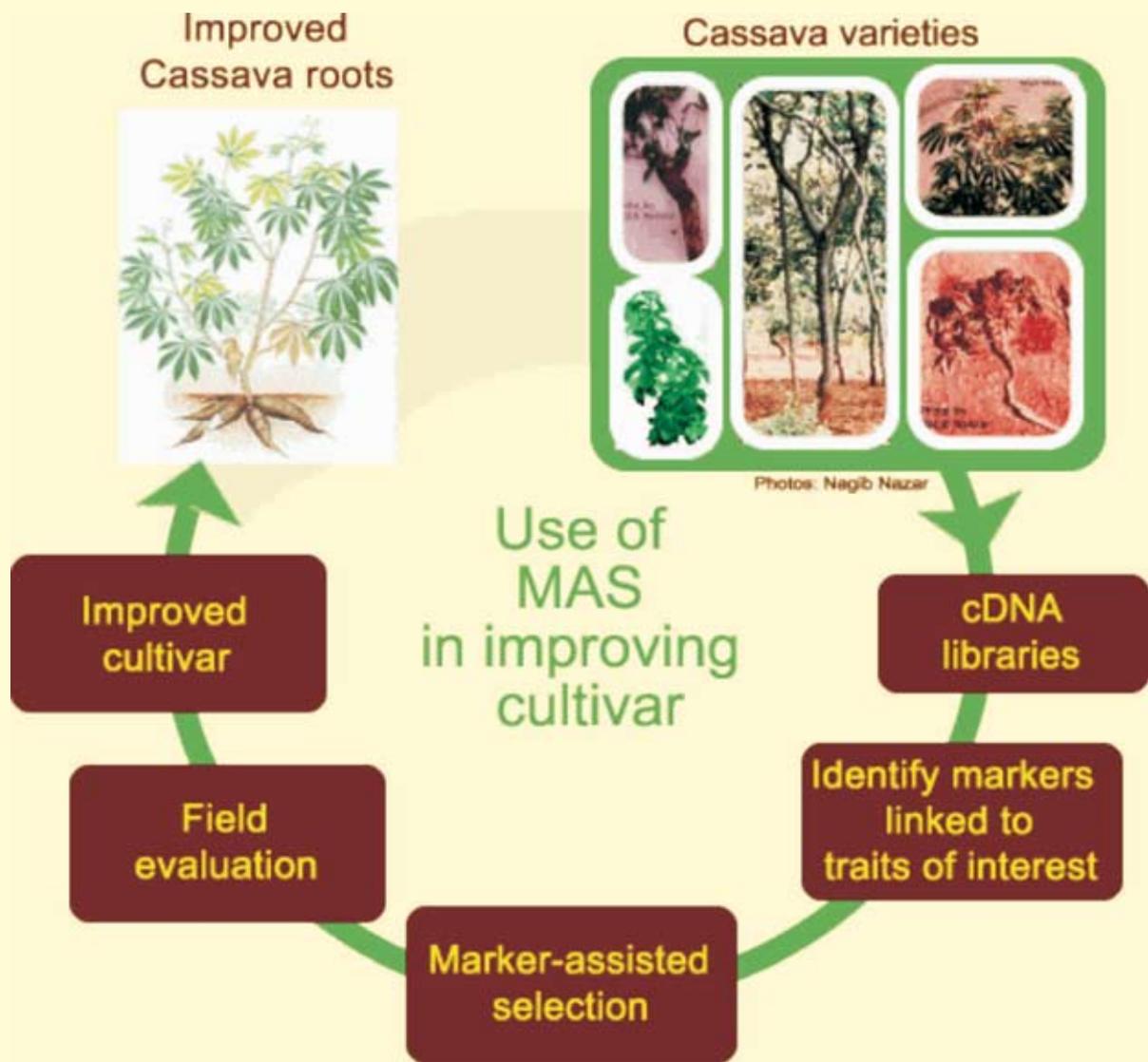
## *Wednesday morning*

*Scene Thirteen:* Wednesday morning

*Place:* Classroom

*Characters:* Efia, Ekow, all other students and the biology teacher

*Situation:* The biology class continues with discussion on molecular markers and MAS



### **Genetic markers**

*The purple section indicates the presence of a desirable gene in an organism's genetic code. This gene is associated with a marker shown by the red flags.*

*Scene Thirteen:*  
Wednesday morning

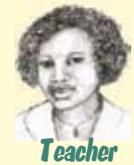
*Place:*  
Classroom

*Characters:*  
Efia, Ekow, all other students and the biology teacher

*Situation:*  
The biology class continues with discussion on molecular markers and MAS

**Teacher:**

Good morning, class. Today we will talk about markers. Ekow, can you tell me what MAS stands for?



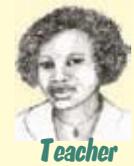
**Ekow:**

MAS or marker-assisted selection is a biotechnology tool that uses molecular markers to select a specific trait in a plant.



**Teacher:**

Is MAS alone enough to choose a specific trait in a plant?



**Ekow:**

No. In order to make a selection decision we must consider the information from available performance records as well as the information from MAS.



**Teacher:**

Wonderful! So, today I will tell you about the types of markers. There are many types of markers and marker associated techniques. You will all like this because almost all of these markers and associated techniques have acronyms, like abbreviations. They are called RFLP, RAPD, AFLP, SNP and SSR.



**Ekow:**

Oh, my goodness! I will surely be lost now.



**MAS** technique can be used once traits have been mapped and a closely linked marker has been found. It is then possible to screen large numbers of samples for rapid identification of progeny that carry desirable characteristics.

**Restriction Fragment Length Polymorphisms (RFLPs)** often pronounced "rif-lip" is used in two related contexts: firstly, as a characteristic of DNA molecules (arising from their differing nucleotide sequences) by which they may be distinguished, secondly, as the laboratory technique which uses this characteristic to compare DNA molecules. The technique is utilized in genetic fingerprinting.

**Random Amplification of Polymorphic DNA (RAPD)** - It is a type of PCR reaction, but the segments of DNA that are amplified are random.

**Amplified fragment length polymorphism - (AFLP)** is a technique that is a highly sensitive method for detecting polymorphism in DNA

**"Microsatellites"** are defined as loci (or regions within DNA sequences) where short sequences of DNA (nucleotides; adenine - A, thiamine - T, guanine - G, cytosine - C) are repeated in tandem arrays. This means that the sequences are repeated one right after the other. The lengths of sequences used most often are di-, tri-, or tetra-nucleotides.

**Microsatellites** sometimes referred to as a variable number of tandem repeats or **VNTRs** are short segments of DNA that have a repeated sequence such as **CACACACA**. They tend to occur in non-coding DNA.

**Simple Sequence Repeats (SSRs)** are polymorphic loci present in nuclear DNA that consist of repeating units of 1-4 base pairs in length.

**A Single Nucleotide Polymorphism or SNP** (pronounced snip) is a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome differs between members of a species or between the paired chromosomes in an individual.

**Teacher:**

No, Ekow, there are easy ways to know what these acronyms mean. But you don't have to worry about them now. I have hung a chart on the wall explaining all these acronyms. You can start learning them slowly for the moment. What is important to know, is that there are many types of markers and these markers may differ in their technical requirements. The ones I have mentioned are just a few examples.



The number of genetic markers that can be detected throughout the genome may be different and the amount of genetic variation found at each marker in a given population may also be different.

**Kunto:**

There are six types of markers. The specific characteristics of a plant are identified by several different methods used for identifying the genes that are responsible for a specific trait.



**Teacher:**

Very good. Let us use microsatellites as an example as they are very popular for constructing genetic maps for QTL analysis and MAS. We can use this technique to understand all the steps involved.



**Dhakiya:**

Teacher, can you explain the genetic map and QTL again, please?



**Teacher:**

Yes, of course. If you remember, we talked about quantitative trait loci or QTL. The genetic map is developed by assaying a number of DNA markers in closely related individuals; each marker identifies a position on the map called a locus.



In crop species, linkage maps are most often used to identify specific chromosomal regions controlling traits of economic importance, such as disease resistance, density or growth. These traits vary quantitatively in crop species, and the identified regions are called QTL.

## Equipment used in DNA analysis

Plant tissue can be ground using a mortar and pestle.



Plant tissue can also be macerated in a genogrinder, which processes two plates at one time.



After extraction, DNA is transferred onto plates for the PCR process. Plates are sorted and organized into groups, based on the marker loci of interest. The last plate contains checks and controls.

Extracted DNA is transferred from sample plates to storage plates using robotics.

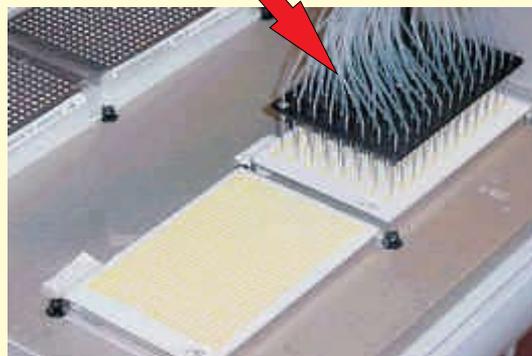


Extracted DNA is transferred from storage plates to PCR plates using robotics.



PCR is performed to amplify the regions of the genome that are to be detected. The post-PCR product is then transferred to a nitrocellulose membrane.

Robotics are used to transfer the PCR product from the PCR plate wells to the nitrocellulose membrane.



Membranes are hybridized with the appropriately labeled allele specific oligonucleotide probes. See page 106.

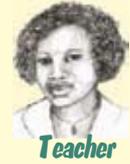
**Ekow:**

Teacher, I have a question; is MAS a biotechnology tool?



**Teacher:**

Ekow, don't you remember? We talked about this before. MAS is the biotechnology tool used to construct the genetic map for QTL analysis.



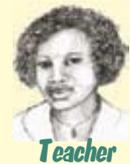
**Efia:**

Besides generating genetic maps for QTL analysis, does MAS have any other uses?



**Teacher:**

The practical application of MAS is well known in plant breeding, especially in crossbreeding. However, you must remember that knowledge of MAS is very important for collecting, conserving and using PGR.



**Ekow:**

Teacher, can you tell us more about how scientists use the MAS technique to conserve cassava PGR?



**Teacher:**

Once again let me give you some basic information before we talk about cassava. In a crop species, some traits are controlled by one or a few genes, whereas other traits may be controlled by many genes. The expression of any particular gene can be modified by the presence of other genes or by the environment in which the organism grows. The content of cassava roots may be determined by the environment where the cassava is grown.



**Efia:**

Cassava! Ekow, we can tell our grandmother about this. Teacher, could you please give us an example using MAS and QTL in cassava?

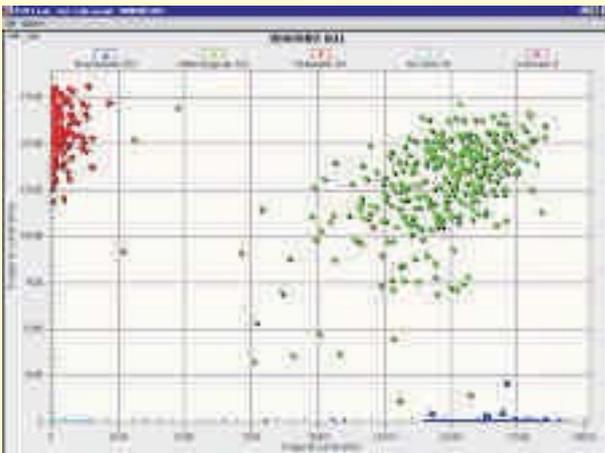


## ***Equipment used in DNA analysis (continued)***

Images are developed on X-ray film and scanned into a computer for scoring. A solid black dot on a single image would indicate that the plant sampled is homozygous for that particular marker allele; a gray to dark dot on both images would indicate that the plant sampled is heterozygous for the marker allele; no dot (no hybridization) would indicate that the plant sampled does not carry the marker allele.



16X (16 96-well plates) autorad images of complimentary alleles at one locus. The images are analyzed and the results are converted into a tabular form for upload into a database. Sample results are electronically communicated to the breeders and are used to select individual plants for advancement to the next level of testing.



This is a graphical representation of sample analysis. The red data points represent samples homozygous for marker allele 1, green data points are heterozygous samples, and blue represents homozygous samples for marker allele.



Bar code readers are used to identify plants for advancement, which are then directly threshed into planting trays (in the background).

Source:

[http://www.cropscience.org.au/icsc2004/symposia/3/4/133\\_schmidtdh.htm](http://www.cropscience.org.au/icsc2004/symposia/3/4/133_schmidtdh.htm)

**Teacher:**

Before I tell you about them, you must understand some fundamental facts related to MAS and QTL.

First, MAS is a fast and efficient method to analyze QTL.

Second, MAS can be a successful tool to analyze QTL without performance records.



But it is always better to have both historical performance records and MAS data, to come to a successful conclusion.

**Ekow:**

I also believe that besides QTL, MAS is useful for other analyses such as genotyping, right?



**Teacher:**

Very good, Ekow! You see, the traits that breeders observe in the field are a result of gene combinations in the plant and the influence of the environment on the expression of those genes. Thus, breeders evaluate genetic lines at different locations and in different years to make sure that they consistently out-perform commercial plants across a diversity of environments and growing conditions. The trait is usually not evaluated until after several generations of self-pollination in the breeding process when excess seed is available. This takes a long time.



**Dhakiya:**

However, MAS takes a much shorter time than obtaining an historical performance record.



**Teacher:**

Yes. Having a genetic marker associated with a gene allows one to identify the desired form of the gene or the allele from the onset of the selection process using DNA from almost any tissue of the plant. This technology allows us to rapidly identify genetic lines that have the desired allele and discard those without it. The outcome will be the development of new cultivars that have a unique processing quality. Whereas it generally takes 7-10 years to develop a cultivar, under normal practice, MAS has decreased the development time of these cultivars by several years.



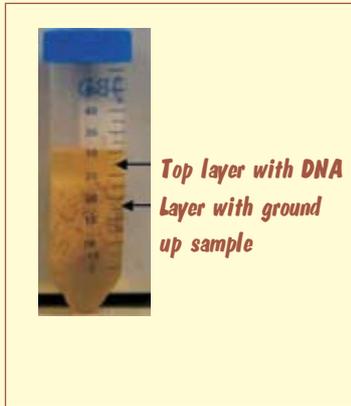
**Kunto:**

Wow! In that case MAS is a very important biotechnology tool.

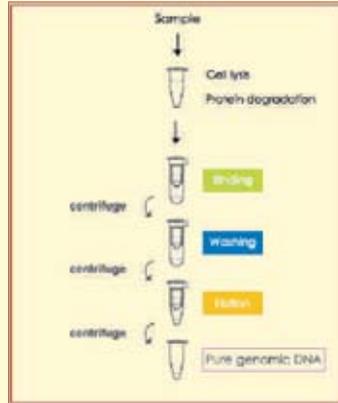


# Biotechnology tools!!!!

**DNA extracted from a large sample (50 ml)**



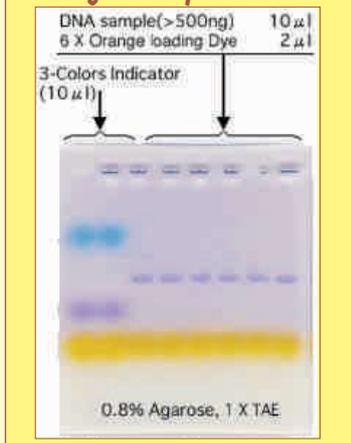
**Steps involved in DNA extraction**



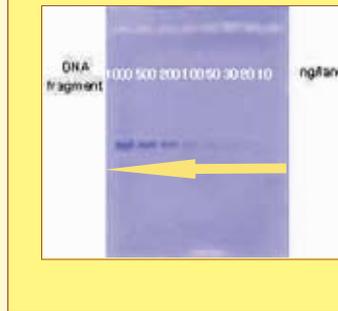
**DNA separated in a gel according to their size**



**Standard way of setting up the gel to separate DNA**



**Different concentrations of same DNA samples (in ng/ml) are run in an increasing concentration showing that while the DNA fragment size remains the same, the band becomes thicker with increased concentration**



**The DNA band can be cut off the gel and purified for further use**



**The separated DNA can be visualised using ethidium bromide under ultraviolet rays**



**Ekow:**

What do we need to know to perform MAS for plant breeding?

**Teacher:**

The essential requirements for MAS in a plant-breeding programme are:

one - the marker should be closely linked with the trait

two - an efficient screening mechanism for the molecular marker or markers, such as PCR, should be available

three - the screening technique should be successfully replicated in any laboratory and finally, it must be economical to use and also user-friendly.

**Efia:**

So, are there any problems associated with this biotechnology tool?

**Teacher:**

Efia, you are always cautious about everything. It is a good question. There are no problems associated with this biotechnology tool itself. But, there can be problems associated with how we use biotechnology tools. It is important to know that any tool we use could result in something that is beneficial to all human kind.

Very well, we will continue with the discussion in our next biology class. We will see how MAS has been applied in real life experience with cassava.



***A cultivar is a cultivated plant that has received a name under the International Code of Nomenclature for Cultivated Plants (the ICNCP, commonly known as the "Cultivated Plant Code"). For this, it must be distinct from other cultivars and it must be possible to propagate it reliably, in the manner prescribed for that particular cultivar.***

***Advantages of molecular markers in plant breeding:***

***Decreased number of breeding generations***

***Uniform method for scoring***

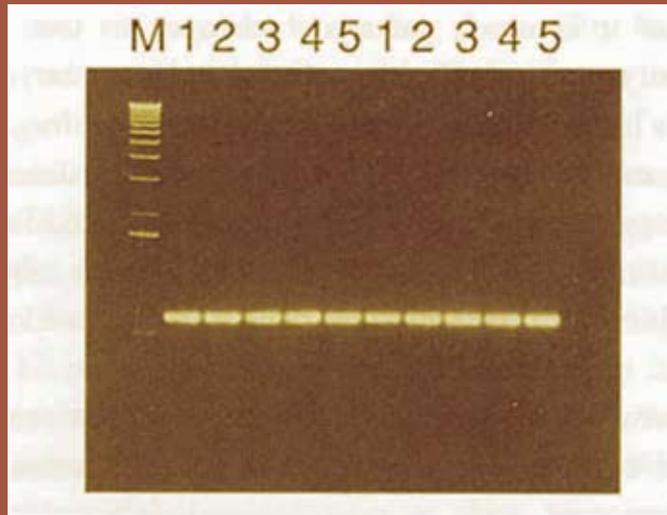
***No need to use phenotypic scoring until the end***

***Tells % of genome from each parent***

***Tells WHICH PARTS OF EACH CHROMOSOME come from each parent***



# Scene Fourteen



*Photograph of a gel showing PCR analysis of DNA isolated from 5 different rice tissues (#1 to 5), All 5 samples are repeated to eliminate human error. The gel is stained with ethidium bromide. Molecular weight marker (M) is one Kb ladder.*

## *Friday morning*

*Scene Fourteen:*

Friday morning

*Place:*

Classroom

*Characters:*

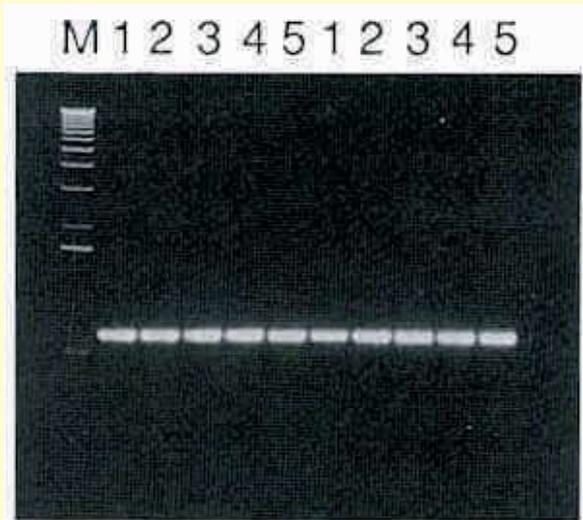
Efia, Ekow, all other students and the biology teacher

*Situation:*

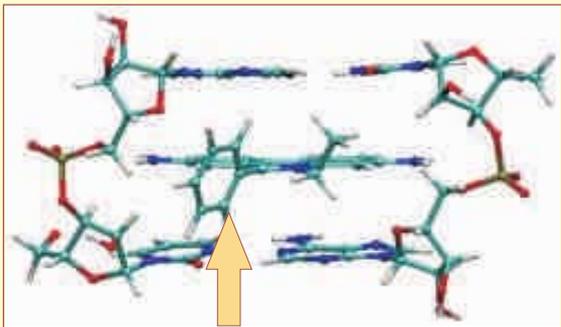
The biology class continues with discussions on the application of MAS

## DNA analysis:

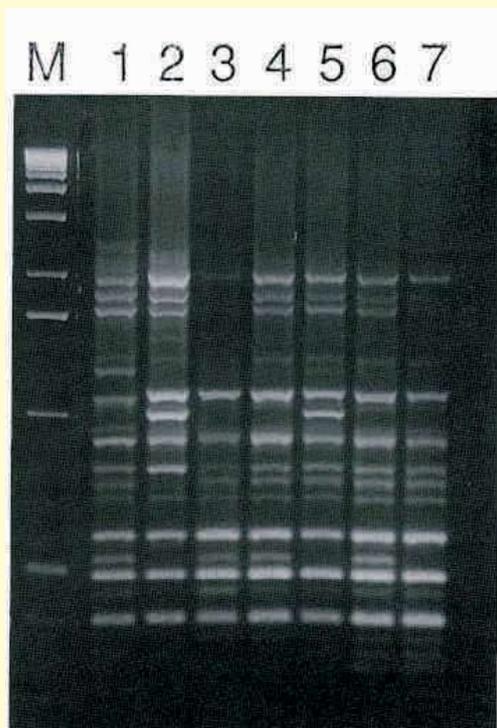
### Visualization of DNA in an agarose gel using ethidium bromide stain



A small sample of DNA is amplified using **Polymerase Chain Reaction (PCR)** and isolated from 5 different tissues of rice plant; young leaf (1), old green leaf (2), green panicle (3), panicle before flowering (4) and root (5). The samples are repeated for verification. "M" is the molecular weight which confirms the size of DNA. The gel is stained with ethidium bromide and visualized under fluorescent light.



Ethidium bromide is an intercalating agent commonly used as a nucleic acid stain. When exposed to ultraviolet light, ethidium bromide will fluoresce with a red-orange color, intensifying almost 20-fold after binding to DNA.



RAPD analysis of DNA rapidly isolated from healthy leaves of different rice plants using primers. The gel was stained with ethidium bromide.

Source:

Rapid DNA isolation for marker assisted selection in rice breeding

Kangle ZHENG, Prasanta Kumar SUBUDHI, Jessica DOMINGO, Gerard MAGPANTAY and Ning HUANG  
Plant Breeding, Genetics and Biochemistry Division,  
IRRI, P. O. Box 933, Manila, Philippines

*Scene Fourteen:*  
Friday morning

*Place:*  
Classroom

*Characters:*  
Efia, Ekow, all other students  
and the biology teacher

*Situation:*  
The biology class continues  
with discussions on the  
application of MAS

**Teacher:**

Good afternoon students. Shall we discuss further about marker-assisted selection or MAS?



**Efia:**

Yes. MAS is used to identify and select a specific trait and therefore must be a good biotechnology tool. Is there an example you could give us about the use of MAS?



**Teacher:**

Yes, Efia. Molecular markers are especially advantageous to detect agronomic traits. These traits can be either a resistance to pathogens or tolerance to stress. Besides this, markers are also useful for setting quality parameters and quantitative traits.



**Ekow:**

But is there an example where MAS has already been used?



**Teacher:**

Yes, Ekow. How about cassava? Would you like to know an example of the use of MAS to select a trait in cassava?



**Ekow:**

Yes! It will be interesting to know how MAS has been used to grow better cassava.



**Teacher:**

Let me tell you about a real example of the use of MAS in cassava trait selection. Let us see whether we can explore the way of breeding

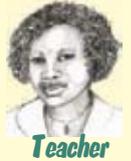


cassava that is resistant to cassava mosaic disease or CMD. Well, you all know now that the cassava mosaic disease or CMD is an economically important disease of the food crop cassava in Africa. In the research lab, scientists identified QTLs associated with resistance to CMD.



**Efiya:**

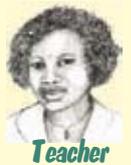
So, how did they do this experiment teacher?



**Teacher:**

Well, they used an F1 progeny derived from a cross between two parents.

F1 progeny is the first "filial" generation or first-generation "hybrid" produced by cross-pollinating two compatible parent plants. Filial is the name given to the generation following the parental generation. Therefore, it is called F1 progeny.



**Teacher:**

One parent is a CMD resistant accession and the other is a susceptible landrace. There are two new terms here; one is accession and the other is landrace.

Any new trait is recorded as an accession of that plant. So the CMD resistant trait of cassava is an accession. An accession is a new variety added to the gene bank. In simple words it is a new addition of a particular species which has improved value.

Landrace means a crop that has evolved and genetically improved through conventional agricultural methods. It also means that it has not been influenced by modern laboratory breeding practices.



**Ekow:**

So one parent is from the laboratory and the other is grown in the field under natural conditions.



**Teacher:**

Yes, Ekow! These two parents were crossed to identify QTLs associated with resistance to CMD.



**Dhakiya:**

So the two parents here are the CMD resistant accession and the CMD susceptible landrace. The CMD resistant cassava plant is resistant to

cassava mosaic disease while the CMD susceptible cassava plant can be infected by cassava mosaic disease.

**Teacher:**

That is correct Dhakiya. The landrace sample is collected from the field. The CMD resistant accession is obtained from the gene bank.



**Ekow:**

This is so interesting. So what happened to the F1 population teacher?



**Teacher:**

The F1 population was evaluated in the field for two growing seasons in Nigeria.



**Efia:**

That is a long experiment!



**Teacher:**

Yes! But then from this population the average disease severity scores were calculated and used for QTL analysis.



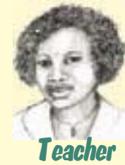
**Kunto:**

Did they find anything?



**Teacher:**

Yes! They detected five highly significant marker-associated QTL effects.



**Dafina:**

What is the most significant information they observed from this experiment?



**Teacher:**

Well, the significant information they observed was that the marker trait associations were due to markers donated by both parents. This means that the CMD resistance is polygenic. Polygenic means it is controlled by or associated with more than one gene. Also the CMD resistance is recessive in nature which means it will be expressed only when the determining allele is present in the homozygous condition in that plant. This also means that both parents must have at least one recessive gene for CMD resistance.





Efiya

**Efiya:**

This is so exciting. It will be wonderful to study plants like this to understand their characteristics. I think biotechnology tools are cool. We can learn so much about a plant by using biotechnology tools and that is besides being able to conserve PGR as accessions for future use.



Ekow

**Ekow:**

Efiya, you are showing off by using the new term we learnt today. Accessions eh? Very good Efiya



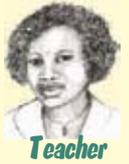
Efiya

**Efiya:**

So what was the final conclusion of this study?

**Teacher:**

They identified the QTL associated with the main source of CMD resistance in Africa.



Teacher

**Kunto:**

I really thought that I would not understand anything about biotechnology tools that can be used for conserving information about plant varieties. But I am wrong. It is so interesting to know that we can pin point a specific trait of a plant and store it and use it for breeding if necessary.



Kunto

**Teacher:**

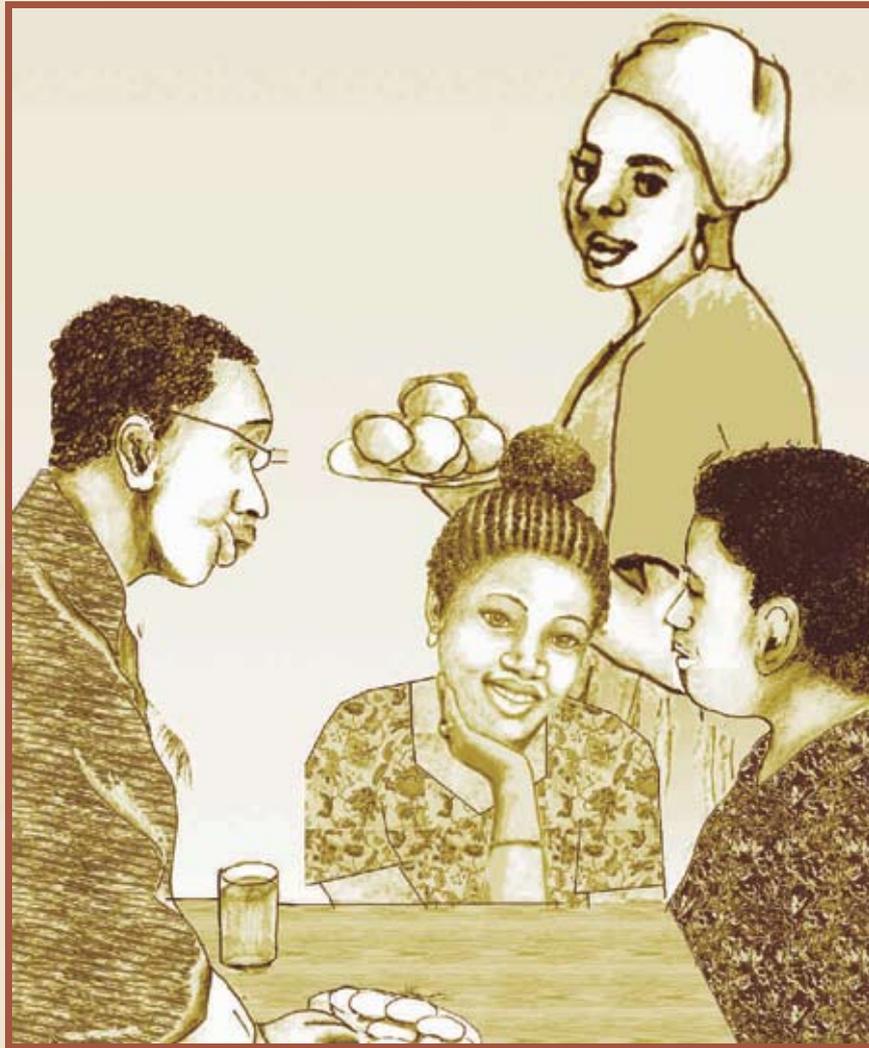
Kunto, I am very happy to hear that, especially from you, because you are the quietest one in the class.



Teacher

Students, it is time to close our discussion. Remember that in future, once a week, in the biology class, you will have a chance to say what you have understood from our discussions. You can share what you have heard or read about biotechnology tools and how they can be used to conserve PGR for future use. At the end of the year the three best presentations will win prizes. Tomorrow is Saturday. Enjoy your weekend and I will see you next week.

# Scene Fifteen



## *Saturday morning*

*Scene Fifteen:*

Saturday morning

*Place:*

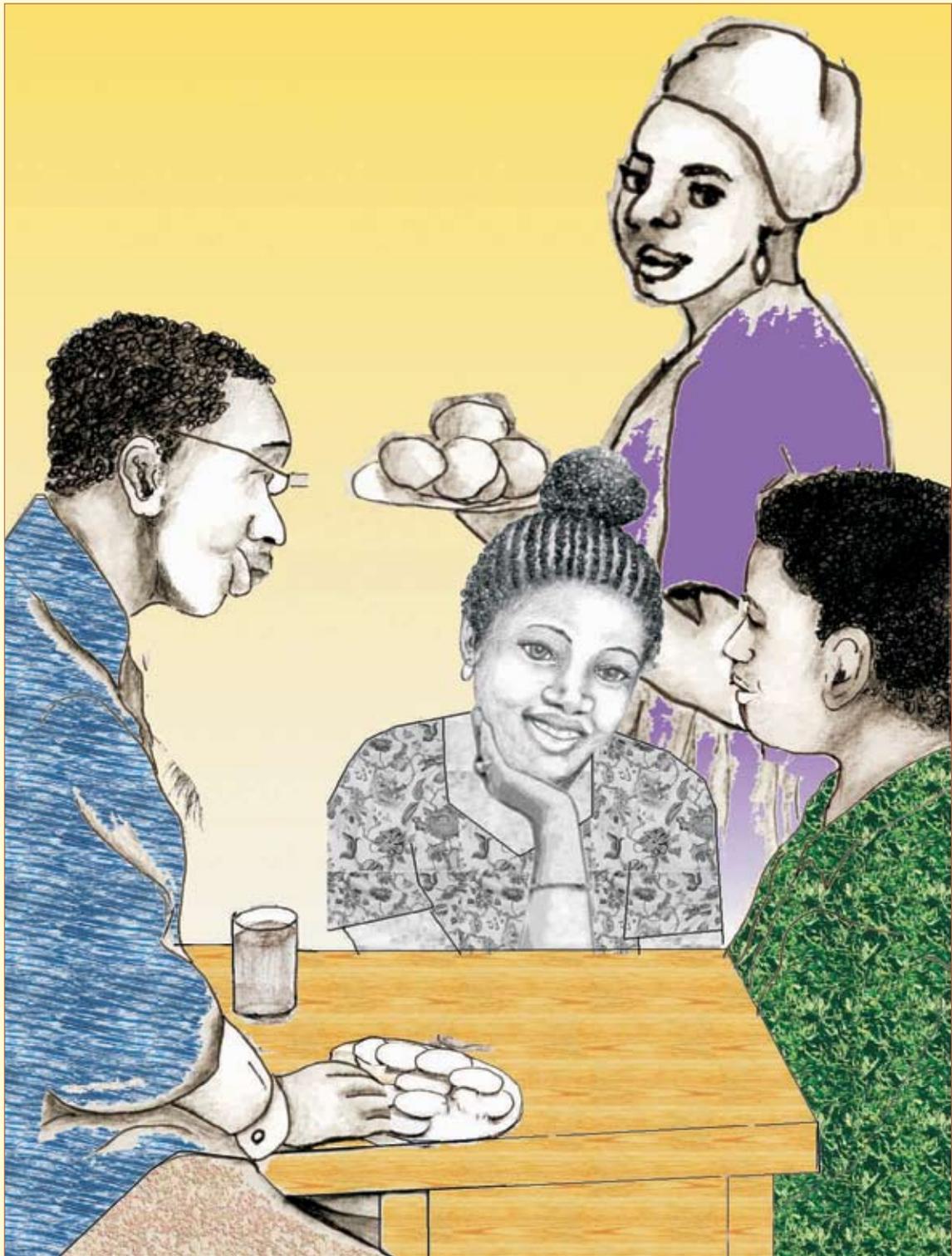
Home

*Characters:*

Efia, Ekow, their parents and grandparents

*Situation:*

Grandparents' visit



*Ekow! “You can ride your bike in the evening; now, stay home and talk to your grandparents”*

Scene Fifteen:

Saturday morning

Place:

Home

Characters:

Efia, Ekow, their parents and grandparents

Situation:

Grandparents' visit

Mum, may I go out and play?

**Ekow:**



Ekow, it is too hot now and your grandparents will be here any minute. Eat your breakfast now and get ready to tell your Grandma what you have learned in your biology class about cassava. You can go out and play in the evening.

**Mother:**



Oh, Mum, it is Saturday. I want to ride my bicycle. Efia is the one who wants to become the conservation specialist. She can explain to Grandma about the "lost" but "not lost" cassava.

**Ekow:**



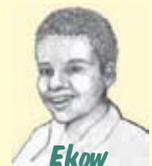
Ekow, does this mean that you know nothing about what you have learned in your biology class and so you are passing on the responsibility to your brilliant sister? Is that it?

**Mother:**



No, not at all! I can explain things just as well as Efia. OK, you got me. I will talk to Grandma.

**Ekow:**



**(When the grandparents arrive, Efia and Ekow explain to Grandma what they have learned about the new biotechnology tools that are used for cassava genetic resource conservation and use.)**



# Scene Sixteen



## *Monday evening*

*Scene Sixteen:*

Monday evening

*Place:*

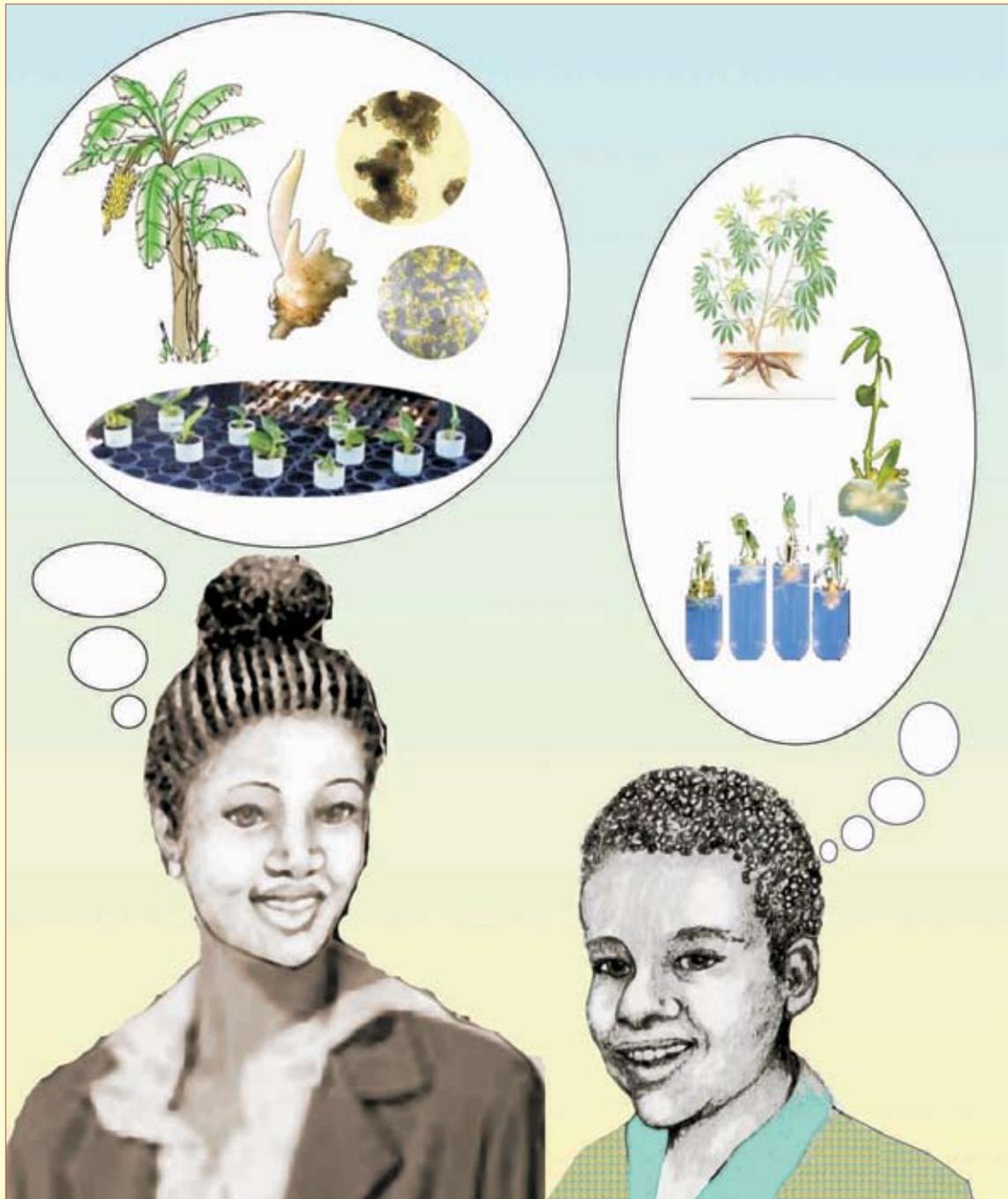
On the way home

*Characters:*

Efia and Ekow

*Situation:*

Efia and Ekow discuss their potential presentations



***What do Efia and Ekow plan to do for their project work in their next biology class?***

*Scene Sixteen:*

Monday evening

*Place:*

On the way to their home

*Characters:*

Efia and Ekow

*Situation:*

Efia and Ekow discuss their potential presentations

**Ekow:**

Efia, have you thought about a theme for your presentation? Will you prepare a poster? What kind of presentation do you have in mind?



**Efia:**

Ekow, I do not have a specific project in mind, but I am hoping to prepare a presentation that will have new information for our classmates. I think I will ask dad to organize a trip to his office in the plant genetic resources center and find out what other major crops they conserve and distribute.

I am interested in knowing more about micropropagation as it seems to be a simple technique but very efficient in helping farmers. So my project will be to identify a crop that is useful for everyone in our country and write a report on how micropropagation is used to increase this crop type so that it can be available to farmers.



I could make some dishes out of this crop produce and prepare a dish for Grandma and Grandpa and also take one to school. Have you thought about a project for yourself?

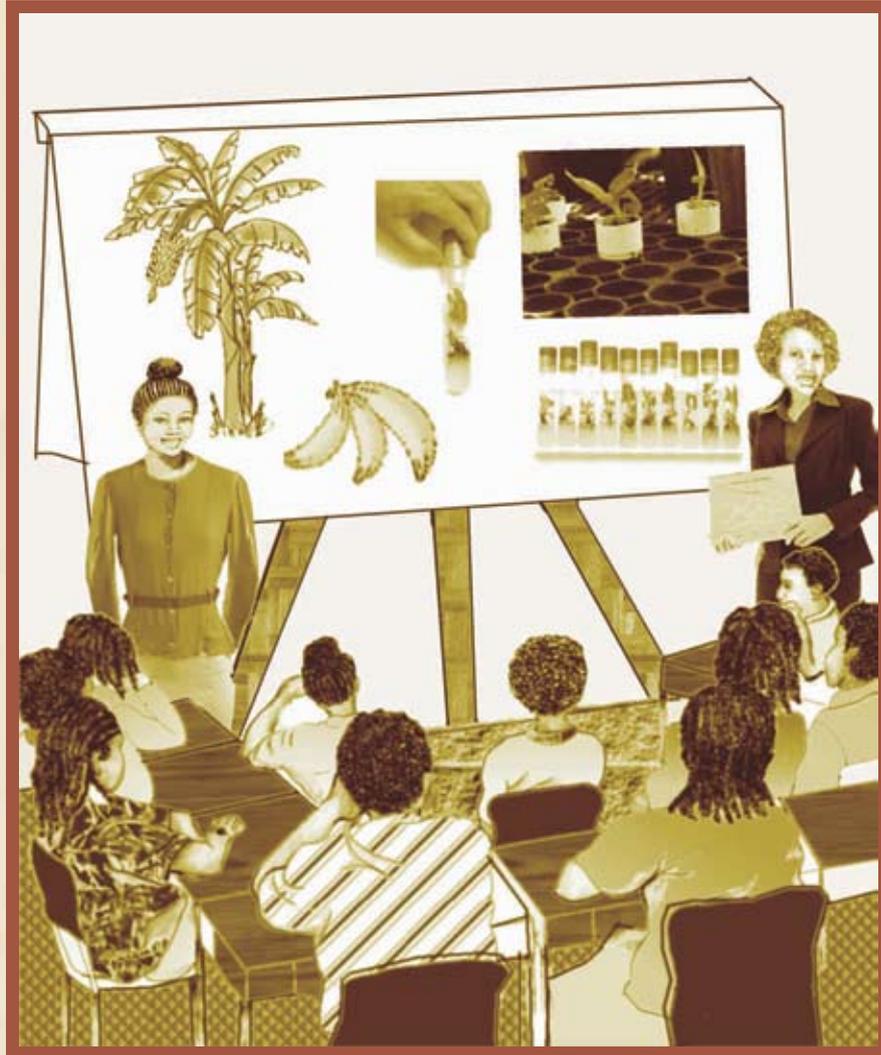
**Ekow:**

I am thinking of asking Uncle Sasi to take me to the plant tissue culture distribution centre where I could get small cassava plants to show to the class. I could prepare a display to show the steps in growing healthy plants.





# Scene Seventeen



*Friday of the following week*

*Scene Seventeen:*

Friday of the following week

*Place:*

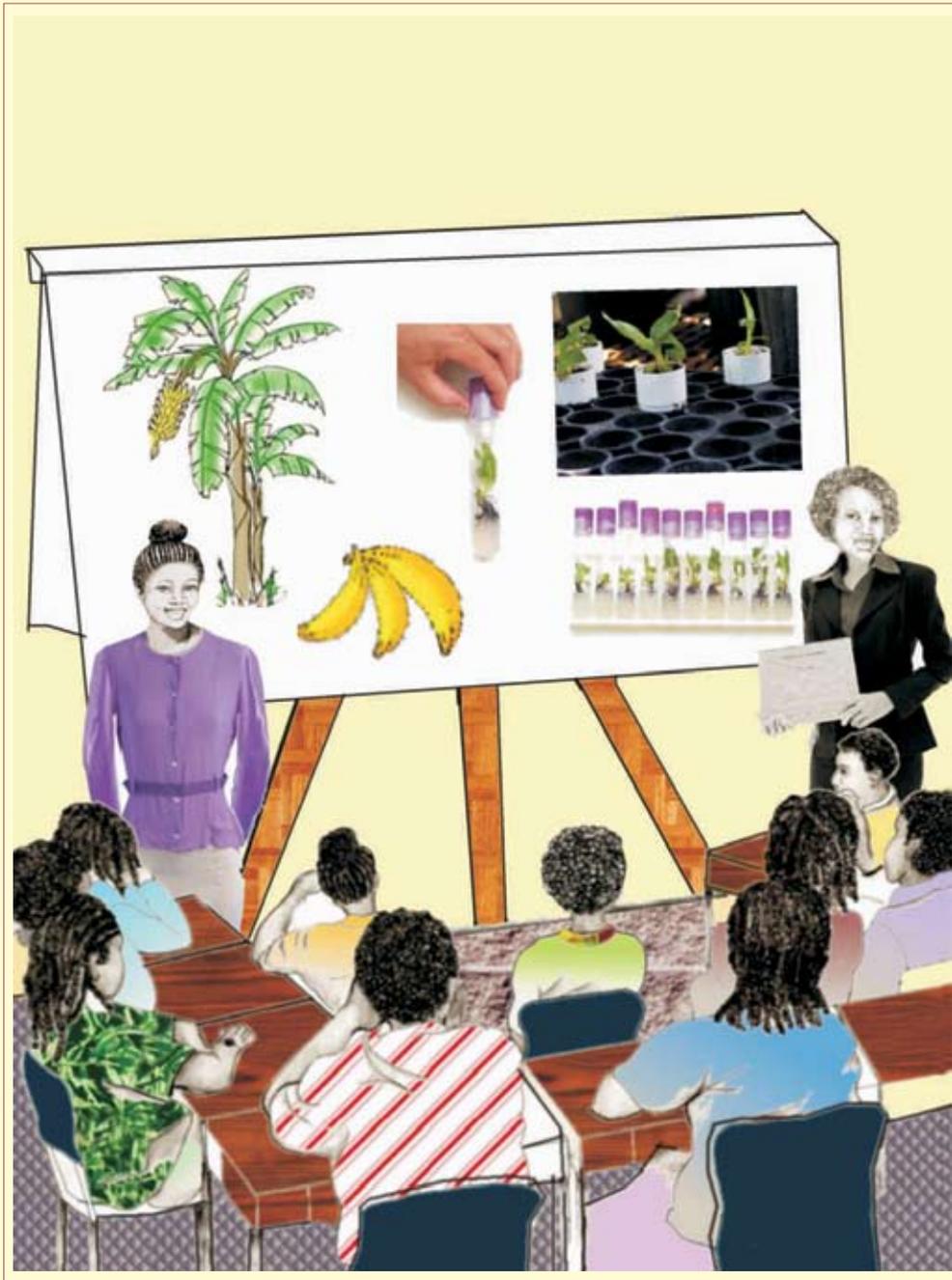
Classroom

*Characters:*

The students and the biology teacher

*Situation:*

The students present their projects



*Effia presents her project work on banana micropropagation*

*Scene Seventeen:*

Friday of the following week

*Place:*

Classroom

*Characters:*

The students and the biology teacher

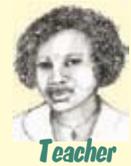
*Situation:*

The students present their projects

***Teacher:***

My dear students, I am very impressed with your spectacular presentations! Efia, your banana cake is delicious. Ekow, your display is extremely creative. Dhakiya, I liked your painting; it is very artistic. Dene, your slide show was most interesting. Thandiwe, your poster is very original. All of you did an excellent job.

We will now vote on the best three projects. Take a piece of paper and write down the three projects that you think are the best. As we agreed at the beginning, the top three projects will win prizes. Those who do not win a prize, please remember that you have another chance to try your best during our annual school day exhibition and win a prize. I am very proud of all of you.



**Teacher**



# Annex I

## ***Stages of plant tissue culture:***

The plant tissue culture technique involves three main stages in the tissue culture process:

### ***STAGE I is the initiation phase.***

It concerns the establishment of plant tissue in vitro by sterilising the material and initiating it into the culture.

The steps involved are:

1. A small amount of parent tissue or a number of cells are taken.
2. The tissue or cells are then transferred to plates containing sterile nutrient agar jelly. This is a gel made from algae which provides an ideal growth medium.
3. Auxins (plant hormones that trigger growth) are added to stimulate the cells to divide by mitosis.
4. Cells grow rapidly into small masses of tissue.

### ***STAGE II is the multiplication phase.***

At this stage, the in vitro plant material is re-divided and placed in a medium with plant growth regulators that induce the proliferation of multiple shoots. This process is repeated many times until the number of plants desired is reached. This is micropropagation.

5. More growth hormones are added to stimulate the growth of stems.

### ***STAGE III is the root formation phase.***

It involves the introduction of hormones to induce rooting and the formation of complete plantlets.

More growth hormones are added to stimulate the growth of roots.

Following these three stages, the plants are then moved from the laboratory to the greenhouse for acclimatization and further development.

The tiny plantlets are transferred into potting trays where they then develop into plants. This process is called the “regeneration” of plant material.



# Annex II

## ***Application of plant tissue culture:***

### ***1. Conservation, maintenance, transfer and distribution of plant germplasm:***

Cell culture offers enormous opportunities to collect, handle, manage and store germplasm in sterile conditions. Cell culture is used for the study of single cells, groups of cells and for the isolation of protoplasts. It is also used for the development of cell lines for the improvement of various types of resistance such as salt or drought tolerance cell lines and toxin resistant cell lines.

### ***2. Micropropagation:***

This is rapid vegetative multiplication of valuable plant material for agriculture, horticulture, and forestry. This process assures the good result that is expected from the germplasm during multiplication and also allows fast and efficient distribution.

### ***3. Production of disease-free plants:***

When the apex of a shoot is used for multiplication by tissue culture, we get disease-free plants because the shoot apical meristem (a group of dividing cells at the tip of a stem or root) is free from pathogens.

### ***4. Plant breeding:***

Tissue culture has also been successfully used in plant breeding programmes.

### ***5. Production of disease- and pest-resistant plants:***

Plants grown from tissue culture usually pass through the callus phase and show many variations. These show some agronomic characteristics such as tolerance to pests, diseases, etc.

### ***6. Cloning:***

Propagation by tissue culture also helps in producing clones. By using the shoot tip, it is possible to obtain a large number of plantlets. Unlimited numbers of plants that are genetically similar, (clones) can be produced in a short span of time by tissue culture.

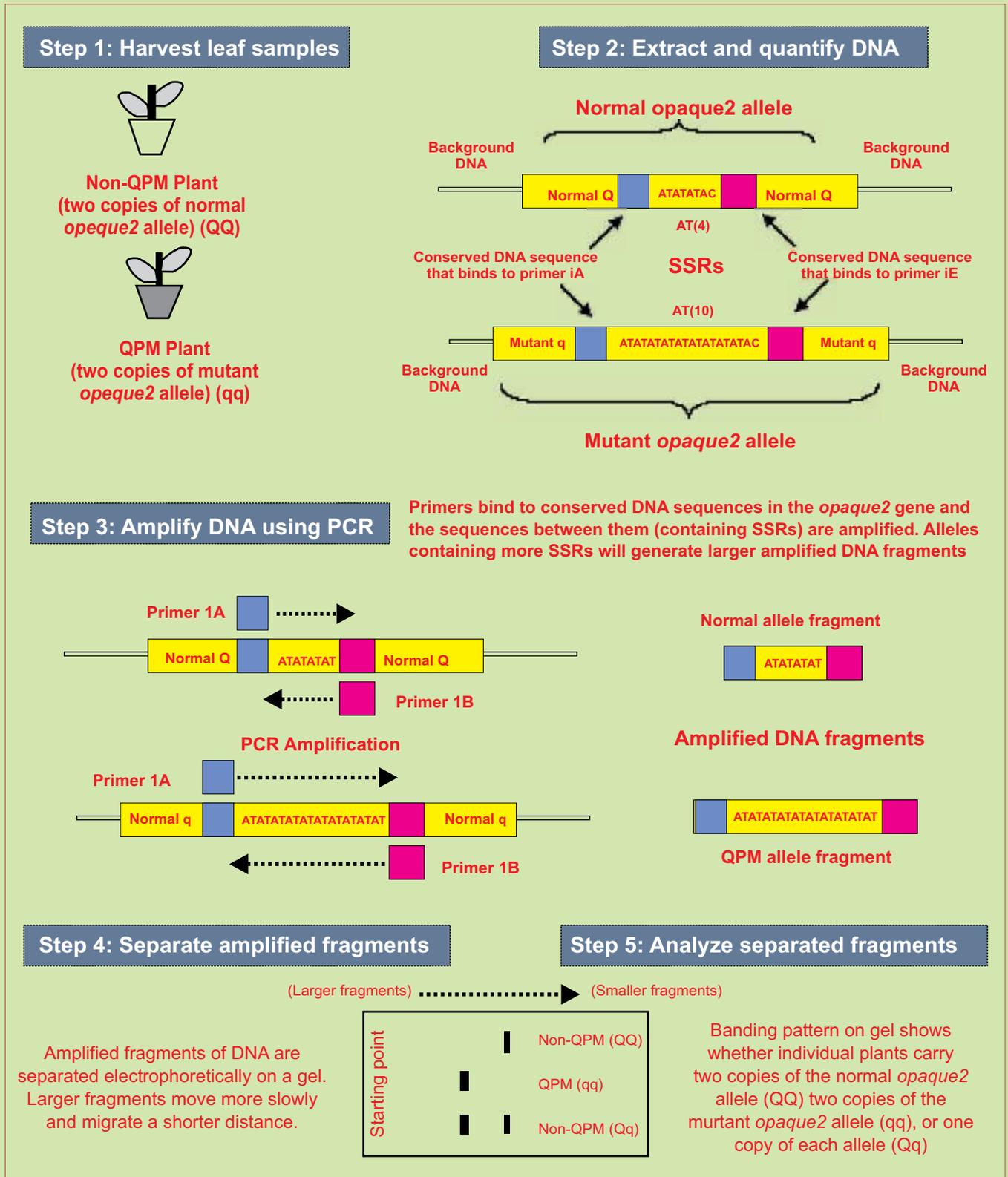


# Annex III

## An example of “Molecular marker analysis using SSR markers”

Source: IS MARKER-ASSISTED SELECTION COST-EFFECTIVE COMPARED TO CONVENTIONAL PLANT BREEDING METHODS? THE CASE OF QUALITY PROTEIN MAIZE by

Kate Dreher, Michael Morris, Mireille Khairallah, Jean-Marcel Ribaut, Shivaji Pandey and Ganesan Srinivasan from International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico





# Annex IV

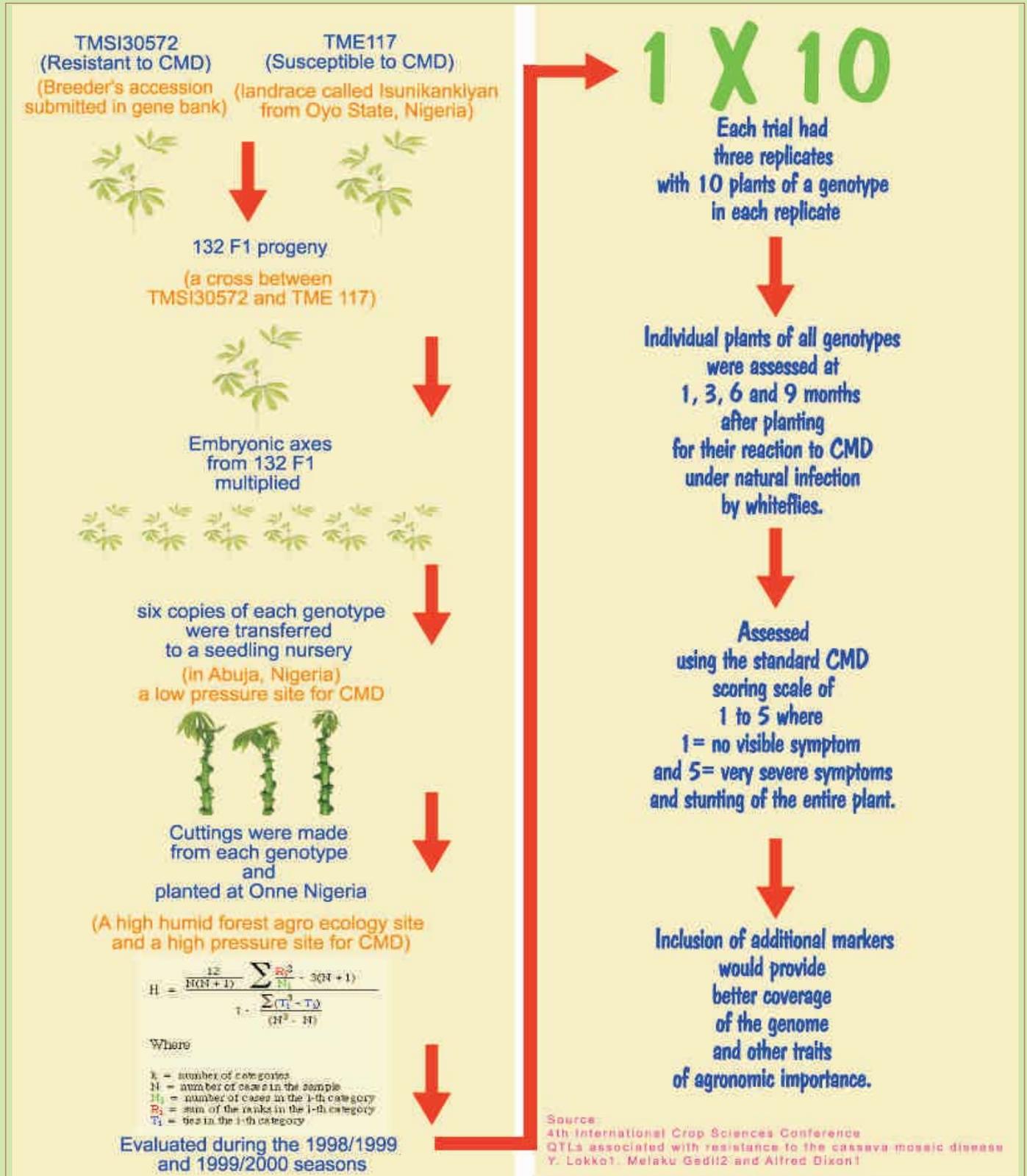
## Steps in identifying QTL associated with CMD.

Source: Proceedings of the 4th International Crop Science Congress: QTLs associated with resistance to the cassava mosaic disease

Y. Lokko<sup>1</sup>, Melaku Gedil<sup>2</sup> and Alfred Dixon<sup>1</sup>

<sup>1</sup>International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria

<sup>2</sup>Georgetown University Medical Centre





# Glossary

**Accession:** A new variety that is added to the gene bank.

**Acronym:** A word formed from the initial letters of a name, such as PGR for plant genetic resources.

**AFLP:** Amplified Fragment Length Polymorphism (AFLP) is a Polymerase Chain Reaction (see PCR) - based method of generating molecular markers. With this technique, the DNA sample treated with restriction enzymes is amplified. This technique allows selective amplification of restriction fragments giving rise to large numbers of useful markers, which in turn can be located on the genome relatively quickly and reliably.

**Allele:** A variant form of a gene. In a diploid cell there are two alleles for every gene (one inherited from each parent and they could be identical). Within a population there may be many alleles for a gene. Alleles are symbolized by a capital letter (upper case) to denote dominant character, and by a small letter (lower case) to denote recessive character.

**Aseptic:** Sterile, free of contaminating organisms such as bacteria, fungi and algae.

**Asexual propagation:** Vegetative, somatic or non-sexual reproduction of a plant without fertilization.

**Asexual reproduction:** Reproduction that does not involve the formation and union of gametes from the different sexes. It occurs mainly in lower animals, micro-organisms and plants. In plants, asexual reproduction is by vegetative propagation (e.g. bulbs, tubers, corms) and by formation of spores.

**Axenic:** Disease free

**Base pair:** A pair of nitrogenous base (a purine and a pyrimidine) held together by specific hydrogen bonds. The length of a nucleic acid molecule is often given in terms of the number of base pairs it contains.

**Biotechnology:** Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

**Biotechnology tools:** All techniques that use biological material to make, modify or improve plants, animals or microorganisms.

**Cassava:** A shrubby tropical plant (*Manihot esculenta*) widely grown for its large, tuberous, starchy roots.

**Cell Culture:** A population of plant or animal cells or micro-organisms grown under controlled conditions.

**Chromosome:** In eukaryotic cells, chromosomes are the nuclear bodies containing most of the genes largely responsible for the differentiation and activity of the cell. They contain most of the cell's DNA in the form of chromatin. Each eukaryotic species has a characteristic number of chromosomes. Bacterial and viral cells contain only one chromosome, which consists of a single or double strand of DNA or, in some viruses, RNA, without histones.

**Clone:** Group of plants genetically identical in which all are derived from one selected individual by vegetative propagation.

**CMD:** Cassava mosaic disease, a disease caused by a white fly that carries the virus within it.

**Conservation:** saving plants so that they can be used when needed in the future.

**Crossbreed (v.):** To produce (an organism) by the mating of individuals of different breeds, varieties or species; otherwise also known as hybridize.

**Crossbreed (n.):** An organism produced by mating of individuals of different varieties or breeds.

**Cryopreservation:** The preservation of germplasm resources in a dormant state by storage at ultra-low temperatures, often in liquid nitrogen. Currently applied to storage of plant seeds and pollen, micro-organisms, animal sperm, and tissue culture cell lines.

**Cultivar:** An internationally accepted term denoting a variety of a cultivated plant. A cultivar must be distinguishable from other varieties by stated characteristics and it must retain its distinguishing character when reproduced under specific conditions.

**Database:** An organized body of related information.

**Diploid:** The status of having two complete sets of chromosomes, most commonly one set of "paternal origin (from the father)" and the other of "maternal origin (from the mother)". Somatic tissues of higher plants and animals are ordinarily diploid in chromosome constitution, in contrast with the haploid gametes.

**DNA:** Abbreviation for **D**eoxyribo-**N**ucleic **A**cid. A long chain polymer of deoxyribonucleotides. DNA constitutes the genetic material of most known organisms and organelles. Usually it is in the form of a double helix. Some viral genomes consist of a single strand of DNA, and others of a single- or a double-stranded RNA. See "base pair, genetic code".

**DNA fingerprinting:** The derivation of unique patterns of DNA fragments obtained using a number of marker techniques; historically these were RFLPs, but lately they are generally **PCR** based. Synonym: genetic fingerprinting.

**Encode:** The gene product specified by a particular nucleic acid sequence.

**Enzyme:** A protein which, even in very low concentration, catalyses specific chemical reactions but is not used up in the reaction. Generally enzymes are named by the addition of the suffix - "ase" to the name of the substance they digest (the substrate). They are classified by a standard numerical system: the Enzyme Commission (EC) number. Enzymes are classified into six major groups, according to the type of reaction they catalyse: 1. oxidoreductases; 2. transferases; 3. hydrolases; 4. lyases; 5. isomerases; 6. ligases.

**Enzyme cutting:** Cutting a DNA at a specific place in a sequence with a specific enzyme.

**Explant:** A portion of a plant aseptically excised and prepared for culture in a nutrient medium.

**Ex situ conservation:** Literally means "off-site conservation". It is the process of protecting an endangered species of plant or animal by removing it from an unsafe or threatened habitat and placing it or part of it under the care of humans. While ex-situ conservation is comprised of some of the oldest and best known conservation methods known to man, it also involves newer laboratory methods.

**Extinct:** No longer existing or living.

**Extinction:** The ceasing of existence of a species. The moment of extinction is generally considered to be the death of the last individual of that species

**F1 progeny:** First-generation "hybrid" resulting from sexual or asexual reproduction.

**Field gene bank:** A facility established for the *ex situ* storage and maintenance, using horticultural techniques, of individual plants. Used for species whose seeds are recalcitrant, or for clonally propagated species of agricultural importance, e.g. apple varieties.

**Gene:** The unit of heredity transmitted from generation to generation during sexual or asexual reproduction. More generally, the term is used in relation to the transmission and inheritance of particular identifiable traits from one generation to the other. The simplest gene consists of a segment of nucleic acid that encodes an individual protein or RNA.

**Gene bank:** The physical location where collections of genetic material, in the form of seeds, tissues or reproductive cells of plants or animals are stored. It may be a collection of cloned DNA fragments from a single genome. Ideally the bank should contain cloned representatives of all the DNA sequences in the genome.

**Gene map:** See "genetic map".

**Gene mapping:** The construction of a localized (around a gene), or broad-based (whole genome) genetic map. More generally, determining the location of a locus (gene or genetic marker) on a chromosome.

**Gene pool:** The sum of all genetic information in a breeding population at a given time.

**Genetic diversity:** The heritable variation within and among populations which is created, enhanced or maintained by evolutionary or selective forces.

**Genetic map:** The linear array of genes on a chromosome, based on recombination frequencies (linkage map) or physical location (chromosomal map).

**Genetic marker:** A DNA sequence used to identify a particular location (locus) on a particular chromosome. See "marker gene".

**Genetic material:** Used to store the genetic information of an organic life form. For all currently known living organisms, the genetic material is almost exclusively DNA.

**Genetic variation:** The phenotypic and genotypic differences among individuals in a population.

**Genome:** It is the entire complement of genetic material (genes plus non-coding sequences) present in each cell of an organism, virus or organelle. The complete set of chromosomes (hence of genes) inherited as a unit from one parent.

**Genus:** (pl.: genera) A group of closely related species, whose perceived relationship is typically based on physical resemblance, now often supplemented with DNA sequence data.

**Genotype:** This can be observed at (1) the locus, (2) trait or (3) organism level.

1. The allelic constitution at a particular locus, e.g. Aa or aa.
2. The sum effect of all loci that contribute to the expression of a trait.
3. The genetic constitution of an organism.

**Germplasm:** 1. An individual, group of individuals or a clone representing a genotype, variety, species or culture, held in an *in situ* or *ex situ* collection. 2. Original meaning, now no longer in use: the genetic material that forms the physical basis of inheritance and which is transmitted from one generation to the next by means of the germ cells.

**Heterozygous:** Heterozygote is an individual with non-identical alleles for a particular gene or genes. The condition is termed "heterozygous". Opposite: homozygote.

**Homozygous:** Homozygote is an individual that has two copies of the same allele for a given gene on its two homologous chromosomes. The condition is termed "homozygous". Opposite: heterozygote.

**Hybrid:** The offspring of two genetically unlike parents.

**Inheritance:** The transmission of genes and phenotypes from generation to generation.

***In situ:*** In the natural place or in the original place. 1. Experimental treatments performed on cells or tissue rather than on extracts from them. 2. Assays or manipulations performed with intact tissues.

***In vitro:*** Outside the organism, or in an artificial environment. Applied for example to cells, tissues or organs separated from the plant or animal and cultured in glass or plastic containers

***In vivo:*** Within the living being. An experiment performed in or on the living tissue of a whole organism is called *in vivo* experiment.

**Landrace:** In plant genetic resources, an early, cultivated form of a crop species, evolved from a wild population, and generally composed of a heterogeneous mixture of genotypes.

**Liquid nitrogen:** Nitrogen gas condensed to a liquid with a boiling point of about -196°C. Commonly used as a medium for long-term storage of biological materials. See "cryopreservation".

**Locus:** (pl.: loci) A site on a chromosome.

**Marker gene:** A gene of known function or known location, used for marker-assisted selection or genetic studies.

**Marker-assisted selection (MAS):** The use of DNA markers to improve response to selection in a population. The markers will be closely linked to one or more target loci, which may often be quantitative trait loci.

**MAS:** See "marker-assisted selection".

**Micropropagation:** Miniaturized *in vitro* multiplication and/or regeneration of plant material under aseptic and controlled environmental conditions.

**Molecular marker:** A genetic marker which is assayed at the DNA level. (This assay is to: 1. test or evaluate and 2. Measure the quantity of a given substance in a sample, chemically or by other means.)

**Microsatellites:** Simple DNA sequences, usually 2 or 3 bases long, repeated a variable number of times in tandem. They are easy to detect with PCR. Microsatellites, also known as Simple Sequence Repeats (SSRs), are widespread throughout eukaryote genomes. A typical microsatellite marker has more variants than those from other marker systems.

**Nucleotides:** One of the structural components, or building blocks, of DNA and RNA. A nucleotide consists of a base (one of four chemicals: adenine, thymine, guanine, and cytosine) plus a molecule of sugar and one of phosphoric acid.

**Phenotype:** The visible appearance of an individual (with respect to one or more traits) which reflects the reaction of a given genotype with a given environment.

**Plant genetic resources (PGR):** The reproductive or vegetative propagating material of: 1. cultivated varieties (cultivars) in current use and newly developed varieties; 2. obsolete cultivars; 3. primitive cultivars (landraces); 4. wild and weed species, near relatives of cultivated varieties; and 5. special genetic stocks (including elite and current breeder's lines and mutants).

**Plant-lets:** A small rooted shoot regenerated from cell culture following embryogenesis or organogenesis. Plantlets can normally develop into normal plants when transplanted to soil.

**Plant tissue culture:** See "tissue culture".

**Plasticity:** Plasticity of a cell or tissue means that it is able to adjust to environmental conditions.

**Polygenic:** Character controlled by many genes of small effect. (Polygene is one of a number of genes, each of small effect, which together act to determine the phenotype of a quantitative trait.)

**Population:** A defined group of interbreeding organisms.

**Propagation:** The duplication of a whole plant from a range of vegetative materials; adapted for *in vitro* culture as micropropagation.

**Pubescent:** The leaves of the plant are covered with fine, hair-like structures.

**Quantitative trait loci (QTL):** A locus where allelic variation is associated with variation in a quantitative trait. The presence of a QTL is inferred from genetic mapping, where the total variation is partitioned into components linked to a number of discrete chromosome regions.

**Restriction enzyme:** An enzyme, specifically an endo-deoxyribonuclease that recognizes a short specific sequence within DNA and catalyzes double strand cleavage of the molecule.

**RFLP markers:** These are detected by treating DNA with restriction enzymes which cut DNA at a specific sequence. For example, the EcoR1 restriction enzyme cuts DNA whenever the base sequence GAATTC is found. Differences in the lengths of DNA fragments will then be seen. RFLPs were the first molecular markers to be widely used.

Their use is, however, time-consuming and expensive and simpler marker systems have subsequently been developed.

**RAPD markers:** These are detected using PCR. The analysis for RAPD markers is quick and simple, although results are sensitive to laboratory conditions.

**Repository:** A place where something can be submitted and stored safely.

**Ribonucleic acid (RNA):** An organic acid polymer composed of adenosine, guanosine, cytidine and uridine ribonucleotides. The genetic material of some viruses, but more generally it is the molecule, derived from DNA by transcription, that either, carries information (messenger RNA), provides sub-cellular structure (ribosomal RNA), transports amino acids (transfer RNA), or facilitates the biochemical modification of itself or other RNA molecules.

**Seed bank:** A place where plant seeds can be kept safely for hundreds of years. It has the unique feature as a conservation technique of making plants rapidly and easily available for investigation and evaluation.

**Selection:** A system for either isolating or identifying specific genotypes in a mixed population.

**Single base pair:** A set of two nucleotides bound by hydrogen bonds.

**SNPs:** Single Nucleotide Polymorphisms is a genetic marker resulting from variation in sequence at a particular position within a DNA sequence. SNPs are commonly the result of transition changes (A for G, T for C), but also transversions (G or A for T or C) and single base deletions. Such variation is extensive throughout all genomes, and offers the particular advantage of being detectable without the need for gel electrophoresis.

**Stock plant:** The source plant from which cuttings or explants are obtained. Stock plants should be well maintained to optimize explant and cutting quality.

**Tissue culture:** The *in vitro* culture of cells, tissues or organs in a nutrient medium under sterile conditions.

**Totipotency:** The ability of a cell or tissue to be induced to regenerate into a complete organism.

**Totipotent:** Plant cells that are capable of generating a whole new plant

**Trait:** One of the many characteristics that define an organism. The phenotype is a description of one or more traits.

**Variant:** An individual that is genetically distinct from others in the population.

**Variety:** 1. A naturally occurring subdivision of a species, with distinct morphological characters. 2. A defined strain of a crop plant, selected on the basis of phenotypic (sometimes genotypic) homogeneity.

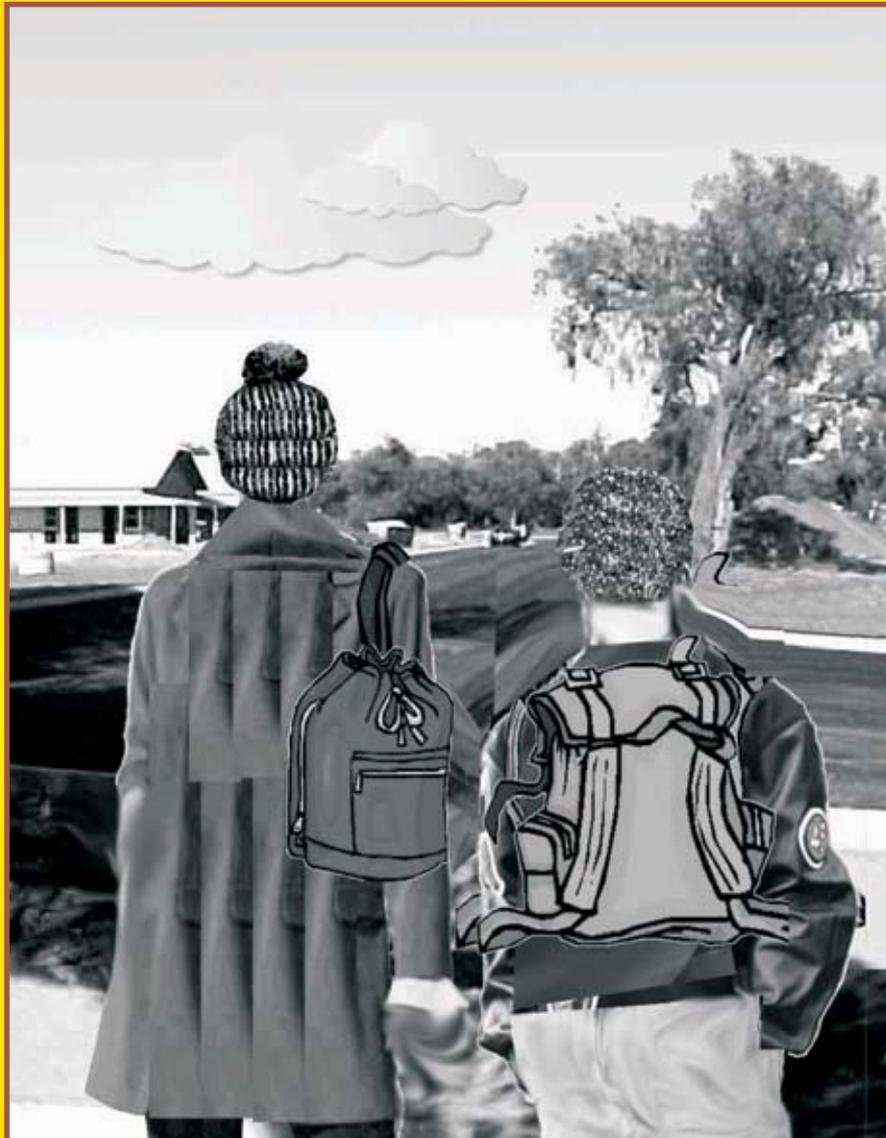
**Vegetative propagation:** See "asexual propagation".

**Vegetative reproduction:** See "asexual reproduction".

**Viability:** The capability to live and develop normally.



# Participate and Learn



For further information, please contact:  
Food & Agriculture Organization (FAO)  
Plant Production and Protection Division (AGP)  
Viale: 00 100 Rome, Italy  
Email: [AGP\\_repishn@fao.org](mailto:AGP_repishn@fao.org)



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