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# Abstract

There are few people in the world who are not familiar with bananas. With an annual production of 145 million metric tons in over 130 countries and an economic value of 44.1 billion dollars, bananas are the fourth most important food crop in the world. The banana originally came from Asia, but was imported into Africa long ago, where it now constitutes a significant source of food security. One third of all bananas are cultivated in Asia, another third in Latin America, and the other in Africa. 20% of the world's production of bananas comes from Burundi, Rwanda, the Democratic Republic of the Congo, Uganda, Kenya, and Tanzania, where they are grown on fields of 0.5 to 4 hectares. Only 15% of the worldwide production of bananas is exported to Western countries, which means that 85% of bananas are cultivated by small farmers to be consumed and sold at local and regional markets. Given that bananas serve as a basic food source for 20 million people in East Africa and for 70 million people in West and Central Africa, Africa is highly dependent on banana cultivation for food, income, and job security. Even so, yields fluctuate at around nine percent of their maximum capacity, for reasons including suboptimal conditions for agriculture such as drought and lack of soil nutrients, but above all because of the multitude of diseases and pests that attack the plants. These infections can cause partial or even total destruction of fields. The *Fusarium oxysporum* fungus is an especially big threat to Cavendish export bananas. As a result, the production on large plantations in Asia and Southeast Africa is at risk, and it threatens to bring the export of bananas to a complete halt. In Latin America, this fungus has not yet appeared. Other significant pathogens are the fungus *Mycosphaerella fijiensis* which causes the leaf-spot disease black sigatoka, the bacterium *Xanthomonas campestris*, viruses, nematodes, and weevils.

To continue to guarantee food security in Africa, Asia and Latin America, as well as the worldwide export of bananas in the future, there is an urgent need for improved banana varieties with an increased yield and nutritional value, which are resistant to all pests and diseases. At the moment, these improved varieties of banana are primarily developed through classical breeding (crossbreeding), but this breeding process is not easy and takes up a lot of time. The most commonly consumed types of banana are triploid (with three sets of chromosomes), seedless, and sterile. The process of selecting parent plants, crossbreeding, picking out the best hybrid offspring, and providing these to farmers, is estimated to take at least 17 years. Moreover, in the new hybrids, traits such as taste and aroma will not always be identical to those of the current banana varieties, which implies they are not that popular with consumers. Efforts are therefore also being made to introduce resistance to disease through biotechnology and genetic transformation of bananas. These techniques ensure not only that new, resistant varieties can be developed more rapidly but also that the banana is given a specific trait while retaining all of its original traits. Furthermore, a number of diseases can only be fought in this way. In the case of the bacterium *Xanthomonas campestris* and the Banana Bunchy Top Virus, there is no known variety with resistance, making the introduction of resistance genes from bananas impossible. At present, the different banana genomes are being researched as extensively as possible and the different traits linked to their genes. In the future, this will open the way for the generation—through both classical breeding and biotechnology—of new and improved banana varieties that contribute to sustainable, environmentally friendly, and economically viable agriculture.

# Facts and figures

*In 2013, around 145 million metric tons of bananas were produced in over 130 countries on more than 11 million hectares of land.*

*Annually, more than 1,000 billion bananas and plantains are consumed, making bananas the fourth most important food crop in the world, after maize, rice and wheat.*

*Bananas are a staple food in the diet of over 400 million people, representing an average of 15% to 27% of their daily calorie intake.*

*85% of banana production is sold locally and only 15% is exported. Commercial plantations are mainly to be found in South America. Bananas are primarily exported to North America and Europe.*

*The two top banana producers, India and China, hardly export at all, but together they still represent 35% of worldwide production.*

*The East African Highlands produce as many bananas for local consumption as all the multinational plantations together produce for export.*

*West and Central Africa produce 50% of all plantain in the world.*

*Bananas imported to and consumed by the West are the result of years of human selection, which started with the original “wild” varieties that produced only seeds and no flesh. The only traces that now remain are the small, black, flavorless dots in the edible seedless varieties.*

*There are hundreds of varieties of “wild” banana but 99% of bananas sold in Western supermarkets are genetically identical Cavendish fruit.*

*The fungus *Fusarium oxysporum*, which completely wiped out commercial production of the Gros Michel variety, now also poses a threat to export of the Cavendish banana. Most local banana varieties consumed in Africa are resistant to *Fusarium* but susceptible to the bacterium *Xanthomonas campestris*, the Banana Bunchy Top Virus, nematodes, and weevils.*

*Most edible bananas are triploid, sterile, and seedless. Developing resistance to disease is therefore very difficult via classical breeding, although biotechnology can help.*

*73% of Ugandan children under five suffer from anemia. For this reason, genetically modified bananas with higher vitamin A and iron content are being developed.*



## Bananas, the green gold of the South

The banana we know today is a fleshy, soft, and sweet fruit that appears in almost every fruit bowl in Western homes. However, the original banana was hardly edible at all and contained many seeds. Thanks to human selection, the banana of today has few or no seeds, which is mainly an advantage in terms of its consumption as a fruit or vegetable. However, the lack of seeds also proves to be a great disadvantage because bananas can no longer reproduce by way of their seeds, making them fully dependent on vegetative propagation. The genetic basis has therefore become fragile.

(Figure: Banana diversity in Tanzania (Source: R. Swennen, IITA)



Figure 1.1: Shrub, raceme, and fruit of *Musa acuminata*  
(Source: H. Mduma, M. Batte, R. Swennen, IITA)

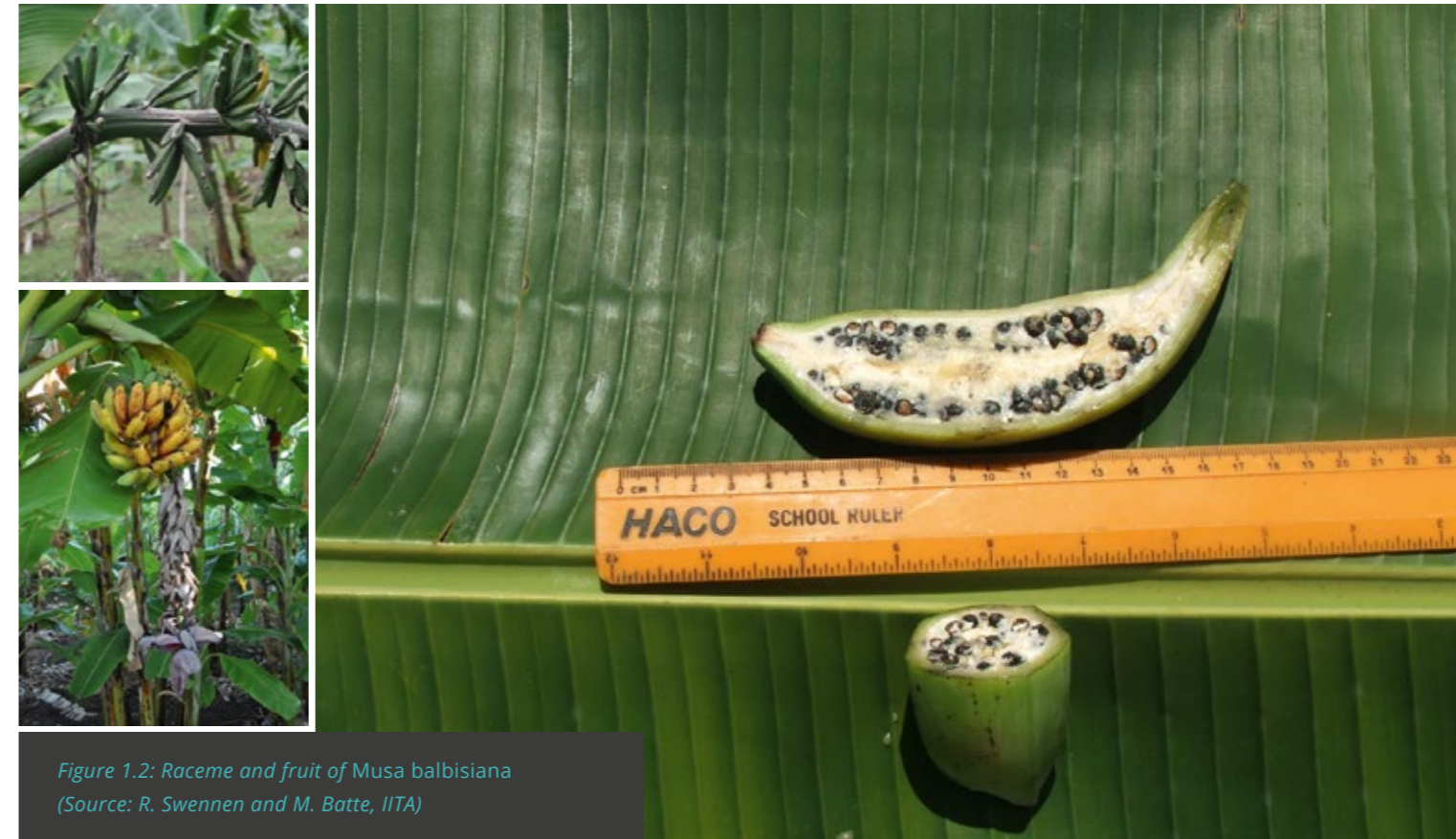


Figure 1.2: Raceme and fruit of *Musa balbisiana*  
(Source: R. Swennen and M. Batte, IITA)

## The ancestors of the modern banana

According to plant systematics, the banana belongs to the *Musa*<sup>1,2</sup> genus. Linguists are not fully in agreement as to the origin of the name: either Carl Linnaeus came up with the name as a tribute to *Antonius Musa*, physicist and doctor of Emperor Augustus (1st century BCE), or the name came from the Arabic word “Mauz”. Almost all modern edible bananas originate from two seed-producing ancestors, *Musa acuminata* and/or *Musa balbisiana*<sup>3</sup>.

The *Musa acuminata* comes from the region of Malaysia, Indochina, and Australia but was probably actively grown for the first time in Papua New Guinea around 8000 BCE. This sort favors

a tropical climate and propagates in the same way as a typical plant: via seeds. Banana plants can, however, also propagate by asexual—or vegetative—reproduction by forming suckers. The *Musa acuminata* produced edible bananas with a sweet taste, an important food source for wild animals such as bats, birds, mice, rats, and monkeys.

The *Musa balbisiana* originates from Southeast Asia and grows primarily in ravines and tropical evergreen forests up to a height of 1,100 meters. The fruit are small and bluish grey, and contain a lot of seeds.

Neither *Musa acuminata* nor *Musa balbisiana* are consumed by humans because of the non-digestible seeds and lack of flesh. The fruits are very small and only weigh between 2 and 5

grams, in contrast to the modern, edible bananas, which can reach up to 200 g. Spontaneous cross-pollination between the wild varieties *Musa acuminata* and/or *Musa balbisiana* led to seedless plants<sup>4</sup>. This very appealing trait for humans did not go unnoticed. Subsequently, specific crossbreeding programs were set up with the fertile “wild” banana plants, which led to the cooking banana and plantain. Hundreds of seedless banana varieties were selected in this manner<sup>4</sup>.

## Why are bananas bent?

The banana plant, sometimes inaccurately referred to as a “tree”, is a herb, albeit the biggest in the world. The stem of the banana plant is in fact a pseudostem or false stem made up of the sheaths of the banana leaves that sprout from

the rhizome. All leaf sheaths together form a pile of vertical, almost concentric layers. Banana plants grow on almost all types of soil as long as the soil is at least 60 cm deep and not too compact, and allows sufficient water transported. Banana plants also favor warmth (27°C) and moisture (at least 2,000 mm/year). There are, however, also varieties that can grow in temperate climates, although they tend to be ornamental or fiber bananas.

Every pseudostem forms one inflorescence. Gravity causes the floral stem to bend and a bud is formed at the end, which is velvety red in the case of export bananas. The typical purple cone of the bud remains unchanged, but the bracts fall off and small white flowers are formed in double rows. The first 5 to 15 rows are female flowers from which the fruit grow. The other male



Figure 1.3: Young bunch of Cavendish bananas  
(Source: FABI, South Africa)

flowers are completely superfluous because bananas are formed from the female flowers with no pollination.

The developing fruit grow towards the sunlight, meaning that they turn against gravity—as the floral stems hang upside down—and take on that familiar bendy shape. This is why bananas are bent. As soon as the fruit are ripe, the pseudostem is cut down and the bunch can be harvested. At the base of the plant, however, new shoots have already been formed and the daughter plants can take over production, while the rhizome remains. The mother plant is thereby replaced, while the shoots or suckers remain genetically identical. This lateral shoot formation

means that banana plantations can last for an average of 6 to 30 years. The individual plants, however, will change position during that period.

Because edible bananas contain no seeds, the shoots on the stem must be selected by the grower to plant a new field. Flowering is also not dependent on seasons, so fresh bananas can be harvested throughout the year. Cultivated banana plants vary in height depending on their variety and growing conditions. Most varieties, such as the Cavendish banana, reach heights of around 3–4 meters, whereas the former export banana, the Gros Michel or Big Mike, reached heights of 7 meters. The leaves of a banana plant can grow to 2.7 meters long and 60 cm wide but are easily torn by the wind.

## Bananas: from the hand or from the pan?

All over the world, an enormously diverse range of bananas are grown and consumed by small-holders in the South. Bananas can be divided into cooking bananas, plantain, beer bananas, and dessert bananas, although this division and interpretation can differ from place to place. In Southeast Asia, there is less of a distinction made between cooking and dessert bananas because there, the fruit are eaten both raw and cooked<sup>5</sup>. There are also fiber and ornamental bananas.

Plantain originally comes from the Philippines and Indonesia. It now grows primarily in the tropical climates of West and Central Africa and Latin America. The plantain is sometimes called the pasta or potato of the South, because it is a staple food in the day-to-day diet. The taste of plantain is different at each stage of development

and the color of the fruit ranges from cream to yellowish to pale pink. When the peel is between green and yellow, the plantain is very starchy and when the color progresses to brown and black, plantain has a much sweeter-acid taste and banana-like flavor. Plantains are generally longer and have a thicker peel than the dessert bananas we are familiar with. They are mostly eaten as a vegetable. They are very nutritious and a good source of potassium, vitamin A, and vitamin C. Plantain can also be found in shops in the West.

The sweet fruit is the dessert banana. It is much sweeter, has a thinner peel, and is smaller than the plantain. This soft and fleshy fruit contains no fat and consists of 70% water and 27% sugars or carbohydrates (90 kcal/100 g). The dessert banana is also rich in potassium, phosphorus, calcium, iron, and vitamins A, B6, and C. People who are allergic to latex may suffer a reaction after eating bananas<sup>6</sup>. Ripe bananas also contain serotonin, dopamine, and norepinephrine<sup>1</sup>. These components have a positive influence on people's mood.

In South America, Asia, India, and Africa, all parts of the banana plant, including the rhizomes, pseudostem, leaves, and flowers, are used in

local and traditional medicine<sup>7</sup>. Banana peel is used in the treatment of burns and to help heal wounds. The inside of the peel is claimed to calm the itch of mosquito bites, and the flower extracts are used for eye problems. Bananas are also applied in treatments for inflammation, rheumatism, diabetes, and fever<sup>1, 7</sup>. They are also recommended for people with high blood pressure, because they are low in salt but high in potassium.

Besides classifying bananas according to their physical traits, banana types are also classified according to their genetic basis. The genetics are complex, however. Types of banana are grouped depending on the number of chromosomes they have and their origin (see box). Most types of banana come from the ancestors *Musa acuminata* with the A-genome and *Musa balbisiana* with the B-genome. Most edible bananas are triploids and therefore contain three sets of chromosomes. They are sterile with AAA-genomes (dessert and highland bananas), AAB-genomes (plantain and some dessert bananas), and ABB-genomes (cooking bananas). However, exchanges often occur between the A- and B-genomes.

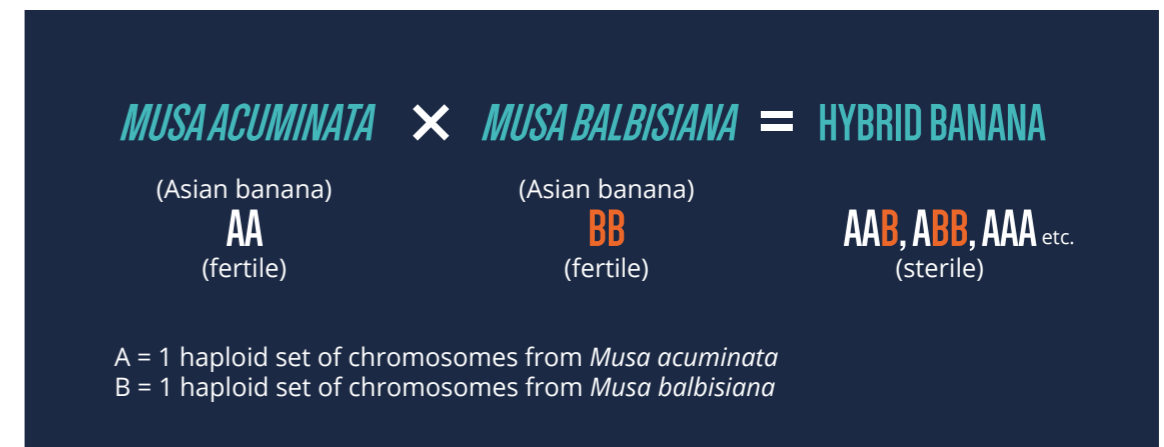


Figure 1.4: Crossing design for triploid hybrid bananas

## A CLOSER LOOK AT THE A- AND B-GENOMES

Knowing the genome, or the DNA sequence, of bananas is very important. This allows the identification of genes that are responsible, for example, for the quality or drought-resistance of the fruit, as well as genes that provide natural resistance to diseases caused by bacteria, viruses, fungus, and nematodes. Furthermore, it provides an overview of the full development process. Bananas are harvested when they are green, and only after being transported and handled they are further ripened with ethylene. It now appears that 597 genes, which are responsible for the changes in the cell walls, the starch and sugar concentrations, and other characteristics, are involved in this ripening process.

In the *Musa* family there are four genomes, which all come from wild varieties: *Musa acuminata* [A-genome, diploid ( $2n = 2x$ ) with 22 chromosomes], *Musa balbisiana* [B-genome,  $2n = 2x = 22$  chromosomes], *Musa schizocarpa* [S-genome,  $2n = 2x = 22$  chromosomes], and *Musa textilis* [T-genome,  $2n = 2x = 20$  chromosomes]<sup>8</sup>. Because most bananas are crossbred from the wild diploids *Musa acuminata* and *Musa balbisiana*<sup>9,11</sup>, different combinations of the A- and B-genomes<sup>12</sup> occur.

In 2012, the A-genome of the DH Pahang cultivar was sequenced<sup>13</sup>. The DH Pahang banana is a homozygote or double haploid (AA) of the *Musa acuminata* genotype. A haploid genome has 11 chromosomes, so  $2n$  has 22 chromosomes. Around 90% of the 523-megabase sequence of this A-genome was studied. A total of 36,542 protein-coding genes were identified. In addition, half of the A-genome is made up of transposons or jumping genes<sup>13</sup>.

One year later, in 2013, the B-genome was also sequenced<sup>8</sup>. For this sequencing, the diploid genome of the *Musa balbisiana* cultivar *Pisang Klutuk Wulung* was used. This cultivar shows partial resistance to black leaf streak disease. The aim of this sequencing is to identify resistance genes. This B-genome is 21% smaller than the A-genome and contains 36,638 protein-coding genes, which is more or less equal to the number of genes in the A-genome. The B-genome primarily contributes to the strength of the plant and tolerance of biotic and abiotic stress factors.

## East African Highland bananas

A very specific type of banana grows in the area around the African Great Lakes, called the East African Highland banana (AAA group). This group is composed of around 70 varieties that are subdivided into beer and cooking—or matoke—bananas. The plantations of these Highland bananas are in the region around Lake Victoria in Uganda, in the Kilimanjaro regions of Tanzania, in Burundi, Rwanda, and the highlands of eastern Congo and western Kenya. These Highland bananas are easily recognized by their many black spots on the pseudostem. These medium-sized bananas are harvested when green. The flesh is white and rather hard, but more creamy and yellow-colored once the banana is ripe. East African Highland bananas are so important for the local population that the local name for

them, “matoke”, is synonymous with the word “food” in Uganda. They are one of the main sources of food, especially in periods in which other harvests are scarce. They are primarily cultivated and prepared by women. Because these fruit cannot be stored for a very long time, the women dry and grind part of them to make a type of flour, which is then used as baby food.

In Uganda, “matoke” is one of the national dishes. It is made by peeling Highland bananas, wrapping them in leaves, and putting them in a pan laid on top of the stalks of a banana plant, which ensure that the fruit remains above the water level. After a couple of hours of steaming on a wooden fire, and adding water every now and again, the flesh becomes soft and orange. The flesh is puréed and served with a piece of meat, a vegetable sauce, or crushed peanuts.



Figure 1.5: East African Highland bananas from Uganda (Source: R. Swennen)



Figure 1.6: Matoke bananas after being steamed (Source: R. Swennen)

## 2 Bananas, a vital part of the world's economy

(Figure: Cavendish bananas harvested in Suriname (Source: R. Swennen))

Bananas: the green gold of the South

### Banana export and production

In 2013, more than 145 million metric tons of bananas were produced in over 130 countries on more than 11 million hectares of land<sup>14</sup> (Table 1, Figure 2.2). The top producers are India, with around 27 million metric tons (amounting to 19% of total production), and China with 12 million metric tons (8% of total production). Uganda is the third-largest producer with around 9.5 million metric tons (cooking and beer bananas), followed by the Philippines (8.6 million metric tons) and Brazil (6.9 million metric tons).

It is worthy to note that the largest producers, India and China, export almost no bananas. Only around 15% (or 20 million metric tons) of the global production was exported in 2012<sup>14</sup>. The large majority of bananas are cultivated on the land or in the gardens of smallholders for personal consumption or local sale. Thanks to the great range of varieties, a rich diversity is maintained. Bananas can be harvested throughout the year, making them a constant source of food, and for smallholders, a constant source of income. In Uganda for example, more than thir-

teen million people depend on bananas for food, income, and work.

The Cavendish banana (AAA group) is the most cultivated type and makes up 28% of fruit consumed locally. After this, it is primarily the AAB subgroup of plantain that is cultivated for consumption in West and Central Africa and South and Central America (21%). In other words, 2 of the 50 recognized subgroups are responsible for more than 60% of total production<sup>15</sup>.

Despite this great diversity, export production is entirely dominated by the Cavendish banana (see below: the favorites of the West), a group of sweet banana varieties that look very much alike in the field and cannot be distinguished once packaged. Commercial banana plants are often cultivated on large plantations. Almost 70% of the entire export market is in the hands of only 10 countries. The largest exporter is the South American country Ecuador<sup>14</sup> (Figure 2.2, Table 1) with around 5.2 million metric tons of bananas or 26% of total exports. Ecuador produces around 6.5 million metric tons of bananas, which means that around 80% of its production is exported. The Philippines, with around 2.6 million metric tons or 14% of total exports, is the second-largest exporter. The top five is completed by Guatemala (2 million metric tons or around 10%), Costa Rica (1.9 million metric tons or around 10%), and Colombia (1.8 million metric tons or around 9%). For commercial purposes, dessert bananas are primarily grown in South America and the Caribbean, cooking bananas in Asia, and plantain in Central and West Africa and in South and Central America<sup>4</sup>. In 2013, five companies were responsible for 44% of international trade: Chiquita (13%), Del Monte (11%), Dole (11%), Fyffes (6%), and Noboa (2%).



Figure 2.1: Export bananas (Cavendish) being packaged for transport in South Africa (Source: FABI, South Africa)

The other 56% was traded by smaller, national companies that either sell their fruit to the large companies or directly to retailers (www.bananalink.org.uk).

Naturally, completely different countries appear in the list of import countries. In 2012, the United States imported around 4.6 million metric tons of bananas, Belgium 1.32 million metric tons, and the Russian Federation 1.26 million metric tons. Germany and Japan completed the top five, each importing over one million metric tons. Antwerp (Belgium) is the main port for banana imports. After the ripening process, 94% of imported bananas are exported again from Antwerp, with

a total value of 1.3 billion euro, with 26% going to other countries of the European Union (18% to Germany, 12% to Great Britain, and 8% to Italy). The banana seems to be the most exported and consumed fruit in the world. The annual consumption per capita of bananas comes to 12 kg in the USA, 8 kg in Japan, 5 kg in China, and 3 kg in Russia. In the European Union, bananas represent around 11% of all fruit consumed and are primarily prized by those who live in northern Europe. The Swedes eat around 19 kg of bananas per year per capita, the Brits 12.5 kg, the French 8.5 kg, and the Belgians an average of 8 kg.

Figure 2.2: Overview of worldwide production, export, and import of bananas and plantain

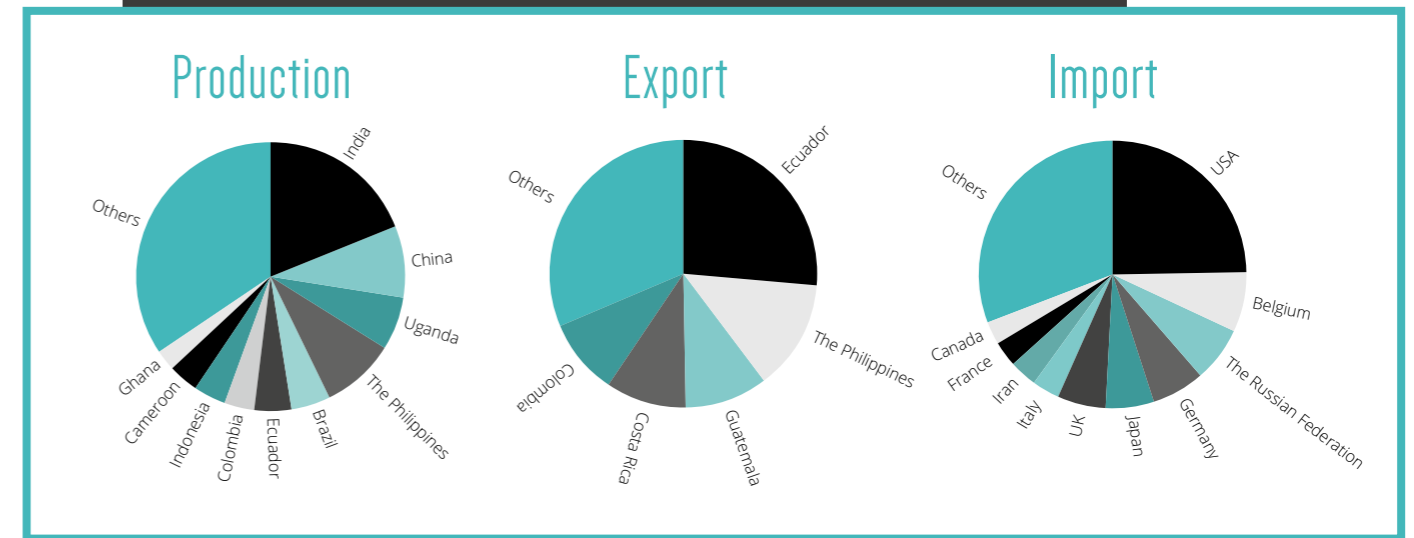


Table 1: Overview of worldwide production, export, and import of bananas and plantain

Country	Production (million metric tons) in 2013	Country	Export (million metric tons) in 2012	Country	Import (million metric tons) in 2012
India	27.6	Ecuador	5.2	United States	4.6
China	12.4	The Philippines	2.6	Belgium	1.3
Uganda	9.5	Guatemala	2	Russian Federation	1.3
The Philippines	8.6	Costa Rica	1.9	Germany	1.2
Brazil	6.9	Colombia	1.8	Japan	1.1
Ecuador	6.5			United Kingdom	0.6
Colombia	5.4			Italy	0.6
Indonesia	5.4			Iran	0.6
Cameroon	5.2			France	0.6
Ghana	3.8			Canada	0.5
Total worldwide production	145	Total worldwide export	19.7	Total worldwide import	19.7

Because not all countries make a clear distinction between quantities of banana and plantain produced, the total quantities here include both<sup>14</sup>.



Figure 2.3: Export bananas are harvested for packaging when green (Source: R. Swennen)

### Picked when green and ripe in the shops

Export bananas start off a greenish-yellow color, and turn yellowish-brown during ripening. As with all fruit, the taste and texture of a banana ripened on the plant are the best. When determining the best time for harvesting, however, the

journey from the field to the consumer should also be taken into account. Fruit harvested when ripe is softer than unripe fruit and is therefore more easily damaged during transport. The shelf life of bananas harvested ripe, which is only between 7 and 10 days, is also an important factor. This period is too short to cover the long distance between tropical countries and the consumer in the West. This is why export bananas are always picked unripe and green, the fruit is handled carefully and stored cool (13.5°C to 15°C), and the transport time is kept as short as possible. The primary aim is to prevent the hormone ethylene, which is produced by the ripening fruit, from triggering the ripening process.

After transport, bananas are ripened in special chambers filled with ethylene at a temperature of 17°C. Imported bananas are ripened as close as possible to the consumer, in the country of import. The pleasant yellow color of bananas in Western supermarkets is therefore achieved by an induced ripening process, which determines the taste and texture of the banana. This treat-





Figure 2.4: Gros Michel bananas at a market in Tanzania (Source: R. Swennen)

ment is essential because green bananas that are never treated with ethylene will not fully ripen before rotting.

## Gros Michel and Cavendish, the favorites of the West

Since the 1870s, only two varieties of bananas have been cultivated for the export industry, the Gros Michel and the Cavendish<sup>16</sup>. Gros Michel, also called Big Mike, is a triploid variety of the AAA subgroup from the ancestor *Musa acuminata*.

Gros Michel bananas were cultivated and traded as far back as the 1800s. It is a very sweet and flavorful variety of banana with a creamier texture than today's commercial bananas. The plant can grow to a height of seven meters and the fruit are around 18 to 23 cm long. It is favored by producers because this fruit has a very strong and thick peel, meaning that it needs little extra care during the growing and transport process. These plants also have very high yields. Some rich Americans came up with the idea of setting up a banana trade. Consumption rose enormously and there were even advertising campaigns proclaiming that bananas were good for

everyone, even babies, and that they could be consumed at any time of day.

Until the 1950s, the Gros Michel was a major export product, primarily to the United States and Europe, but had already been threatened since the beginning of the 1900s by the fungus *Fusarium oxysporum* (see Chapter 3). This fungus first emerged in Panama and quickly spread across the whole world<sup>17, 18</sup>. The entire production of Gros Michel came to a standstill in South and Central America and Africa and almost all growers went bankrupt. Nowadays, the Gros Michel is hardly cultivated at all, except in some parts of Thailand and by smallholders in Africa, the Caribbean, and South and Central America.

In the period when the Gros Michel was disappearing as the export banana, a new variety, the Cavendish, which did appear to be resistant to the fungus, appeared on the market. The Cavendish banana was discovered in Vietnam. It is also a triploid banana from the AAA group and was cul-



Figure 2.5: Local banana "Sukali Ndiizi" trade in Tanzania (Source: R. Swennen)

tivated for the first time in 1836.

The Cavendish plant grows to a height of three to five meters and the fruit are less sweet and smaller than those of the Gros Michel plants. Since the 1960s, when the Gros Michel disappeared from the market, the Cavendish has taken over the role of export banana. Since then, all bananas traded worldwide have come from the Cavendish variety, which also has no seeds, so is infertile and has to be propagated through vegetative reproduction. As a result, all Cavendish bananas are genetically identical and the fruit that were sold in the 1960s in the West were identical to those that we find in the supermarket today.

## Banana: from staple food to alcoholic drink, shoes, and carrier bags

It is clear from the production and export figures that the majority of bananas produced in the South are intended for consumption and trade on local and regional markets. Bananas and plantain appear to feed more people per unit of area than any other crop in the world<sup>19</sup>. Above all, they are much cheaper to produce than rice

and wheat<sup>20</sup> and cultivation is less sensitive to global price fluctuations than is the case with rice, maize, and wheat<sup>19</sup>. Indeed, the banana is a significant staple food throughout the world with an average of 21 kg/inhabitant/year. These averages are, however, much higher in developing countries. In Uganda, Burundi, and Rwanda, people eat up to 300–400 kg of bananas/year, which comes to an average of 3–11 bananas/day/person<sup>21</sup>. Almost every mealtime consists of mashed and seasoned bananas. Studies showed that in countries such as Uganda, Rwanda, and Burundi, bananas are responsible for 30% of calorie intake, in certain areas even reaching as much as 60%<sup>22</sup>. In Uganda, it is largely thanks to banana cultivation that famine is not widespread.

Bananas can be eaten in several different ways: fried, steamed, baked, or raw. They can also be made into jam, beer, cookies, and rusks. In Leuven (Belgium), a banana liqueur called "Musa Lova" has been created. Banana pancakes are also very popular in South and Southeast Asia. Unripe plantain is largely used to make banana chips. These are thin, dehydrated slices of banana with a dark color and intense banana taste. Making a juice from bananas requires extrac-



Figure 2.6: (A) Banana liqueur ([www.musaLova.be](http://www.musaLova.be)), (B) Banana beer Tanzania (Source: R. Swennen), (C) Uganda Tonto banana beer (Source: Y. Lokko, UNIDO), (D) Banana chips (Source: D. Amah, IITA), (E) Fried bananas Tanzania (Source: R. Swennen)



Figure 2.7: (A) Roof made from banana leaves (Uganda) (Source: R. Swennen), (B) Clothing made from banana fiber (Tanzania) (Source: M. S.R. Byabachwezi, ARI Maruku, Tanzania)

tions because of the texture of the fruit, but the fruit can be used in flour, soup, breakfast cereals, and even in commercial fruit salads and smoothies. In South and Southeast Asia, the heart of the banana blossom is often consumed; it is vaguely reminiscent of artichoke, because the fleshy parts of the bracts and the heart of the blossom are edible. In East African countries such as Burundi, Rwanda and Uganda, traditional uses of the cooking banana matoke include a staple mash, juice, beer, local gin, wine and liqueur.

In tropical countries, banana leaves are used as packaging material, roof material, umbrellas, and even disposable plates. The fibers from the banana leaves are made into bags, shoes, paper, rope, baskets, teabags, and banknotes. For a long time, Japanese kimonos were made with banana fiber<sup>23</sup>. In some areas and especially in the dry season, banana leaves and cut stalks are a major component of animal feed.

The banana plant also has another important function in certain areas of Africa, such as Ivory Coast. Banana plants are cultivated to give shade to cocoa or coffee plants<sup>24</sup>. Both plants benefit from being cultivated together: when the cocoa

plant is still young, the shade from the banana plant protects it, and the cocoa plant, which has roots that grow much deeper into the ground, ensures that nutrients for the banana plant are transported from deep underground to the surface. The fallen cocoa leaves keep the ground moist and weed-free. Thanks to this symbiosis between the cocoa and banana plants, greater yields can be expected from both plants. Banana plants also have an important ecological role. They reduce land erosion, and remnants from their harvesting also return nutrients to the ground after each production cycle<sup>24</sup>.



Figure 2.8: Growing banana plants and coffee plants together in Tanzania (Source: R. Swennen)

## SUPPORTING SMALLHOLDER FARMERS EARN MORE FROM BANANA PRODUCTION THROUGH BANANA VALUE ADDITION IN UGANDA

Bananas are Uganda's leading staple crop supporting the food security of about 13 million people. The main banana types produced are the cooking banana matoke, dessert bananas as well as plantain. In general, most of the bananas produced in Uganda are sold and consumed fresh. Whole bunches are collected on the farms and transported to the local markets, which are quickly saturated. Therefore, most production has traditionally been transported to the urban areas. There is also some export of fresh matoke to Europe, USA and regionally, particularly to South Sudan, where prices on many different types of food are higher.

Without much value addition, the bananas are susceptible to over-ripening and rotting, during storage and transportation, resulting in substantial losses in income to farmers (who already receive very little for their products) and shortages of supply on the market. Processing of bananas is vital not only in extending their shelf life but also in transforming the crop into alternative products, which opens secondary markets. Processing adds value to green bananas and increases the quality of food commodities. In addition, by engaging in banana value addition activities the farmers can earn more from banana production. Traditional banana processed include banana beer (tonto) and local gin (waragi).

Recently value added products such as vacuum-packed peeled versions of matoke, matoke flour, banana juice and banana wine as well as dried fruits and plantain chips are available on supermarket shelves and for the export market. Traditionally, the juice is extracted from ripened fruits through a laborious mechanical process. Although enzymatic treatment which yields higher quantities of juice is available, the traditional is the most preferred. It is estimated that two million liters of waragi/alcohol and 1.4 million liters of banana beer are sold annually.

Although most of the banana value addition is on cottage industry scale, some of the institutions are involved in the production of high quality value added banana products in Uganda. The Presidential Initiative for Banana Industrial Development (PIBID) produces banana flour under the brand name tooke flour, Afri Banana Products Ltd is an agribusiness incubation company producing vacuum packed matoke, banana juice and wine, Forest Fruit Foods Ltd is a small-scale enterprise (SME) producing banana juice, and Fruits of the Nile supports smallholder farmers in producing dried fruits for export.

Also the residues from banana plantations are good sources of material for value addition. Traditional uses of banana waste include banana fibres, leaves, peels and pulp. Banana fibre is used domestically in thatching

houses and also for making loose ropes for carrying tea. Banana leaves are also extensively used in preparing meals throughout the country. Indeed a flourishing commercial market for banana leaves exists in the country. Banana leaves also have a strong cultural significance and contribute to the flavor of meals, particularly in central Uganda. The peels and pulp from juice extraction as well as rejected bananas are often available for animal feed. Recently, the Uganda Industrial Research Institute (IRRI) has developed a prototype for the extraction of fiber from the stems for the manufacturing of paper and textiles have been developed. Finally, banana waste can be used to generate biogas to serve various energy needs, thereby, reducing the dependency on firewood and charcoal. The resulting sludge, a by-product of the biodigester system, can be used as compost.

With funding from the Global Environmental Facility (GEF) under its Least Developed Country Fund (LDCF) for interventions climate change adaptation, UNIDO is assisting the Government of Uganda in its banana value addition program. The project aims at supporting vulnerable communities in Western Uganda to better adapt to the effects of climate change through banana value addition activities, to provide greater opportunities for income generation, poverty reduction and food security.

## THE BIGGEST BANANA COLLECTION IS IN LEUVEN

Belgium has already been researching bananas for over 100 years. Edmond de Langhe, professor emeritus at KU Leuven, started studying and collecting bananas in the 1950s. Since 1976, the Belgian government has been funding research into plantain at the International Institute of Tropical Agriculture (IITA). In 1979, PhD student Rony Swennen headed the IITA team and also contributed to building the research program. Currently, Prof. Dr. Rony Swennen, director of the Laboratory of Tropical Crop Improvement at KU Leuven, administers the largest banana collection in the world. The laboratory run by Edmond de Langhe and Rony Swennen is also behind the creation of the "International Network for the Improvement of Banana and Plantain" (INIBAP), a global network that safeguards the biodiversity of the banana, and which celebrated its 30th anniversary in 2015. INIBAP, now part of "Bioversity International", is a network that operates in Africa, Asia, and Latin America. In each of these continents, the network receives backing from regional and national networks made up of members of farmers' associations, NGOs, cooperatives, universities, and the private sector.

The banana collection in Leuven is under the supervision of the United Nations and Bioversity International and consists of more than 1,500 disease-free types of banana—and this number continues to grow. There are still a great number of unknown types of banana in the wild and researchers often embark on jungle expeditions in search of new varieties. Once a year, all germinated seeds are re-cultured in test tubes and placed in a cool



Figure 2.9: World banana collection in Leuven (Source: R. Swennen)

Figure 2.10: Banana tissue is also stored in liquid nitrogen (Source: B. Panis, Bioversity International)

chamber (15°C) with low light intensity to ensure slow growth. In the last 30 years, banana cuttings have travelled from Leuven to over 355 locations in 100 different countries, hence the collection is called the International Transit Centre. The plants are then taken out of dormancy to create new banana plants from tissue cultures, which are then placed in cultivation chambers at 27°C until they are ready to be sent. Every applicant receives five specimens per type. Of these shipments, 75% are intended for local researchers and 25% for growers, NGOs, and agricultural organizations. The plants are given for free but recipients are not permitted to patent them. After the genocide in Rwanda and the subsequent mass migration from Rwanda and Burundi to Tanzania, 70,000 plants were sent to Tanzania, which were cultivated locally to create 6 million healthy banana plants. Small farmers saw their yields and income triple as a result, keeping famine at bay.

The scientists in Leuven also keep, for each type, data on growing conditions, traits, and the different environments in which they develop. The aim is also to safeguard biodiversity by storing all types of banana at -196°C in liquid nitrogen. At the moment, 800 types have already been frozen, but it will likely take another 12 years to freeze the other 700. This group of scientists is applying this technique (cryopreservation) not only to bananas but also to 27 other plant families such as date palms, sweet potatoes, chicory, sugar beets, cassava, and strawberries. The banana collection is also duplicated in order to be sure that a type can never be lost—for example, in case of fire. The Laboratory of Tropical Crop Improvement, IITA, and Bioversity International also work closely with small farmers in Africa, primarily to encourage them to cultivate multiple varieties of banana at the same time and heighten their chances of a successful harvest.



### 3 Threats to banana production lurk around the corner

The bananas we find in the shops today are primarily cultivated on large plantations. Because they have no seeds, virtually all banana plants are the result of vegetative reproduction, which means they are genetically identical and have the same positive and negative traits. The entire plantation is, as a result, highly susceptible to attacks from bacteria, viruses, fungus, nematodes, and insects if its banana plants do not have the right forms of resistance. The banana types cultivated by smallholders for local consumption are often also genetically identical and highly susceptible to potential attackers. Once a certain type of banana is afflicted by a disease or pest, its spread and the subsequent loss of the harvest also occur very rapidly.

Banana plantation affected by Panama disease (Source: A. Viljoen, Stellenbosch University, South Africa)

Bananas, the green gold of the South

### Panama disease almost completely wiped out Gros Michel and now also poses a threat to the Cavendish banana

Panama disease was caused by the soil-borne fungus *Fusarium oxysporum f. sp. cubense* (Foc), Tropical Race 1 (TR1)<sup>18, 25, 26</sup>. This soil-borne fungus infects the banana plant through the roots and spreads via the sap flow in the xylem tissue throughout the entire banana plant. In certain areas of the vascular bundles, the fungus forms a type of gel that obstructs the flow of fluids and nutrients through the plant, causing the plant to dry out<sup>27</sup>. When the pseudostem of an infected plant is cut open, the vascular bundles appear to have turned completely brown/black. Other symptoms are yellowed leaves, and root and rhizome rot. The disease was first identified at the beginning of 1900 in Panama, from where it got its name, and subsequently spread from there to neighboring countries.

Fungi spread mainly by way of spores (microscopic survival and propagation structures). These fungal spores return to the ground from the plant that has died, to infect other plants. Because this fungus first completely ravages the

plant from inside, the disease is detected at a very late stage. The infection and spread to other plants is often too far advanced at that stage to be able to intervene.

The spores of *Fusarium oxysporum* are able to germinate for longer than a year and can survive in the soil for 20 to as many as 40 years, even in the absence of the host plant (banana)<sup>28, 29</sup>. Panama disease Foc-TR1 primarily affected the Gros Michel variety, resulting in the complete discontinuation of its commercial cultivation since 1960. In the 1960s, Panama disease was already regarded as one of the most devastating plant diseases. At that time, the economic losses were estimated at 2.3 million dollars and many growers went bankrupt. The fungus also appears to be deadly for a whole range of local banana varieties and therefore also constitutes a threat to banana cultivation in developing countries.

Given that the Gros Michel variety could no longer be cultivated on a large scale, a new commercial banana was explored: the Cavendish (see Chapter 2). The Cavendish plant appeared to be immune to the *Fusarium oxysporum* fungus Race 1 and became increasingly important for trade from the 1960s onward. As a result, the infection disappeared from the spotlight, although in 1986 it was warned that new



Figure 3.1: Banana plantation affected by Panama disease (Source: A. Viljoen, Stellenbosch University, South Africa)



Figure 3.2: Cross-cut of the pseudostem after infection by the *Fusarium* fungus (Source: A. Viljoen)

infectious diseases were threatening to break out as a result of the cultivation of so many genetically identical plants in monocultures<sup>30, 31</sup>.

In 1992, a new and highly aggressive variant of the *Fusarium* strain was discovered: *Fusarium oxysporum* Tropical Race 4 (Foc-TR4), which was first identified in Asia at the end of the last century and has now also appeared in Taiwan, Indonesia, Malaysia, the Philippines, China, and Northern Australia. In these areas, the cultivation of the Cavendish banana has dropped significantly, which is problematic given that China, the Philippines, and Indonesia are among the top producers for banana (see Chapter 2). In October 2013, the outbreak of this strain was reported in Jordan<sup>32</sup>, where relatively few bananas are cultivated, around 40,000 metric tons per year, but where 80% of plantations are already infected<sup>32</sup>. More recently, Foc-TR4 was identified in Mozambique, Lebanon, Oman, and Pakistan<sup>17, 18, 32</sup>. This disease is clearly a new and real threat for the entire global production of bananas<sup>33, 34</sup>.

The *Fusarium oxysporum* soil-borne fungus spreads primarily through contaminated soil and water but also through footwear, clothing, tools, insects, irrigation water, and infected cuttings<sup>35</sup>. When planting material comes from tissue culture, it is possible to work with disease-free plants (see Chapter 4), but they can also very quickly come under attack once they are planted in infected fields. It's now a case of waiting with trepidation to see when this Foc-TR4 strain will reach Central and South America and how quickly the spread of this disease will threaten the Cavendish in America and Africa, with the resulting worldwide impact on international trade.

## Black leaf streak causes a sharp drop in yields

Black leaf streak, also called black sigatoka, is caused by the fungus *Mycosphaerella fijiensis*. This disease was first identified in the Sigatoka valley in Fiji in 1912 but was probably also present in other areas of Southeast Asia<sup>36</sup>. Because

infected banana leaves were very often used as packaging material, this disease spread very quickly outside Asia. The fungal spores are also very quickly spread by wind or rain<sup>36</sup>. In 1972, the disease was first identified in Honduras, followed by Belize, Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica, and many other Central and South American countries<sup>37</sup>. Almost at the same time, in 1973, this disease also emerged for the first time in the African country of Zambia, but has now been identified throughout Sub-Saharan Africa.

The fungus *Mycosphaerella fijiensis* develops spores on the banana leaf at high humidity and a temperature of around 27°C<sup>36</sup>. Two to three days after infection, the spores enter the banana leaf through the stomata\* and continue to multiply. The infection starts on the underside of the leaf and forms small black spots, which gradually spread and form black stripes parallel to the leaf veins, as a result of which photosynthesis\*\* can no longer occur<sup>38</sup>. The production of energy drops and the leaf finally dies. The reduced energy supply causes early ripening of the fruit, making it unsuitable for export. A banana plant normally has 11–16 leaves but if fewer than five remain as a result of disease, the fruits do not develop sufficiently<sup>39</sup>. In sick plants, the fruit harvest is halved. The fungus infects all banana, Highland, and plantain varieties—not only the commercial Cavendish variant. In East Africa, banana production fell by over 40% as a result of this disease<sup>24</sup>. In the 1970s, Uganda still produced 15 to 20 metric tons of bananas per hectare, but in areas where this fungus is present, the production is now only 6 metric tons per hectare.



Figure 3.2: Destruction caused by sigatoka. There is little fruit and healthy leaf tissue to be seen (Source: J. Kimunye, IITA)

\* Stomata are structures on the surface of leaves of terrestrial plants. A stoma consists of a microscopically small opening formed by two guard cells. Stomata are very important for plants because they are the means by which the plant takes in carbon dioxide from the air and releases the oxygen and water produced in the leaf.

\*\* Photosynthesis is a vital process in plants through which energy from light is used to convert carbon dioxide into energy-rich compounds (carbohydrates), such as glucose. This is how the plant is able to produce energy for itself. Most photosynthesis takes place in the leaves of a plant.

## Viruses threaten worldwide banana cultivation

Plantain and bananas are under threat from several viruses<sup>40</sup>. These are largely spread through sap-sucking insects such as aphids, or through vegetative reproduction of the banana plant. The Banana Bunchy Top Virus (BBTV) in particular leads to very serious economic consequences.

BBTV is a small virus with circular single-stranded DNA<sup>41</sup>. The spread from plant to plant occurs through the aphid *Pentalonia nigronervosa*<sup>42</sup>. During feeding, the aphid spreads the virus from diseased to healthy plants. The aphids bring the



Figure 3.4: A banana plant infected with the Banana Bunchy Top Virus (BBTV) in DR Congo (Source: R. Swennen)

virus into the vascular bundles of the plant, after which it multiplies and spreads throughout the entire plant. The first symptoms become visible around 20 to 25 days after infection. The virus causes irregular dark-green spots and stripes of variable length and size on the leaf sheath and petioles as well as on the main and side veins<sup>40, 41</sup>. The new young leaves that develop on an infected plant are much smaller and shorter, with yellow edges<sup>43</sup>. Most of the time, the plant produces either no fruit, or misshapen fruit. The disease was first identified in Cavendish plants in 1889 in Fiji<sup>44</sup>, although the first infection occurred in 1901 on the African continent, in Egypt<sup>45</sup>. Currently, BBTV infection in banana plants has been identified in 36 countries, including 14 in Africa and 22 in Asia and Oceania<sup>40, 46, 47</sup>. Losses are sometimes so incredibly drastic (50% to 100%) that banana cultivation comes to a halt.

The banana streak virus (BSV) is globally widespread and was identified for the first time in 1958 in Ivory Coast<sup>40</sup>. This virus has one double-stranded DNA molecule and is transferred to other plants either by the mealy bug or by the vegetative reproduction of infected plants. This virus produces irregular yellow spots and stripes on the leaves and destroys the pseudostem by splitting it, usually resulting in the death of the plant. This virus infects banana plants in 43 countries, across Africa, Asia, Europe, Oceania, and tropical America.

Banana bract mosaic disease was only identified for the first time in 1979 in the Philippines<sup>48</sup>, but is now also widespread in a number of other Asian countries, including India, Sri Lanka, Vietnam, and Thailand. In Latin and Central America, the infection occurred for the first time in Colombia and Costa Rica, but recently also in

Ecuador<sup>49</sup>. This RNA virus causes fusiform purplish stripes on the leaves and pseudostem at the level of the main veins, and even on the fruit. Infection with this virus, which is spread between banana plants by an aphid, can lead to yield losses ranging from 30% to 70%<sup>40</sup>.

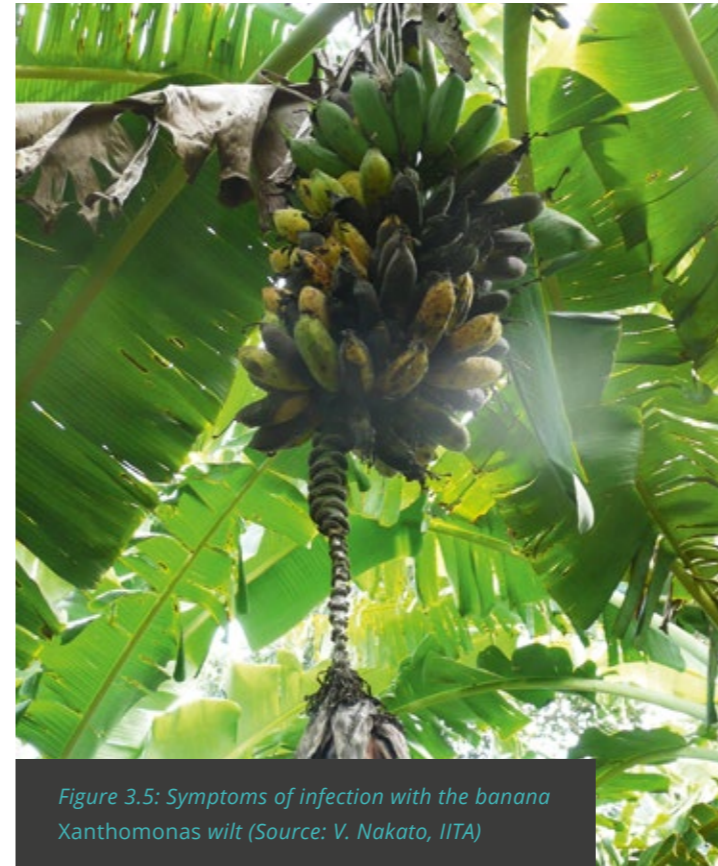


Figure 3.5: Symptoms of infection with the banana *Xanthomonas wilt* (Source: V. Nakato, IITA)

## The *Xanthomonas* bacterium is the greatest threat to bananas in Africa

The banana disease called Banana *Xanthomonas* wilt (BXW) is a disease caused by the bacterium *Xanthomonas campestris* cv. *musacearum*<sup>50</sup>. This disease was identified for the first time in the 1960s in Ethiopia, in a close relative of the

banana, namely the *Ensete ventricosum* or the “Ethiopian” or “false” banana<sup>51</sup>. In 2001, this disease was also identified in Uganda, where it has now spread across the whole country<sup>50</sup>. Other Central and East African countries such as the Democratic Republic of the Congo, Rwanda, Kenya, Tanzania, and Burundi have since then been affected<sup>22</sup>. The bacterium infects most cultivated banana varieties but the beer banana “Pisang Awak” appears to be the most susceptible<sup>52, 53</sup>.

When a plant is infected by the bacterium, the leaves yellow and wither, the fruit ripen unevenly and incompletely, and the flesh presents yellow and dark-brown spots<sup>22</sup>. When the pseudostem is cut, a pink bacterial exudate can be seen. The symptoms spread very quickly throughout the plant within three to four weeks of infection and depend on the variety, the stage of growth, and the method of infection. Additionally, the symptoms develop more quickly during the rainy season. The main sources of infection are infected banana plants and remnants on the ground, insects, wind, rain, and contaminated tools. When the plants are affected by nematodes or weevils, the bacteria can also penetrate through the roots. *Xanthomonas* can survive in the ground in the absence of the banana plant for three to four months<sup>54</sup>. As a result, an infected field cannot be used for a new harvest for at least five to six months<sup>22</sup>.

BXW is a threat to banana production and the source of income and means of subsistence of smallholders in Central and East Africa, given that the entire plant is affected and wiped out. As a result, no fruit or daughter plants/suckers are formed and the plantation is lost<sup>53</sup>. This disease has already caused economic losses of two to eight billion dollars and the loss of

production has already caused major increases in price<sup>19</sup>. Moko is a related disease caused by the *Ralstonia solanacearum* bacterium. The loss of harvest and symptoms are highly comparable to those of BXW. However, Moko only occurs in Latin America<sup>55</sup>.

## Tiny worms, huge problems

All over the world, but primarily in Africa, banana production is very much under threat from infection with nematodes<sup>56</sup>, the main ones being the migratory *Radopholus similis*, *Pratylenchus goodeyi*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus*, and the root-knot nematode *Meloidogyne spp.*<sup>57-59</sup>. Nematodes are microscopically small parasitic worms that feed on plants. The banana plant is infected via the roots, where the migratory nematodes feed on the cell content and form large cavities. As a result, the plant's ability to take in water and nutrients from the soil is inhibited, causing the leaves to yellow and fall off early, and reducing and slowing the production of fruit. Because the root system is weakened, the plant falls over<sup>24, 56</sup>.

Nematodes need a living host to survive and

reproduce but can spread from one host to another through the soil over a certain distance. Female nematodes lay several eggs at a time in infected roots and the lifecycle of a nematode is around 20 to 25 days from egg to egg<sup>56</sup>. The young nematodes will either stay in the root and infect it further or penetrate neighboring healthy plants through the soil. The spread of nematode infections occurs primarily through contaminated soil—for example, through flooding, contaminated tools or shoes, or the use of diseased plant material.

Infection of banana plants with *Radopholus similis* was first identified on Fiji in 1891<sup>60</sup>. Infected plant material then appears to have been brought into New South Wales in 1910 and into Jamaica in 1915<sup>60</sup>. There are indications that this nematode was not present in Africa before 1960. The sharp fall in banana production in Uganda and Tanzania since 1970 is probably primarily attributed to the nematode *Radopholus similis*, even though this nematode is often found together with other types<sup>60</sup>. *Radopholus similis* primarily affects the Highland banana in Uganda, but almost all banana varieties are prone to

infection via other types of nematodes. Given that infections in bananas often go hand in hand with additional infections from fungus and bacteria, it is difficult to determine the impact of nematode infections, but the economic losses are estimated at 30% to 60%<sup>20, 56</sup>.

fruit are formed, and the plants fall over easily in wind storms<sup>61, 63</sup>. As a result, an infection with weevils can completely wipe out a plantation. Plant material that has fallen to the ground then forms another breeding ground for the weevils. Weevils seldom fly, but they spread by crawling to the different plantations<sup>61</sup>. To combat the spread of a weevil plague, it is very important to till the fields properly in order to quickly curb infections and it is best to plant disease-free plant material obtained from tissue culture. However, a period of two years must pass before a new plantation can be planted on previously infected land<sup>61, 64</sup>.



Figure 3.8: Damage caused by weevils. Cross-cut of the pseudostem (Source: D. Coyne)

## Weevils: an underestimated threat?

The banana weevil *Cosmopolites sordidus* is a threat to banana plants in areas of Central Africa, Central America, Brazil, the Caribbean, and Australia<sup>61</sup>. This weevil is associated with a sharp drop in yields in banana plantations in East Africa and with the yield decline syndrome in West Africa<sup>62</sup>. The weevil lays its eggs at the rhizome or pseudostem of the banana plant. The growing larvae dig tunnels, which can reach up to eight millimeters in diameter, and fully penetrate the rhizome<sup>63</sup>. These tunnels are then taken over by fungi and the rhizome rots. The flow of sap and intake of nutrients from the soil decrease considerably, the leaves wither, only small

Figure 3.6: Banana roots infected by *Meloidogyne spp.* (Source: D. Coyne, IITA)



Bananas, the green gold of the South

Figure 3.7: Damage caused to banana plants by nematode infection (Source: D. Coyne)





# 4 Biotechnology can protect the banana against attacks

Various diseases caused by bacteria, fungi, viruses, nematodes, and insects are a threat to worldwide banana production. This is a major problem not only for the Western world, which primarily imports bananas, but especially for small local farmers in developing countries who cultivate bananas as a source of food and income. There are many routes to combat these diseases. Methods such as prevention and/or better cultivation management seem simple, but are not always so efficient. Spraying with fungicides and/or pesticides, if these are available, can have a negative effect on people and the environment, especially with regular use. Moreover, regular use can considerably reduce their effectiveness because of the possibility of resistance being built up by the pathogens. For each disease, attempts are made to produce resistant banana types through breeding, which is easier said than done. This is why obtaining resistant banana plants through biotechnology is a reliable method that could offer effective solutions.

Sebastien Carpentier, KU Leuven

Bananas, the green gold of the South

## The farmer as the protector of the field and banana plants

The most obvious way to combat the spread of disease in the field is to eliminate infected plants as quickly as possible. However, this is not always a simple matter. Certain diseases such as Panama disease primarily manifest themselves inside the plant and are only visible once the infection has already spread to several plants. Moreover, fungal spores can spread very quickly, meaning that the disease propagates so rapidly that by the time the farmer reacts, it is already too late. The only solutions are to fully destroy the plantation or to eliminate all roots from the ground and either leave the soil unplanted for a number of months or plant other, insensitive plants in the soil until all pathogens have disappeared<sup>29</sup>. That is why it is important to supply growers with sufficient information on the potential impact and early identification of symptoms of infection<sup>65</sup>.

To prevent or limit plagues, it is also important to plant the banana plants far enough away from one another for better water drainage and weed control, remove the male buds, and work with disinfected tools. However, this does not appear to be viable for local farmers because it entails additional labor and costs<sup>66</sup>. In 2001, a strategy was developed in Uganda to curb BXW: all infected plants had to be removed and burned, the banana plants could only be transported and moved under strict conditions, and the male fruit buds were removed. Farmers received additional information to enable them to recognize the disease more quickly, and had to use properly sterilized equipment on their fields. The disease was reduced to 10% of its original level as a result. However, it transpired that the

measures were not viable for farmers because they were labor-intensive and too costly<sup>66, 67</sup>. The control of banana disease, and especially BXW, additionally appears to be very dependent on the country and especially its political stability. In Uganda and Tanzania, countries with strong political leadership and stability, BXW is more than 90% under control. In the eastern regions of the Democratic Republic of the Congo, on the other hand, the disease has quadrupled.

To combat the spread of plagues and diseases, it is very important that farmers always work with disease-free planting material. Nevertheless, smallholders usually use young shoots from old plantations to save costs, which only exacerbate the spread of the banana diseases. Currently, IITA and Bioversity International are working alongside national programs in Africa to

## TISSUE CULTURE TO DEVELOP DISEASE-FREE PLANT MATERIAL

*Tissue culture is a method used to propagate plants under sterile conditions based on the ability of individual plant cells to grow into an adult plant (totipotency). Tissue culture is very important for many different types of banana because of their almost sterile nature and the impossibility of reproduction via their seeds. Through tissue culture or vegetative reproduction, the plant can propagate easily, generating genetically identical offspring (clones). As soon as new shoots develop, they can be cut off from the plant and, after treatment in sterile conditions with filtered air, grown on a culture medium that contains salts, nutrients, vitamins, and plant hormones for optimal plant development. Introducing the plant hormone auxin to the culture medium, ensures that the shoot will form roots. Once the shoot is large enough, it can be planted in soil.*



eliminate as many infected plants as possible from the field and plant new, healthy plant material in selected areas ([www.rtb.cgiar.com](http://www.rtb.cgiar.com)). Obtaining disease-free plant material is not a simple task. Currently, the International Transit Centre, located in the Laboratory of Tropical Crop Improvement (KU Leuven — Rony Swennen, see box in Chapter 2, page 20) is the primary source of 100% disease-free planting material from tissue culture.

Tissue culture promotes the spread of disease-free plant material and offers a wealth of advantages for local farmers<sup>68-70</sup>. It is crucial, however, to only plant this material in non-infected soil. By using plants obtained from tissue culture, banana farmers in Kenya increased their yields from 10 to 30 metric tons per hectare<sup>68</sup>. The purchase of the plants was expensive but because of the greatly increased

yield, the farmers saw their income go up by at least 145%<sup>68</sup>. The use of shoots from tissue culture also provides access to useful endophytes in the soil. Endophytes are useful microorganisms, such as bacteria or fungus, which live alongside the banana plant and protect the plant from disease, plagues, and unfavorable conditions, such as drought and heat. An important caveat to bear in mind is that banana plants obtained from tissue culture appear to be somewhat more sensitive to Panama disease than shoots that have grown in the field<sup>29, 71</sup>. Nevertheless the banana tissue culture industry is becoming more and more important in banana production systems in Africa. A number of SMEs and government funded research facilities provide the service of commercial production of banana plantlets to meet the demands of farmers.



Figure 4.1: Polyculture of bananas and beans in Tanzania (Source: R. Swennen)

## Monoculture, polyculture, or crop rotation: which of the three?

A major cause of the fast spread of disease in today's (commercial) bananas is the fact that monoculture is the main strategy used on plantations<sup>30</sup>. Monoculture means that the same crop is always grown on the same piece of ground with no crop rotation. Bananas are a rather extreme form of monoculture given that the young banana plants are all genetically identical to the mother plant with all its good and bad/negative traits.

Particularly on plantations on which export bananas are cultivated, only the genetically identical Cavendish banana is present. If these plants are sensitive to a certain fungus, virus, bacteria, and/or nematode found in the ground, it can spread at lightning speed. Smallholders are, however, increasingly opting to grow more than one variety on their land because they realize that growing varieties of banana with different susceptibility to a certain plague delays its spread, thereby increasing their chances of a successful harvest. This is why, on the whole, 4-5 different banana varieties are grown, but this number can go up to as many as 20.

Another step in the right direction is surrounding banana plants with other plants such as coffee, sorghum, potatoes, maize, cassava, rice, sugar cane, or yam<sup>24</sup>. This technique, called polyculture, can lead to a less rapid spread of disease. For export, the Gros Michel has completely been replaced by the Cavendish, because cultivating Gros Michel on large plantations is no longer possible. In many South and Central American

countries, such as Costa Rica and Nicaragua, Gros Michel is still cultivated by smallholders, who grow coffee bushes along with bananas, which means that there is less risk of infection with *Fusarium oxysporum*<sup>72</sup>. Bananas are also often cultivated alongside beans (*Phaseolus vulgaris*)<sup>73</sup>. To cultivate beans, however, the ground requires more tillage, which can be detrimental to the banana plants because of potential damage to the roots<sup>74</sup>.

An efficient way to combat the spread of disease is crop rotation. This is the process of growing different crops, one after the other, on a plot of land. One crop only returns to the plot after a period of several years. In addition, this crop rotation provides for a better soil structure and fertility, and each year the composition of the soil is broken up in terms of both the population of microorganisms and its chemical properties. It was recently shown that cultivating pineapple and banana alternately was more effective in combating the *Fusarium* population in the soil than banana and maize<sup>75</sup>. Farmers in the South, though, are reluctant to engage in crop rotation because the banana plant is a perennial crop with a long cycle and is crucial as a source of food and income.

## Could chemical, biological, or physical pesticides save the banana?

A range of fungicides were tested against Panama disease and black sigatoka<sup>36, 76</sup>. Fungicides against *Fusarium oxysporum* worked well *in vitro* (for example, in a test tube) and in the plant chamber, but not in the field. Even injecting the banana plants with fungicides or dipping the

plants in fungicides did not produce convincing results<sup>77, 78</sup>. Soil decontamination, heat sterilization, or ground application appeared to reduce the fungus contamination but the soil was very quickly infected again by the fungus<sup>29, 79</sup>.

To combat black sigatoka with fungicides, 50 to 70 sprays a year are required, which is not justifiable from an economic and ecological point of view<sup>37, 80</sup>. The costs quickly run up to around 1,800 dollars a year, which is too high for small local farmers<sup>16, 81</sup>. In large plantations, these fungicides are mainly administered with airplanes or helicopters, which require landing spots and fuel. As a result, the cost of combating these diseases can reach 20%–40% of the total production cost of the banana<sup>36, 80</sup>. Repeated use of fungicides also makes the fungus increasingly more tolerant and resistant to these products, leading to the use of increasingly strong fungicides or combinations of fungicides<sup>36</sup>.

Nematodes are primarily combated with chemical soil decontamination products and nematicides<sup>82</sup>, which are nevertheless ineffective and cause all sorts of problems such as soil and groundwater pollution and health issues for humans and animals<sup>83, 84</sup>. Until a few years ago, methyl bromide was frequently used to kill nematode populations but its use is now restricted because of its negative effect on the ozone layer. Because of their high price, nematicides are also only used on commercial banana plantations, where the banana yield can increase by 250%. Nematicides do not destroy nematodes in the soil, which means that the treatment has to be repeated often, increasing the chance of resistance.

To combat viral infections in banana plants as

much as possible, the herbicide 2,4-D is used in combination with an insecticide that eliminates the aphid, the carrier of the virus. These treatments cannot be used by all farmers, however, because of their high cost.

There are currently no pesticides or biological agents to combat the *Xanthomonas* bacterium. Biological pesticides also appear to be somewhat ineffective against other banana diseases<sup>29</sup>. For biological control of a plague, microorganisms or biological materials are added to the soil to combat the attackers. Biological control of the Panama disease still appears impossible at this time. There seem to exist a number of soils that can suppress the spread but it is still unclear which factors are responsible for this effect<sup>85</sup>. It was recently proven that the liquid waste from the production of sisal fibers is very effective in destroying the nematode *Radopholus similis* in the Grand Nain banana plant<sup>86</sup>. Whether this could end up becoming a standard product to fight nematode infections for all banana types remains to be seen. Seaweed or camellia seed extract can also induce a sharp drop in the nematode population in the soil and have a positive impact on the growth of the banana plant<sup>87, 88</sup>.

## Developing a banana resistant to disease and pests through crossbreeding programs: a formidable task

Although bananas and plantain are very important food crops throughout the world, there are currently only seven breeding programs set up to develop better varieties of banana<sup>4, 89</sup>. These programs are located in Brazil, Cameroon,

Ivory Coast, Guadeloupe, Honduras, India, Nigeria, Tanzania, and Uganda. They aim not only to develop plants with increased resistance to disease and pests but also to generate plants with increased yields, resistance to extreme environmental conditions such as drought, and reduce crop losses resulting from early ripening induced by damage during transport or by insects<sup>90</sup>. In most crossbreeding programs, the emphasis lies on developing hybrids (offspring from two different parent types) that show resistance to leaf-spot disease, but also to nematodes and *Fusarium*<sup>91</sup>.

Banana crossbreeding programs have to overcome at least five major obstacles. (1) The bananas most frequently consumed are seedless, sterile, and triploid. As a result, diploid, fertile parents must be used, which mostly have weak agronomic traits and the fruit of which are often not of good quality. (2) Crossbreeding bananas requires a lot of manual labor (see below). (3) Introducing one or more genes often occurs over several generations<sup>92, 93</sup> and, because of the relatively long generation time, the desired hybrid will only be obtained after many years. (4) A banana plant is rather large in size so a great deal of space is needed to properly analyze all hybrids obtained. (5) The genome is still insufficiently well-known to identify all the genes necessary for resistance to a particular pest<sup>4, 89</sup>. Despite this, there have already been a number of successes<sup>4, 85, 89, 94</sup>.

The first banana hybrids were developed in Trinidad, but that program no longer exists. Subsequently, the Fundación Hondureña de Investigación Agrícola (FHIA) in Honduras ([www.fhia.org.hn](http://www.fhia.org.hn)) produced new hybrids. Although the focus initially lay on the dessert

banana, now there is also work done involving plantain and cooking bananas. Because fertile plants are necessary for breeding, the FHIA often uses Highgate (AAA), a mutant of Gros Michel (AAA) as the mother plant. After fertilization, this plant produces a number of fruit with seeds. AAB types such as Dwarf Brazilian/Dwarf Apple are also frequently used. One of the first hybrids, developed in 1988, was FHIA-01 or Goldfinger, a tetraploid dessert banana that shows resistance to leaf-spot disease and *Fusarium* Foc-TR1 and Foc-TR4, as well as increased resistance to the nematode *Radopholus similis*, but not to other nematodes. Initially, this hybrid also appeared to be resistant to black leaf streak but this resistance was quickly broken down by the fungus<sup>4</sup>. In addition, this hybrid also appears to tolerate cold and wind ([www.fhia.org.hn](http://www.fhia.org.hn)). However, the FHIA-01 hybrid has a lower flesh/peel ratio and is slightly less tasty and therefore less well-liked than the Cavendish, Grand Nain, and Williams. As a result, this hybrid is primarily used by small farmers and is not cultivated as an export product. The FHIA-03 hybrid, the Sweetheart cooking banana, is a tetraploid banana that produces no seeds and has a characteristic light-green pseudostem. FHIA-03 shows resistance to *Fusarium* and leaf-spot disease and partial resistance to the *Radopholus similis* nematode ([www.fhia.org.hn](http://www.fhia.org.hn)). This hybrid is commercially cultivated in Cuba on more than 3,500 hectares, without requiring frequent administration of fertilizers and fungicides<sup>85</sup>. This hybrid is also cultivated in Burundi, Cameroon, and Nigeria. The FHIA-17, FHIA-23, and FHIA-25 hybrids were successfully cultivated in the Kagera region of Tanzania after delivering four million shoots. This tripled the income of around half a million people.

IITA (Nigeria) started its breeding program for the banana in the 1980s when leaf-spot disease was identified in the region for the first time. Rapid progress was achieved by crossing fertile plantain types with wild, resistant ancestors<sup>95</sup>. In 1995, IITA started a crossbreeding program in Uganda with the National Agricultural Research Organization (NARO) because the disease had also broken out there, with a special focus on East African Highland bananas. Fertile EAHB types were identified, resistant tetraploid parents were developed, and crossbreeding was carried out to produce resistant triploid hybrids. In 2010, NARO introduced Kabana 6 (Kiwangaazi) to the market. This hybrid has a high yield and shows resistance to leaf-spot disease and partial resistance to nematodes and weevils<sup>89</sup>. The cooperation between NARO and IITA has now generated 27 different NARITA (NARO-IITA) hybrids, of which 25 are now being tested in the field in Uganda and Tanzania<sup>96</sup>. These hybrids all come from a cross between different female triploid fertile EAHB types, different diploids, and the diploid Calcutta 4 banana (AA), which contains very fertile pollen and shows resistance to leaf-spot disease<sup>4</sup>. The selected tetraploids were then crossed with improved diploids to obtain triploid hybrids.

Despite the first successes, the breeding programs still face a number of substantial challenges. For the consumer, it is especially important that the hybrid bananas have the same quality (taste, aroma, absence of seeds) as the current non-resistant bananas. There is also the danger that the pathogens, such as fungus, nematodes, and viruses break down the resistance, especially if resistance is only determined by one gene<sup>97</sup>. Also, a gene sometimes provides resistance to one type of nematode, e.g. *Radopholus*

*similis*, but not necessarily to other nematodes, such as *Pratylenchus* spp.<sup>89, 98</sup>. This implies that several resistance genes have to be introduced into the hybrid, making the crossbreeding program longer and more complex. Finally, there is to date no known banana that shows resistance to the *Xanthomonas* bacterium, or to the Banana Bunchy Top Virus (BBTV).

It is especially important for the crossbreeding programs that the genomes of the different types of banana continue to be studied in detail, to gain better knowledge of the genes and their associated traits. This genomic knowledge can then lead to faster development of breeding procedures, which is called superdomestication<sup>99</sup>. With superdomestication there is close interaction between growers and scientists with genome knowledge, who work together to draw up the profile of the hybrid with all the desired traits on paper, to subsequently determine how the new hybrid is to be developed and which specific genes are necessary to achieve the desired result<sup>90</sup>.

### Banana breeding in practice: easier said than done

Although most types of banana cultivated and consumed today are triploid, sterile, and seedless, there are still a number of types that continue to have a small degree of fertility and can form seed if pollinated with highly viable pollen. Here, the grower plays the role of natural pollinator.

The grower picks the male flowers in the morning and brings them to the female flowers of the plant to be fertilized. Every day, the flowers open

consecutively, meaning that the bunch of flowers have to be pollinated manually every day for around a week. Most pollinated female flowers will produce no seed, some a couple of seeds, and a limited amount will produce many seeds. The big problem is that these seeds mostly do not germinate by themselves, and therefore need help from the growers. For this reason, the growers collect embryos from the sterilized seeds and let these germinate in test tubes with a solid medium that contains all the necessary nutrients. Afterwards, these plants are

transplanted into the soil and are allowed to continue growing and harden off, so that finally they can be planted in the field. In most cases, the triploid offspring are evaluated as potential new varieties and the diploid (two sets of chromosomes) and tetraploid (four sets of chromosomes) plants as new candidate parents for subsequent crossbreeding. Carrying out such crossbreeding in banana plants is clearly a very complex technique, which requires skills and suitable equipment that smallholders do not have at their disposal.

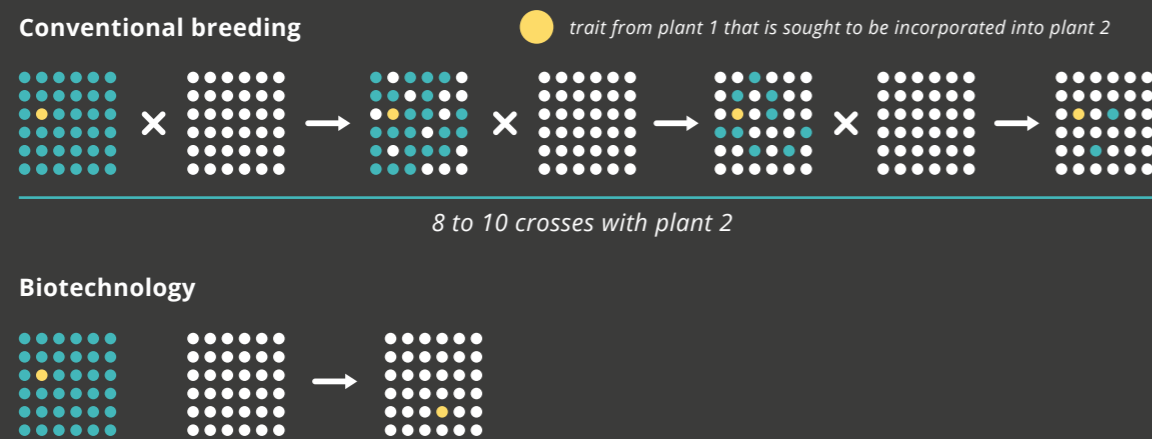


Figure 4.2: Manual pollination of banana plants (Source: M. Batte)

## BREEDING VERSUS GENETICALLY MODIFIED PLANTS<sup>100</sup>

Since the origins of agriculture, people have sought to produce plants that are better and stronger, and have commercial appeal. These new and improved plants are obtained by continuous selection every harvest time. By crossbreeding two parent plants differing in traits, their traits are combined into one plant. Subsequently, the offspring that contains the advantageous combination of the traits of the two parent plants is used. Originally, this crossbreeding and selection occurred with no knowledge of what was happening at the level of the genetic material, or DNA. Now we know that when plants are crossbred, large sections of DNA are exchanged, by way of which half the DNA of parent plant 1 is united with half the DNA of parent plant 2 (see Figure 4.3). This produces offspring each with a unique combination of the desirable and undesirable traits of the parent plants. For centuries, this was how farmers eliminated the less beneficial traits. In some breeding programs only one desirable trait from parent 1 is sought to be transferred to parent plant 2. To achieve this, several consecutive crosses are necessary, where the offspring with the desirable trait is repeatedly crossbred with the original (commercially appealing) parent plant 2. This process is called backcrossing. Backcrossing is repeated several times to obtain a new plant with as many traits as possible from the original beneficial parent plant 2, but with the new trait from parent plant 1. Depending on the duration of the life cycle (from seed to seed) and the plant's reproduction method, this process can take from several years to several decades. For crops that primarily propagate by means of vegetative reproduction, such as potatoes, sweet potatoes, bananas, and cassava, 7 generations are needed; for self-pollinating crops such as rice and wheat, 10 generations; and for cross-pollinating crops, such as maize, it can be as many as 17 generations<sup>93</sup>. With this kind of breeding, traits from only one type of plant or closely related plants can be combined (e.g., banana with banana but not with tomato or rice).

Figure 4.3: Conventional breeding versus biotechnology



Gene technology and genetic modification of plants have the same goal as conventional breeding: to create new varieties with traits that are beneficial for the farmer, industry, or consumer. The great advantage of gene technology is that one or more desirable traits can be incorporated into a plant in a targeted way. The genetically modified (GM) plant will, as a result, be genetically identical to the beneficial parent plant 2 (see Figure 4.3), except for the trait that was added. As a result, the new beneficial plant is obtained much more rapidly than with conventional breeding. Another major advantage of gene technology is that the newly added DNA does not need to come from the same type of plant, but can also be from other types of plant, and even from yeasts, animals, and fungi. In other words, the barriers of species no longer exist.

## GENETIC MODIFICATION OF PLANTS THROUGH THE GENE GUN OR AGROBACTERIUM TUMEFACIENS<sup>101</sup>

Plants can be genetically modified in a number of ways, but the methods most often used to date are the gene gun or *Agrobacterium tumefaciens*. Incorporating DNA into the plant using the gene gun is the mechanical method, which is also called particle bombardment. In this process, minuscule particles of gold are coated with the DNA that will be inserted into the plant. These gold particles are then "shot" under high pressure into the plant tissue and, in some cases, the DNA penetrates the nucleus of the plant cell, where it is spontaneously incorporated into the plant's DNA. However, this method is not particularly efficient and often many copies and/or only parts of the desired DNA are incorporated into the plant DNA. A much more efficient method of transformation makes use of the soil bacterium *Agrobacterium tumefaciens* natural ability to transfer DNA.

Figure 4.4: Crossing design for triploid hybrid bananas

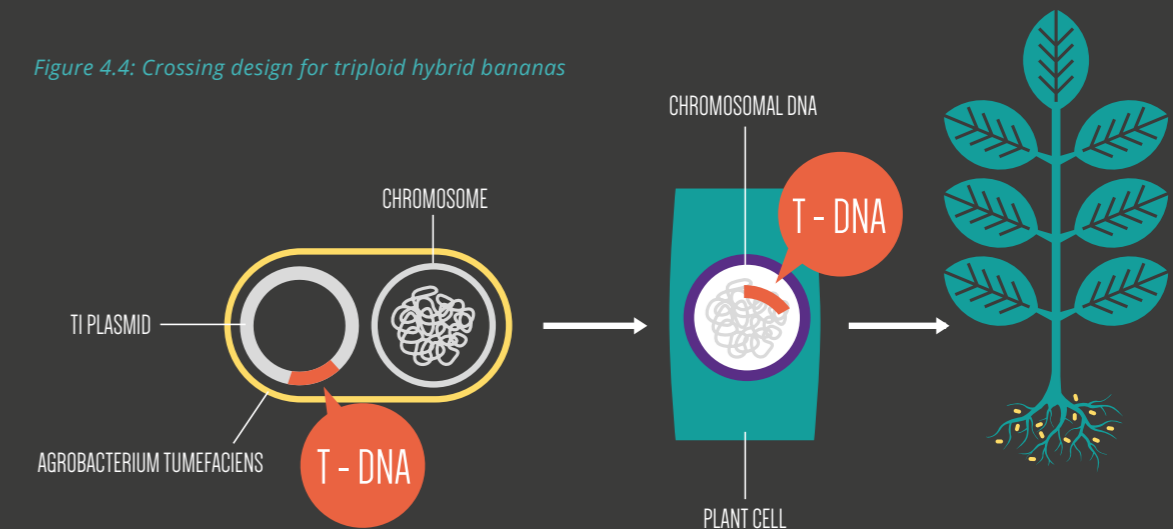


Diagram of *agrobacterium tumefaciens*'s gene insertion mechanism in addition to its chromosomal DNA, the bacterium also has a ti plasmid (represented by the white circle). The genetic information contained in the T-DNA (red segment) is transferred to a plant cell by *agrobacterium* where it is then inserted into the plant's DNA. An adult plant is then formed from this modified plant cell. This plant has an extra piece of genetic information but is otherwise identical to the original plant.

In nature, these bacteria infect certain plant cells and, during the infection process, transfer a piece of their own DNA, the transfer or T-DNA, to the nucleus of the plant cell in which plant DNA is present. This T-DNA is thereby incorporated into the plant DNA. Because this T-DNA carries the information for making opines, molecules on which *Agrobacterium* feeds, the bacterium forces the plant to produce its food. In the 1970s, molecular biologists, including Ghent University professors Marc Van Montagu and Jeff Schell unraveled the DNA transfer mechanisms. They discovered that the genes that lie on the T-DNA are not essential for the transfer of the bacterial DNA to the plant cell. They could replace this DNA with the desired DNA and the transfer to the plant cell continued to work. In this way, the plant no longer made opines for the bacterium but the bacterium transferred information to the plant that was beneficial to humans.

Recently, it was discovered that all 291 cultivated sweetpotato varieties tested contained one or more T-DNA genes, which implies that this transfer of T-DNA genes took place a long time ago, during the process of evolution<sup>102</sup>. In addition to the desired DNA, the DNA of a selection gene can be incorporated during genetic transformation. Genetic transformation of cells does not work with 100% efficiency, however, and the plants that receive the new genetic information must be traced. The selection gene ensures that the cells that have absorbed the new DNA offer resistance to a certain herbicide or antibiotic, as a result of which all plants can simply be grown in the presence of that particular herbicide or antibiotic after genetic modification. Only the plants that survive have the new DNA and the remainder die off. This selection occurs during the development phase in the laboratory, and the antibiotics are not used in the field.

In both methods, the gene gun and transformation with *Agrobacterium tumefaciens*, use the unique feature that individual plant cells are able to grow into a completely new plant. This means that one GM plant cell can be used to produce an adult plant, each cell of which contains the new DNA

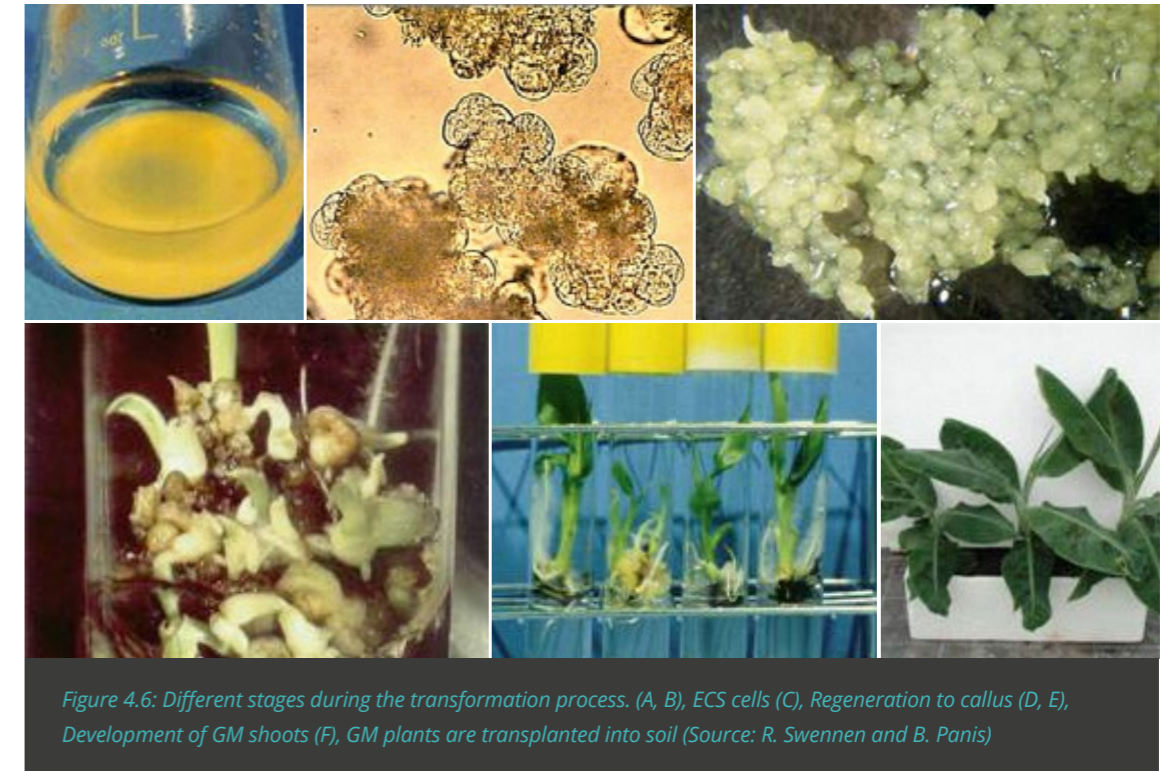


Figure 4.6: Different stages during the transformation process. (A, B), ECS cells (C), Regeneration to callus (D, E), Development of GM shoots (F), GM plants are transplanted into soil (Source: R. Swennen and B. Panis)

## Genetic transformation of bananas in practice

For 20 years, it has been possible to genetically modify bananas by incorporating foreign genes, either with the help of the gene gun<sup>103, 104</sup>, or using *Agrobacterium tumefaciens* (see box)<sup>105-109</sup>. Although the transformation process can vary slightly for each type of banana, it is still structured according to the same principle and occurs in a completely sterile environment. In this transformation method, it is very important to isolate the transformation-competent tissue, namely the cells that, on one hand, are able to take in and incorporate foreign DNA into their own DNA and, on the other hand, have the ability to grow into an adult plant. For this reason,

embryogenic cell suspension (ECS) cells are used for banana transformation<sup>103, 109, 110</sup>.

The first and also the trickiest step in the transformation process is the development and isolation of these ECS cells, which can be obtained from meristems, embryos, or unripe female flowers<sup>80, 111</sup>. Making a suspension can take up to 18 months. These ECS cells are incubated for six to seven days in an *Agrobacterium* suspension, after which they are washed and the bacteria are killed. The cells are then placed on a medium with an antibiotic and are given 2 to 3 months to grow into a GM plant. The ECS cells that have not taken in the new DNA will die off early. The GM plants are subsequently transplanted into soil where they can grow into adult plants<sup>112</sup>. Since the ensuing GM plant has developed from one

transformed cell, all cells in that GM banana plant have the same new DNA in the same place<sup>106</sup>. The entire procedure takes around 6–8 months and is easily reproducible. On average, 20 to 70 different GM banana plants can be generated per ml of ECS cells, depending on the type and the quality of the ECS cells<sup>80, 109, 112–114</sup>.

The GM bananas developed to date show resistance to fungi, viruses, nematodes, or bacteria (see below). This resistance is first always demonstrated *in vitro* and/or in a plant growth chamber and only afterwards in the field. The reasons for this are that (1) screening *in vitro* is quicker, (2) the optimal growth conditions for bananas

can be simulated *in vitro* and in growth chambers (high moisture and high temperature), and (3) this saves space as banana plants can grow to heights of four meters and develop a very large leaf surface area. Given that after genetic transformation a large number of plants have to be analyzed, this analysis cannot be carried out on all full-grown plants<sup>16</sup>. The biotechnological research on bananas originated in Belgium but is now primarily located in Australia, Belgium, Brazil, China, India, Kenya, Malaysia, Nigeria, South Africa, Uganda, the United Kingdom, and the United States<sup>4</sup>.

## RNA INTERFERENCE (RNAI) AS A MEANS OF SILENCING GENES<sup>101</sup>

A widening body of knowledge about the genetic material of organisms lies at the basis of new methods for giving desirable traits to plants. RNA interference (RNAi) can be used to silence genes in a very specific manner. In this process, two types of genetic material, DNA and RNA, are of great importance. Apart from some viruses, DNA can be regarded as the universal carrier of genetic information. In higher organisms (plants and animals), DNA is made up of two strands and each cell of a specific organism contains the same DNA, although each cell does not need the same information. For example, a root cell needs to make different proteins than a leaf cell. Two steps are necessary in order to arrive at the production of protein from specific genetic information. First, the piece of DNA (gene) necessary for the production of a protein is copied into RNA (transcription). This RNA is exported from the nucleus to the cytoplasm of the cell where it is then translated to the appropriate protein. If the RNA is broken down before it can be translated, the message contained in the DNA will never lead to the production of protein. This phenomenon is called gene silencing: literally, silencing the gene function by suppressing the appropriate RNA.

The plant's defense mechanism is based on the presence of double-stranded RNA. In the plant, the messenger RNA (mRNA) is made up of one strand. When viral RNA replicates in the plant cell, double-stranded RNA molecules are present. The plant cell recognizes these as not being its own and, in defense, breaks down the double-stranded RNAs. This process activates a mechanism that breaks down all similar RNA molecules, even if they are single-stranded. These small pieces of broken-down RNA then come into contact with the mRNA of the target gene, recognize it, and attach to this mRNA. This is a signal for the whole to be broken down, which means that the protein for which the target gene codes is no longer produced. RNAi can thus be used to suppress the transcription and/or translation of certain pieces of genetic material. RNAi also appears to work very efficiently in bananas<sup>115</sup>.

## Which GM bananas offer good prospects for the future?

### GM bananas resistant to *Fusarium oxysporum*

**f. sp. cubense:** The fungus *Fusarium oxysporum* poses a serious threat to worldwide production and trade of many types of banana. For this reason, scientists are trying to develop banana plants that show resistance to this fungus by means of genetic modification. One way to develop GM bananas resistant to Foc-TR1 and/or Foc-TR4 consists of transferring resistance genes from “wild” bananas to the desired banana variety. An extended study of eight different wild banana plants demonstrated that resistance variation exists<sup>116</sup>. *Musa balbisiana* shows the most symptoms, while *Musa basjoo* and *Musa itinerans* are fully resistant to the fungus. However, only the genomes of *Musa balbisiana* and *Musa acuminata* have been sequenced (see Chapter 1). Because *Musa acuminata* also shows some resistance to Foc-TR4, this ancestor must contain at least one gene that is responsible for this resistance. Researchers have succeeded in identifying this gene or these genes and transferring it to the genome of the Cavendish banana with the help of genetic transformation<sup>17</sup>. After the first analysis in the plant growth chamber, a number of GM plants were grown on soils contaminated with *Fusarium oxysporum* in Australia for an 18-month field test<sup>17</sup>. The results of this field test have not yet been published in papers.

A second way of obtaining resistance against *Fusarium* is to introduce genes from other organisms into the banana genome. These are primarily genes that code for proteins or RNA with a negative effect on the metabolism of the

fungus<sup>27, 117–122</sup>. They cause the production of pores in the fungal membrane, disrupt the membrane, or silence important enzymes in the fungus, resulting in its death. When the fungus infects the banana plant, it takes in nutrients from the plant cell for a short time but if it also takes in toxic proteins, the metabolism ceases, the fungus dies, and the infection stops. This was the idea behind the development of GM bananas that produce small RNAi molecules (see box) against two different vital genes of the fungus, velvet and transcription factor 1<sup>27</sup>. In addition, it is hoped that genes that work against the *Fusarium oxysporum* fungus also provide resistance to other fungi such as *Mycosphaerella fijiensis*, which causes black sigatoka<sup>119</sup>. The ultimate solution could even be to incorporate several genes into one type of banana that can provide resistance to several pathogens, because they are often all present together in the field anyway<sup>123, 124</sup>. Until now, most resistance experiments against *Fusarium* have only been tested *in vitro* and in the plant growth chamber. There are still no results available from the Race 1 field tests<sup>117–119</sup> and field tests for Race 4 tolerance have not yet been conducted<sup>120–122</sup>.

**GM bananas resistant to black sigatoka:** Gene technology is also being used to try to develop banana plants resistant to the highly destructive leaf-spot disease. In 2013, genes from rice, *rcc2*, and *rcg3*, which are responsible for producing chitinase<sup>125</sup>, were expressed in the Gros Michel banana<sup>16</sup>. Chitinases are enzymes that degrade chitin, an important component of the cell walls of fungus. When the GM Gros Michel produces rice chitinase, it is absorbed by the fungus upon infection and the cell wall of the fungus is broken down. As a result, the fungal infection is eliminated and this GM Gros Michel is resistant to

black sigatoka<sup>16</sup>. A total of 17 GM Gros Michel banana plants were developed, 2 rcc2 lines and 15 rcg3 lines, which were tested *in vitro* for their resistance to *Mycosphaerella fijiensis*. Of these GM bananas, 9 did not show any disease patterns 39 days after infection, and 108 days after infection (end of the study) the areas of necrosis were limited to 16% compared to the non-transformed plant<sup>16</sup>.

**GM bananas resistant to BXW:** Given the rapid spread of BXW throughout Africa and the lack of resistance genes in bananas, genetic transformation is the only way to offer resistance to these bacteria. To combat BXW, the Banana Bacterial Wilt project was set up with the aim of developing BXW-resistant bananas for local farmers. The African Agricultural Technology Foundation (AATF), IITA, and NARO are all involved in this project. In an initial study, two resistance genes from sweet pepper were used: the *Hrap* gene, which produces a protein that protects plants from bacteria, and the *Pflp* gene, a ferredoxin-like protein<sup>126, 127</sup>. Both genes had already shown their effectiveness against other bacteria such as *Erwinia carotovora* ssp. *carotovora* and *Pseudomonas syringae* in transgenic *Arabidopsis thaliana* and tobacco plants<sup>128, 129</sup>. The coded proteins are also not on the list of allergens and can therefore be cleared for human consumption. In any case, these proteins occur naturally in a number of other plants such as rice, tomatoes, and peppers, which are eaten raw. GM plants with the *Hrap* or *Pflp* gene are generated for two banana varieties, Sukali ndiizi (AAB) and Nakinyika (AAA). A total of 40 GM *Hrap* and 25 GM *Pflp* plants showed resistance to the bacteria in the plant growth chamber and were therefore tested during a field test in Uganda<sup>126</sup>.

In the field, the GM banana plants did not differ morphologically from their non-GM equivalents and their growth and fruit development were normal. Before blossoming, the researchers infected all plants with *Xanthomonas*. While all non-GM banana plants showed symptoms and finally wilted, 20% of the *Hrap* GM plants and 16% of the *Pflp* GM plants appeared to show no symptoms of disease in the field<sup>126</sup>. It was also very important that a resistant mother plant was able to pass on the resistance to the daughter plants<sup>126</sup>. There are plans to develop banana plants with both resistance genes, *Hrap* and *Pflp*, in the future, which will make it even more difficult for the bacteria to infect the banana plant. In a second study, the researchers introduced the *XA21* rice gene into the Gonja manjaya (AAB) banana variety<sup>67</sup>. This *XA21* gene makes rice resistant to infection by *Xanthomonas oryzae* and also appears to work against bacterial infections in GM citrus fruits and GM tomatoes<sup>130, 131</sup>. *In vitro*, 12/25 GM bananas showed resistance to *Xanthomonas campestris* cv. *musacearum*. These resistant plants were then transferred to the plant growth chamber, where their resistance was tested again on 90-day-old plants. The resistance of the GM plants varied, but 58% of plants were fully resistant. The *XA21* expression in bananas had no effect on the measurable characteristics of the plant. These resistant GM plants will now be further analyzed in a field test for resistance to *Xanthomonas campestris*, and other important properties, such as fruit quality, will be studied<sup>67</sup>.

**GM bananas resistant to nematodes:** A possible approach for developing banana plants resistant to nematodes is to block the digestive process of the worms during infection of the plant. Cysteine proteinases are important digestive enzymes

in nematodes. There are proteinase inhibitors in plants, cystatins, which block these cysteine proteinases, killing the nematodes. Resistant tomatoes<sup>132</sup>, *Arabidopsis*<sup>133, 134</sup>, rice<sup>135</sup>, and potatoes<sup>136</sup> were generated in this way. In a second approach, nematode invasion in the banana plant is acted upon by expressing two synthetic peptides (a molecule with a limited number of amino acids) in the plant's roots<sup>137, 138</sup>. This combats both the intrusion of the nematode through the root and the development of the nematode population in the root. In GM plantain of the Gonja manjaya variety, maize cystatin, the synthetic peptide, or both genes were expressed<sup>20, 114</sup>. These plants appeared to show resistance in the greenhouse to the nematodes *Radopholus similis* or *Helicotylenchus multicinctus* and even to a combination of both<sup>114</sup>. Afterwards, this resistance was researched in more detail in 12 GM plants during a field test in Uganda<sup>20</sup>. A clear drop in the quantity of nematodes was identified in all the GM plants, of which 8 GM plants were significantly resistant during vegetative development, 10 at the time of flowering, and all 12 at the time of harvest. In addition, there was a clear increase in fruit yield, which was as high as 186% in comparison with the control plant. The plants with the highest yield increase will now also be tested for resistance to other nematodes, especially *Meloidogyne* spp. and *Pratylenchus* spp.<sup>20</sup>.

**GM bananas resistant to weevils:** There is a great deal of controversy regarding the impact of weevils on banana cultivation, as they are considered by some to be an important or serious threat while others rather consider it local, limited, or unimportant<sup>61</sup>. This is why research into resistance to weevils remains rather limited. In the literature on natural resistance

to weevils in bananas, the results are often contradictory<sup>61</sup>. With few exceptions, the plantain (AAB) and Highland banana (AAA-EA) appear more susceptible to weevils than any other genome groups<sup>63</sup>. Of the two ancestors, the *Musa acuminata* (AA) appears more susceptible than the *Musa balbisiana* (BB)<sup>139</sup>. Finally, diploids appear more resistant than polyploids<sup>140</sup>. At present, the main organizations trying to obtain weevil-resistant bananas through breeding<sup>63</sup> are NARO, IITA, and the African Centre for Banana and Plantain (CARBAP) in Cameroon. The AA cultivars Calcutta 4 and Pisang Lilin are predominantly used for this<sup>140</sup>. There is also a need for a simple and quick screening method to test resistance against weevils in the laboratory, growth chambers, and the field. Finally, assistance from biotechnology is needed in order to develop GM bananas resistant to weevils<sup>63</sup>, especially by acting on the weevils' digestive system and more specifically by expressing a combination of serine and cysteine proteinases<sup>63</sup>. This research is the focus of collaboration between NARO, IITA, and the Forestry and Agricultural Biotechnology Institute (FABI) from the University of Pretoria, South Africa.



## 5 Bananas with extra vitamin A

In developing countries, and primarily in Sub-Saharan Africa and southeastern India, many people suffer from a lack of vitamin A. This lack of vitamin A primarily affects children under five and pregnant women and leads to blindness, stunted growth and skeletal development, and even death. More than 80% of vitamin A intake by the population of developing countries occurs via plants, in the form of provitamin A carotenoids (pVAC), which are then converted in the human body to retinol and finally to vitamin A<sup>141</sup>.

Banana nursery in Costa Rica (Source: R. Swennen)

Bananas, the green gold of the South

Bananas are also an important source of pVAC, but quantities vary considerably from type to type. A detailed analysis of 171 types of banana showed that the quantity of pVAC can fluctuate from 0 to 3500  $\mu\text{g}/100\text{ g}$  wet weight<sup>142</sup>, which implies that eating one banana of certain types per day is already sufficient to cover 50% of the daily recommended intake of pVAC. The types of banana eaten in East Africa such as the Highland banana and plantain, however, contain quantities of pVAC that vary from 3.89 to 18.75  $\mu\text{g}/\text{g}$  wet weight<sup>143</sup>. There is a correlation between the color of the flesh and the quantity of pVAC: bananas with a white or cream-colored flesh such as the Cavendish banana produce very little pVAC, while bananas with dark orange flesh, such as the Karat and the Asupina banana, have very high levels of pVAC<sup>1, 144</sup> ([www.promusa.org](http://www.promusa.org)). Furthermore, the AA diploids and the AAB and ABB triploids appear to have higher levels of pVAC than the AAA bananas, but still no clear correlation can be shown between genotypes and quantities of pVAC<sup>21, 145, 146</sup>. It does, however, appear that the levels of pVAC in plantain are higher than in cooking and dessert bananas. Finally, the pVAC content varies between ripe and unripe fruit, depending on the type of banana<sup>146</sup>.

Processing bananas in food also appears to have a negative effect on the quantity of pVAC. Various processes such as exposure to light and air, damage during storage and transport, freezing, baking, and frying cause a drop in pVAC levels<sup>146</sup> ([www.promusa.org](http://www.promusa.org)), with the result that more fruit needs to be consumed per day to meet the recommended daily intake of vitamin A.

Bananas with more pVAC can be obtained in two ways: (1) classical breeding and (2) genetic modification. HarvestPlus (<http://www.ifpri.org/program/harvestplus>) funds the research that aims to obtain, through breeding, bananas with high levels

of pVAC for the population of Sub-Saharan Africa. This primarily involves crossbreeding bananas that produce high quantities of pVAC, show a high yield, and are resistant to disease. Because most (edible) bananas are triploid and sterile, however, this process is not simple.

Since 2007, the Bill and Melinda Gates Foundation has supported a project in which researchers from Australia (Queensland University of Technology) and Uganda (NARO) are attempting to develop GM bananas with increased pVAC and iron content. This involves the *PSY2a* gene from the Asupina banana, a banana with high pVAC content, being incorporated into other types of banana. The *PSY2a* gene codes for phytoene synthase, a protein that is important in the production of  $\beta$ -carotene or provitamin A. This gene appears in all types of banana but the protein produced has different enzymatic activity in the different types, as a result of which some bananas, such as the Asupina banana, contain more pVAC than others (such as the Cavendish banana)<sup>144</sup>. The effect of the additional *PSY2a* gene is first being tested in the Cavendish types Williams and Dwarf Cavendish, and in the Pome type Lady Finger, but the final goal is to transfer the technology to the Highland banana and a disease-resistant hybrid Kabana 6H, obtained from crossbreeding<sup>147</sup>. Field tests have already been carried out in Australia (since 2008) and Uganda (since 2010) with the GM Cavendish bananas, and a number of GM plants with increased pVAC were identified<sup>147</sup>.



## 6 Conclusion

Bananas are vital for millions of people in the southern hemisphere. Not only are banana harvests sold on local markets, making them a source of income, but they are also a major food staple. Bananas are also the number one domesticated crop in the world but, both large and small growers make little use of the wide variation in varieties. Banana cultivation, however, is under threat from a large number of diseases and pests, which can be controlled to a certain extent by sound agronomy or through the development of new varieties by means of breeding. For some threats, only GM bananas offer a sustainable and reliable solution. In the case of bananas, gene technology is perceived by many to be justified, despite the sometimes strong opposition by certain NGOs. Popular types of banana can often not be improved in any other way, owing to the fact that resistance genes to specific diseases are not present in bananas. As a result, resistance genes must be sought from other types of plants or even other organisms. In addition, bananas obtained through breeding rarely meet consumers' expectations in terms of color, taste, or other important characteristics. A major argument from those opposed to GM technology, namely that the transgenes can spread uncontrollably throughout nature, does not apply to bananas. Given that most banana varieties are triploid, seedless, and sterile, the new genes, once incorporated into the banana genome, can no longer be propagated through the pollen and therefore cannot find their way into nature in an uncontrolled manner. If GM bananas are not embraced, the banana industry and local food production in Africa risks coming to a complete standstill. A similar situation occurred with the Hawaiian papaya industry in the 1990s. Infection of the papaya with the papaya ringspot virus threatened the entire production of this fruit. The public sector developed a virus-resistant GM papaya. The seeds were brought onto the market in 1998, and in 2000 papaya production in Hawaii was restored to the level prior to the outbreak of the virus<sup>101</sup>. All GM bananas are currently triploid. In the future, it would also be useful to develop diploid GM bananas—i.e., with two sets of chromosomes. Diploids can be crossbred much more easily, meaning that GM technology could support the further development of new varieties through breeding.

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The Laboratory of Tropical Crop Improvement forms part of the Faculty of Bioscience Engineering at **KU Leuven**. It is led by Prof. Dr. Rony Swennen and has around 40 scientists. Research focuses on the production of tropical crops, primarily bananas, with improved properties by combining techniques from conventional breeding, molecular plant biotechnology, genome analysis, and phenotyping. The ultimate goal is to improve the quality of life of smallholders through sustainable agriculture. Furthermore, the laboratory actively contributes to the protection of biodiversity, manages the world's largest banana collection (both in vitro and deep-frozen) and is the base of Biodiversity International in Belgium.



The National Agricultural Research Organization, **NARO**, has more than 25 years experience in generating and promoting technologies for improving banana productivity in East and Central Africa where producers are facing several constraints including pests and diseases. NARO aims at producing high yielding, pest and disease resistant banana varieties that are acceptable to end-users and developing agronomic practices that improve productivity of the crop. Subsequently, NARO has applied both conventional breeding and genetic engineering in improving East African highland bananas for higher yields, pro-vitamin A, banana bacterial wilt, nematode and weevil resistance. NARO in collaboration with IITA has led to the development of 27 EAHB hybrids. Two of these hybrids were formally released as new cultivars in 2010 by NARO and are already being grown by 15% of banana farms in Uganda.



**IITA** (International Institute of Tropical Agriculture) is one of the world's leading research partners in finding solutions for hunger, malnutrition and poverty. Their award-winning research for development (R4D) approach addresses the development needs of tropical countries, especially sub-Saharan Africa. IITA works with partners to enhance crop quality and productivity, reduce producer and consumer risks, and generate sustainable wealth from agriculture. The non-profit organization was founded in 1967, is governed by a Board of Trustees, and supported by several countries. IITA is a member of CGIAR – a global agriculture research partnership for a food secure future



The laboratory of Plant genetics, breeding and biotechnologies is hosted at the faculty of sciences of the Kisangani University (**UNIKIS**) in the Democratic Republic of Congo (DR Congo). The laboratory was started and is still directed by Professor Benoit Dhed'a Djailo. Located in the center of the Congo basin, the objectives of the lab are the collection, the characterization, preservation and the fast propagation of healthy plants as well as the selection of better plantain bananas of the DR Congo. Since 2005, more than 100 different cultivars of plantain bananas have been collected through the country and preserved in a field collection and 97 of these cultivars have been morphologically characterized. Thus, DR Congo is central for the plantain diversity. Work is now being oriented towards the in vitro conservation, the agronomic, nutritional and molecular characterization as well as towards the post-harvest aspects.



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