Bananas: disease resistance through genetic modification

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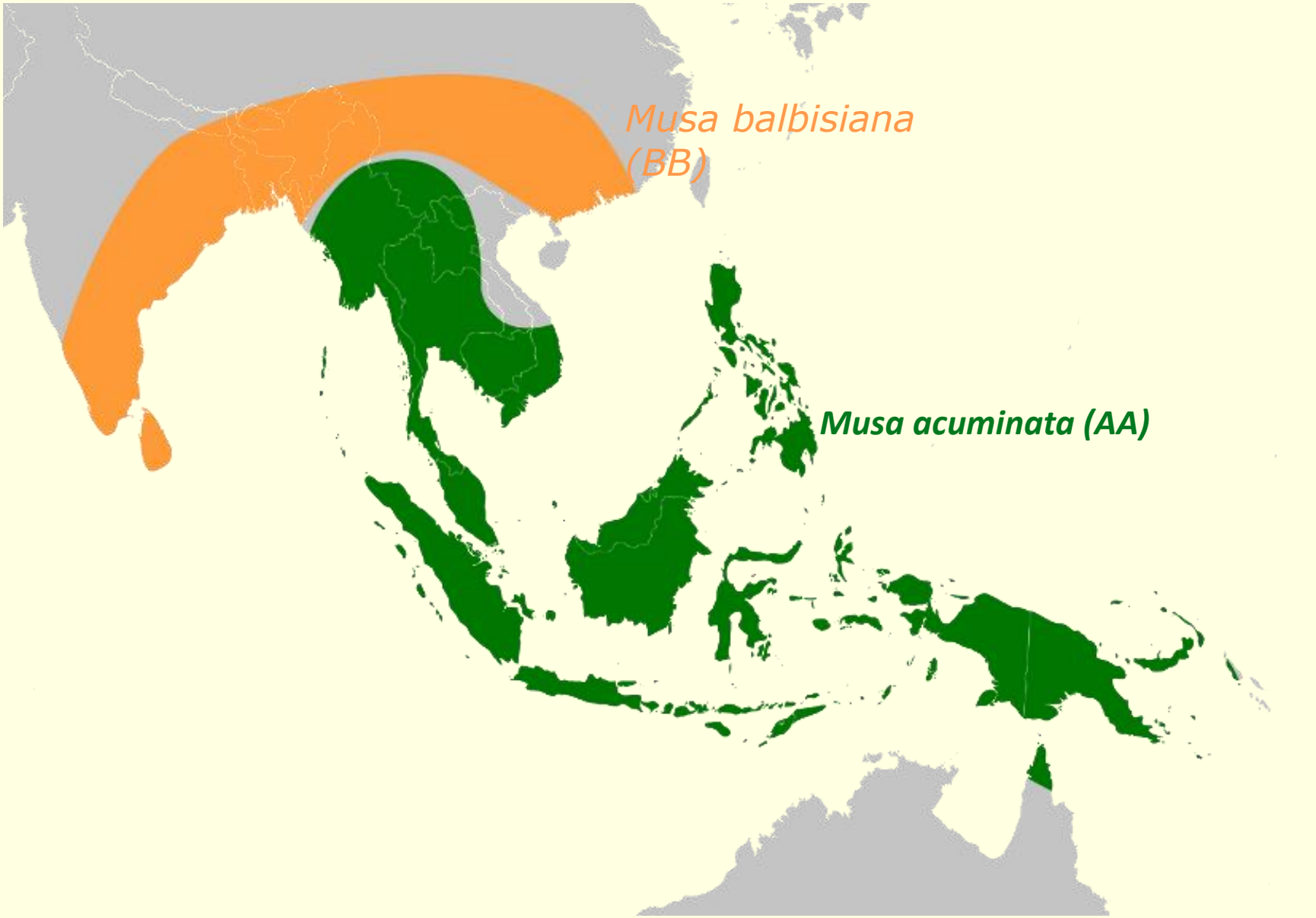
The Top 10 Food Crops

Ranking	Crop	Production mt	Commercial production of GM
1	Corn/maize	823	+++
2	Wheat	690	-
3	Rice	685	-
4	Potatoes	314	-
5	Cassava	232	-
6	Soybeans	230	+++
7	Banana/plantain	122	-
8	Sweet potato	110	(+)
9	Sorghum	65	-
10	Yams	51	-

The Origin of Bananas




Finger bananas
Cavendish
Plantains

Bananas

- Most cultivated bananas are **triploids** derived from two wild progenitors: *Musa acuminata* and *M. balbisiana*
- Non-graminaceous monocots
- Large, herbaceous (non-woody)
  - pseudostem of leaf sheaths
- Cultivated bananas are parthenocarpic, low fertility
- Perennial growth
- Naturally propagated by suckers
- Fruit year round in tropics

Bananas

- In top 10 world crops
- World's largest fruit crop in terms of both volume and value
- a dessert crop in the west
- a key dietary component in nearly all countries in the wet tropics
- ~100 million tonnes annual production
  - 1/3 each from Africa, Asia-Pacific, Americas
  - 85% consumed/traded locally
  - 15% for export trade
- Consumption highest in Africa's great lakes region and Papua New Guinea
  - Up to 1kg/person/day

The problem for bananas

- The vast majority of domesticated bananas are **selections** from the wild, not bred: >300 different varieties and landraces

- Domesticated bananas are essentially sterile

As a result:

- Unlike nearly all other major crops, bananas have not been improved for thousands of years

- Because they are sterile they are exceptionally difficult to breed conventionally

- They have major deficiencies: diseases and nutritional value

Annual Banana Production

Million metric tonnes

India	**26.2**
Uganda	**10.5**
Philippines	9.0
China	**8.2**
Ecuador	7.6
Brazil	**7.2**
Indonesia	**6.3**
Mexico	**2.2**
Costa Rica	2.1
Colombia	2.0
Thailand	**1.5**

Countries in bold non-export bananas

Bananas (matooke) in Uganda: An integral part of the culture

The major banana diseases

- Black & Yellow Sigatoka
- Fusarium wilt race 1 & tropical race 4
- Banana bunchy top
- Bacterial wilt & Moko
- Nematodes

The major banana diseases

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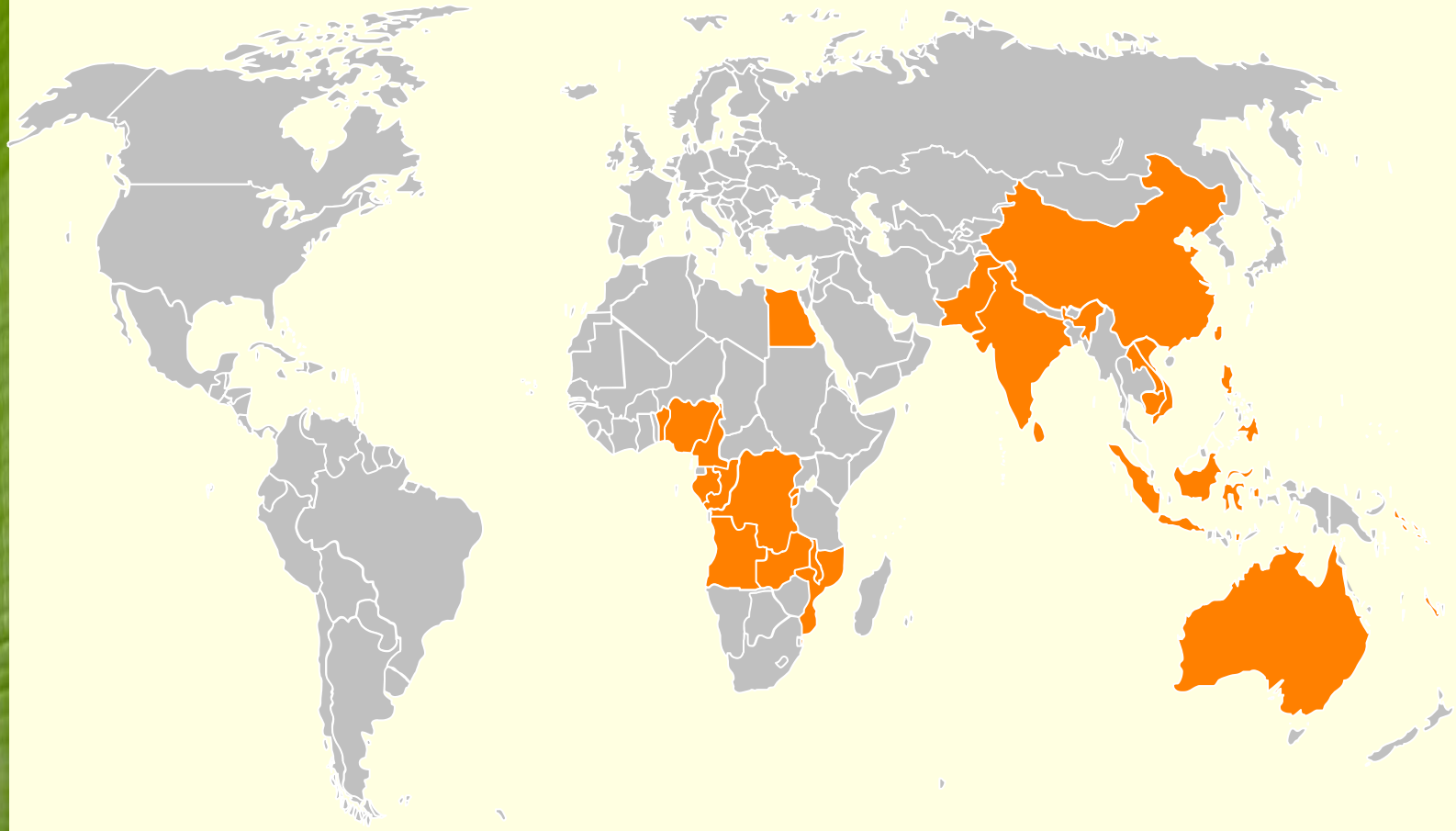
Banana bunchy top virus

Banana bunchy top virus

BBTV worldwide distribution



Bunchy top disease movement

- Infected suckers
- Infected tissue culture plantlets
- Aphids

Progression of Banana Bunchy Top Virus in Africa

- **1901 - Egypt**

Progression of Banana Bunchy Top Virus in Africa

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- **1950s – D.R. Congo**

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  - **Central African Republic**
  - **Equatorial Guinea**
  - **Gabon**
  - **Rep. Congo**
  - **Rwanda**

Progression of Banana Bunchy Top Virus in Africa

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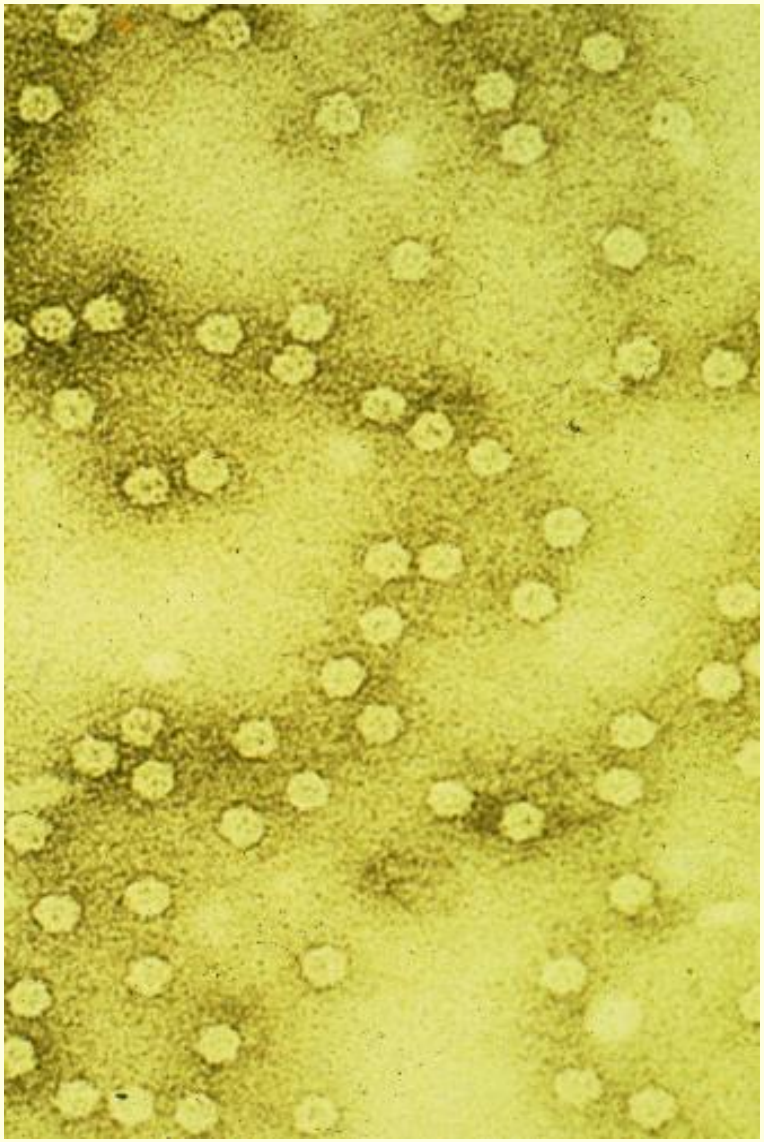
- **2012 – Benin and Nigeria**

Banana bunchy top virus in Malawi

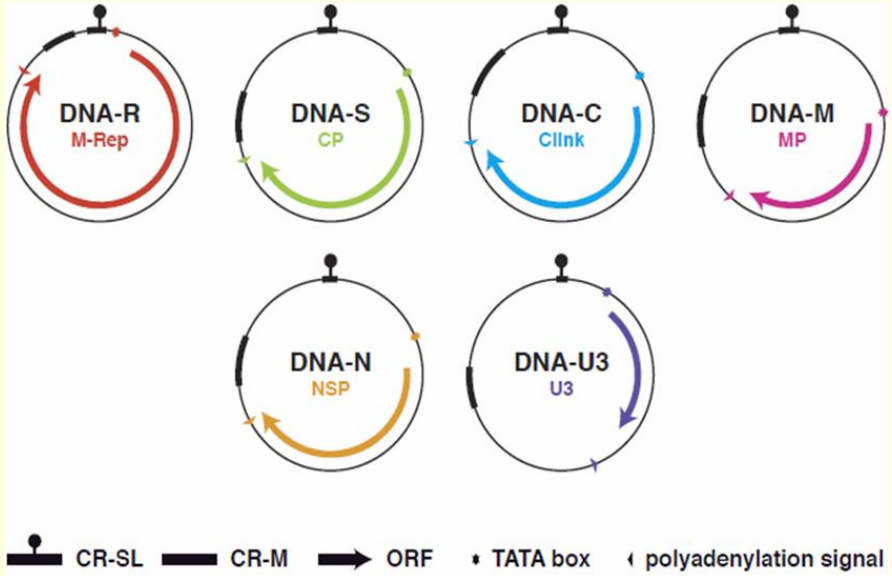


BBTV virions:
18nm isometric
particles

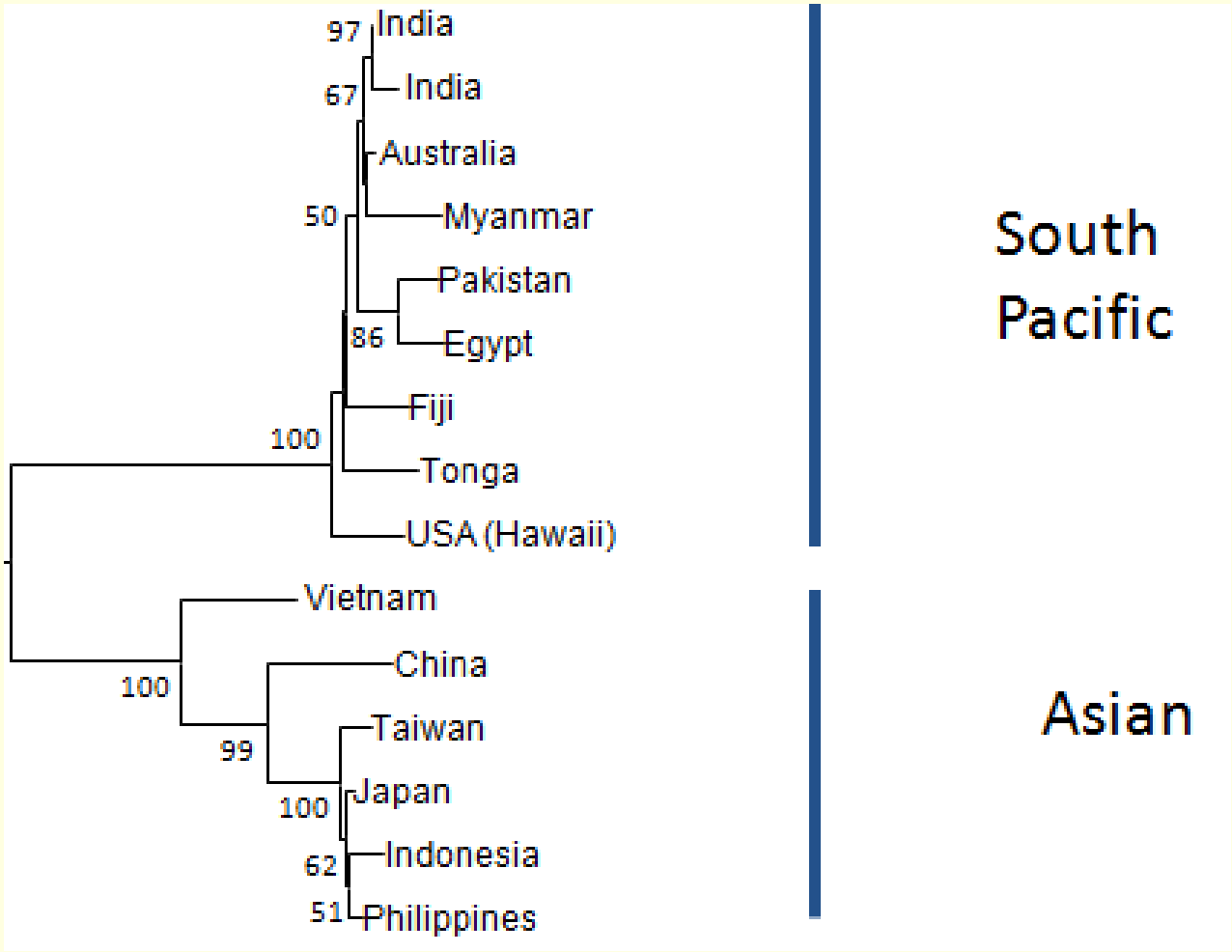
Banana bunchy top virus

Banana bunchy top virus (BBTV)

- ssDNA virus
- Isometric virions (18-20 nm)
- Multicomponent genome
- Each DNA encodes a single ORF except DNA-R
- DNA-M involved in RNA silencing



Banana bunchy top virus isolates subgroups



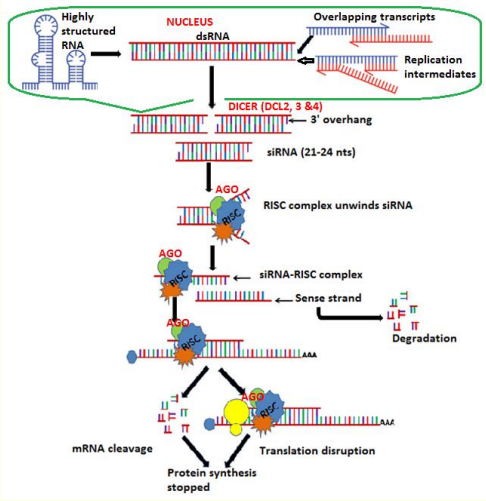
Sequence similarities between the two groups is approximately 90% for DNA-R and 85% for DNA-N

Bunchy top control options

- Avoidance: virus-free planting material, quarantine
- Minimisation: rogueing
- Grow slow infecting cultivars
- **Conventional breeding: no resistance genes have been identified in bananas**
- **Genetic modification**

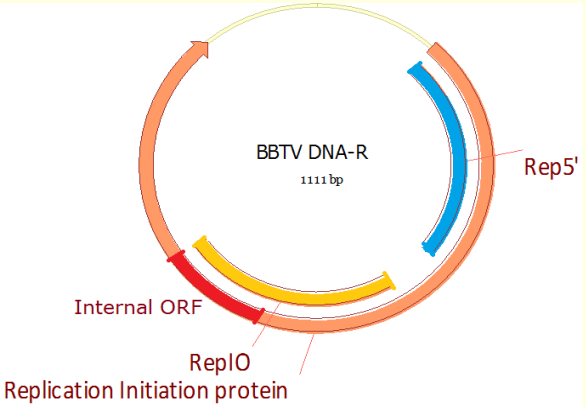
Strategies for GM resistance for BBTV

- RNA interference (natural process)



- The plant equivalent of human immune response
- Plants recognise the invading virus as a pathogen and set up an ongoing defense (often too late)
- As with human vaccination, plants can be “pre-immunised”

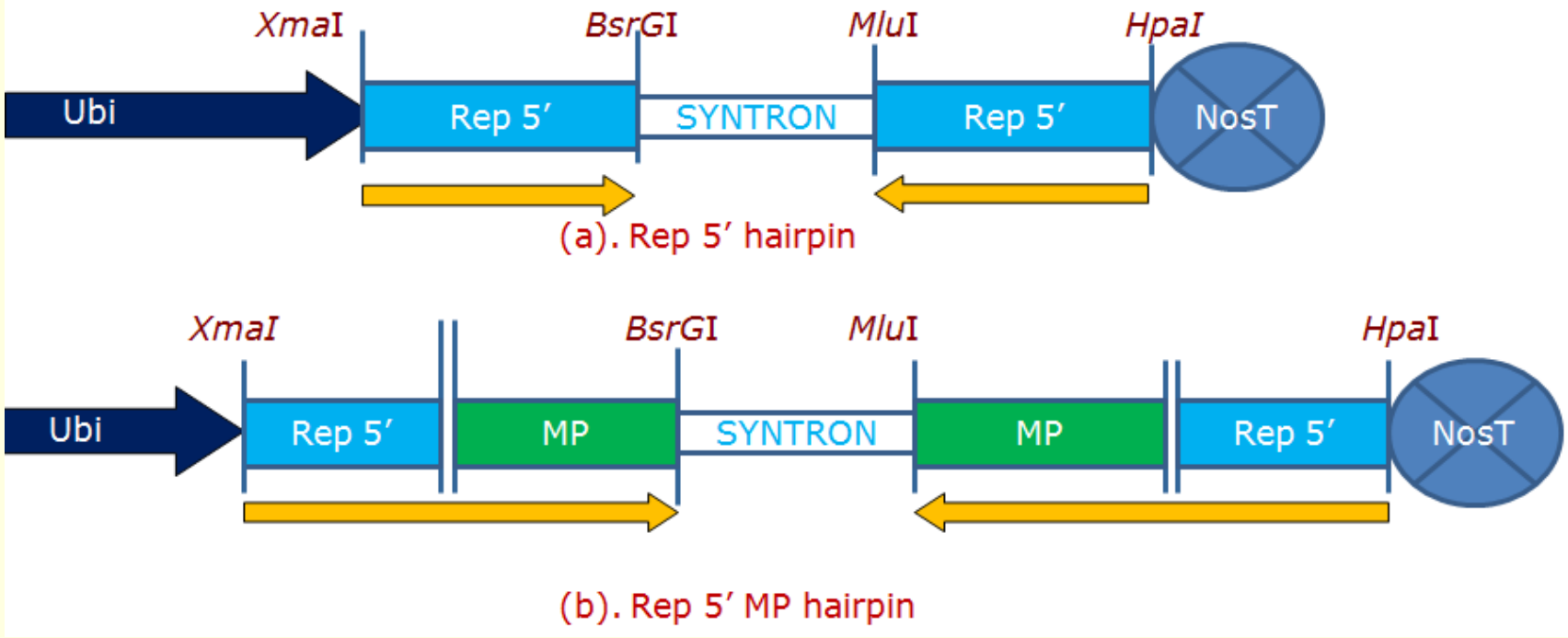
Identification of target BBTV sequences



- Human immune response targets proteins; plant RNAi targets RNA

- Three BBTV genes were selected:
  - Rep gene mRNA: critical function in replication
  - MP gene mRNA: function in cell-to-cell spread and silencing suppression
  - NSP gene mRNA: nuclear shuttle protein moving ssDNA from nucleus to cytoplasm

Hairpin(hp) RNA constructs



BBTV RNAi resistance

Transgenes

- RNAi transgenes targeting: movement protein gene or replication gene

Glasshouse challenge

- Multiple independent lines identified with apparent immunity (all contain MP sequence)

Field trials

- First field trial planned for late 2015 in Malawi

Panama Disease
or
Fusarium wilt

Fusarium wilt or Panama disease of bananas

- One of the most **devastating diseases** of agricultural crops in history
- Caused by *Fusarium oxysporum* fsp *cubense*
- Soil inhabiting fungus that lives more than **50 years** in infested soil; there are no chemical controls
- Infects through the roots and kills susceptible cultivars
- **Race 1** caused the destruction of Gros Michel in Central and South America last century which lead to Cavendish as the replacement
- **Tropical race 4**: kills Cavendish and many other bananas: a repeat of the demise of Gros Michel?

Distribution of Fusarium wilt Race 1

Fusarium in Lady finger

Wild-type Lady Finger challenged with Race 1

GM Lady finger
with a stress tolerance gene

Wild-type

Transgenic plants

Transgenic resistant
Lady finger: inoculated

Non-transgenic susceptible
Lady finger: inoculated

Non-inoculated Lady finger

Fusarium wilt Race 1

Panama Disease tropical race 4

A deadly disease that can kill more than 40% of the bananas in the world and it is one the move

Banana diseases

Yes, we have no bananas

BOGOTÁ

A huge export industry is battling for survival on two fronts

WHEN bananas started to be widely exported in the 1870s, they were an exotic treat. But by the 1950s the fruit (botanically, a herb, but never mind) was a favourite of millions far from the tropics. Then Panama disease struck. The soil fungus swept through Central and South America, killing banana plants in its path. By the 1960s Gros Michel (Big Mike), the variety accounting for virtually all exports, was close to extinction. The export industry approached collapse.

But in the nick of time growers identified a resistant commercial variety, called Cavendish. Compared with Gros Michel, it was small and bland. Gros Michels could be flung into train carriages and ships' holds; Cavendishes had to be packed in cardboard and shipped in pricey refrigerated containers. But there was no other alternative. Soon Cavendish replaced Gros Michel as the world's top banana: the variety now accounts for 95% of all exports.

Bananas are now the world's most valuable fruit. Exports rose from 11.9m tonnes in 2001 to 16.5m in 2012. Americans eat more bananas than apples and oranges together. But once more the export industry is fighting to survive—and this time, on two fronts.

First, Black Sigatoka, a disease which blackens leaves and can halve yields, is showing resistance to the fungicide used to combat it. It is normally controlled by spraying almost weekly, which increases growers' costs considerably. Now growers in some places are having to increase dosage substantially, suggesting that spraying could soon become not just pricey, but ineffective. Second, Foc Tropical Race 4, a strain of Panama disease that attacks the Cavendish, has struck in several countries. Central and South America, which produce four-fifths of exports, have so far escaped. But "it's not a question of whether it will occur there," says Gert Kema, a plant pathologist at Wageningen University in the Netherlands. "It's a question of when."

The race is now on to find a banana that is both resistant to the two diseases and commercially viable. Consumers might turn their noses up at pungent varieties. Thin-skinned ones would not survive weeks in a ship's hold. A candidate may be hidden in the Laboratory of Tropical Crop Improvement in Leuven, Belgium, which houses a big collection of specimens of bananas and plantains (close relatives that must be cooked before eating).

Modifying the Cavendish is another, perhaps quicker, approach. Scientists at the UN and the International Atomic Energy Agency have bombarded plants with gamma rays; three of the resulting mutants have shown resistance in the laboratory to Black Sigatoka. And *Musa acuminata malaccensis*, a wild Asian fruit that is the precursor of edible bananas, is thought to be resistant to Panama disease. A hybrid Cavendish containing some of its genes has grown well in infected ground. But in both cases, field tests are needed.

Cavendish, like other cultivated banana varieties, is seedless and propagated by cuttings. That produces clones, which is efficient for exporters, since the fruit are all similar in shape and size. But it also means a single disease can threaten the entire crop—and the non-tropical world's banana supply. If the export industry is to have a long-term future, it needs to diversify. ■

Correction: In the chart accompanying our article on oceans on February 22nd, we swapped the "over-exploited" and "fully exploited" labels. The correct version is available at http://econ.st/1jrQLIL.

Climate

Law on Mother Earth

Domestic climate legislation is booming

WHEN Tony Abbott became Australia's prime minister in 2013, almost his first acts were to abolish the country's Climate Commission and to promise the repeal of a carbon tax. Soon after, Japan scrapped plans to cut carbon emissions by 25% by 2020, citing its post-tsunami shutdown of nuclear-power plants. Such actions in large countries—Japan is the fifth biggest carbon emitter; Australia the 17th—give the impression that the world as a whole is stepping away from environmental rules and laws.

But according to lawmakers themselves, that impression is false. In a review published on February 27th of national climate legislation in 66 countries, accounting for 88% of carbon emissions, they calculated that almost half of their parliaments passed climate-change or energy-efficiency acts in 2013. Only Japan and Australia went the other way.

The new laws varied hugely. Some were sweeping—take Bolivia's whimsically named Law on Mother Earth and Integral Development to Live Well. Others were narrowly targeted but could have a big impact: Canada introduced rules on exhaust from heavy lorries. Still others, including an environmental-protection law in the poor and violent Democratic Republic of Congo, may have all too little effect. The review considered only the quantity of legislation, not its quality. It says nothing about whether laws are implemented or effective.

All the same, the patient accumulation of domestic decisions is striking. The survey by GLOBE International, a group of lawmakers, and the Grantham Research Institute of the London School of Economics, shows that the world's stock of climate laws has risen steeply, from fewer than 50 in 2000 to almost 500 in 2013. The most active period was in 2008-09, in the run-up to an international climate conference in Copenhagen, at which the signing of a treaty restricting carbon emissions was widely expected. Those hopes were dashed, but have since been rekindled, this time focused on a meeting to be held in Paris in 2015. Countries like to have new climate laws to show off at such gatherings, which may partly explain why the pace of legislation did not slacken much in 2013.

The nature of the laws, though, is changing. During the busy years countries put broad framework legislation on the books. All but five of the 66 passed laws to promote low-carbon energy, for instance. Now, many laws are fleshing out details of that framework legislation.

GLOBE started as a talking shop for a few parliamentarians. It has now set up a formal arrangement, helped by the World Bank, to provide policy advice and swap ideas about good practice among lawmakers. They need help. As the review says, there may be a lot of laws. But this does not necessarily make for better policies—let alone a more stable climate.

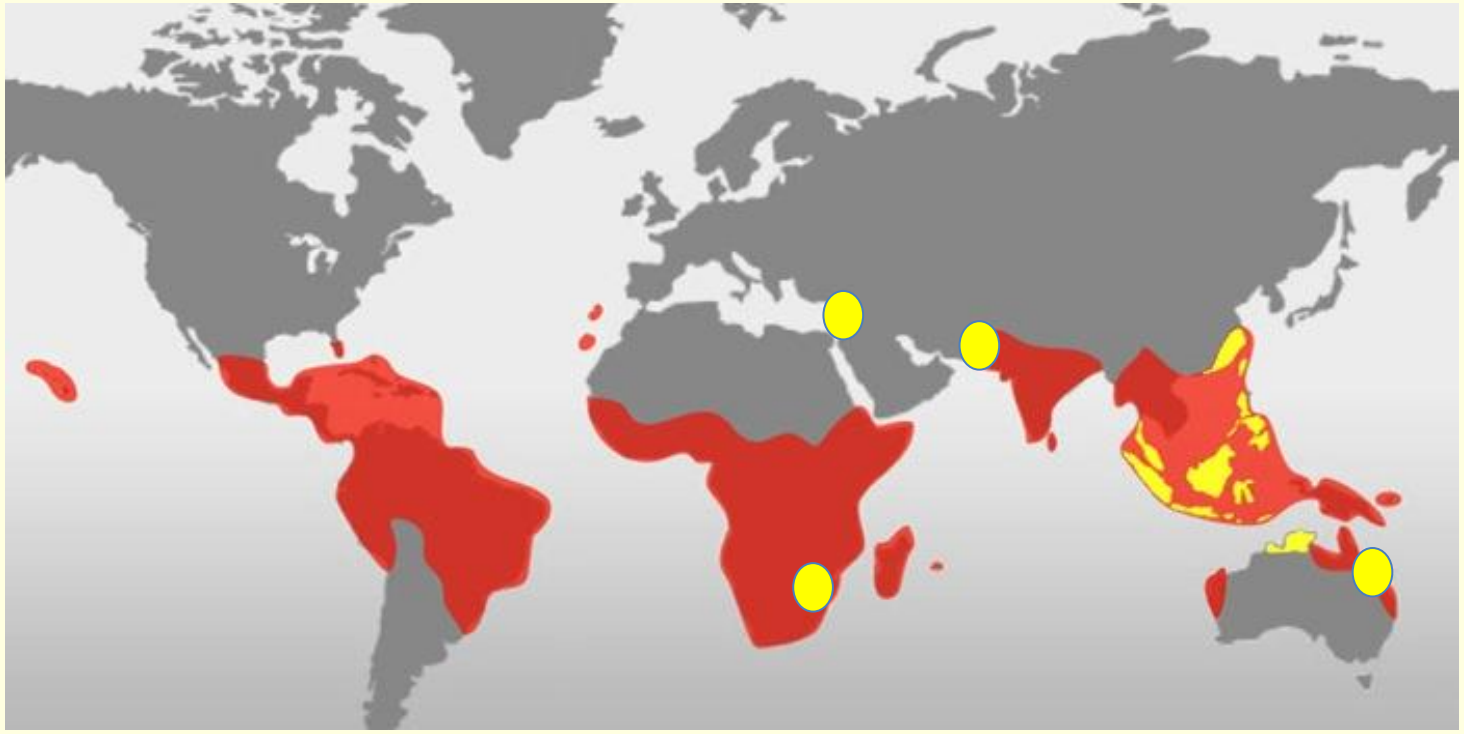
The Economist March, 2014

Fusarium wilt tropical race 4 (TR4)

Fusarium wilt tropical race 4 (TR4) in Mindanao, the Philippines, February, 2014

Distribution of Fusarium wilt Race 1 and Tropical Race 4

TR4: Indonesia, Malaysia, China, the Philippines, Australia, Mozambique, Pakistan, Jordan, Lebanon



Tropical Race 4 in Australia

Tropical Race 4 in Australia

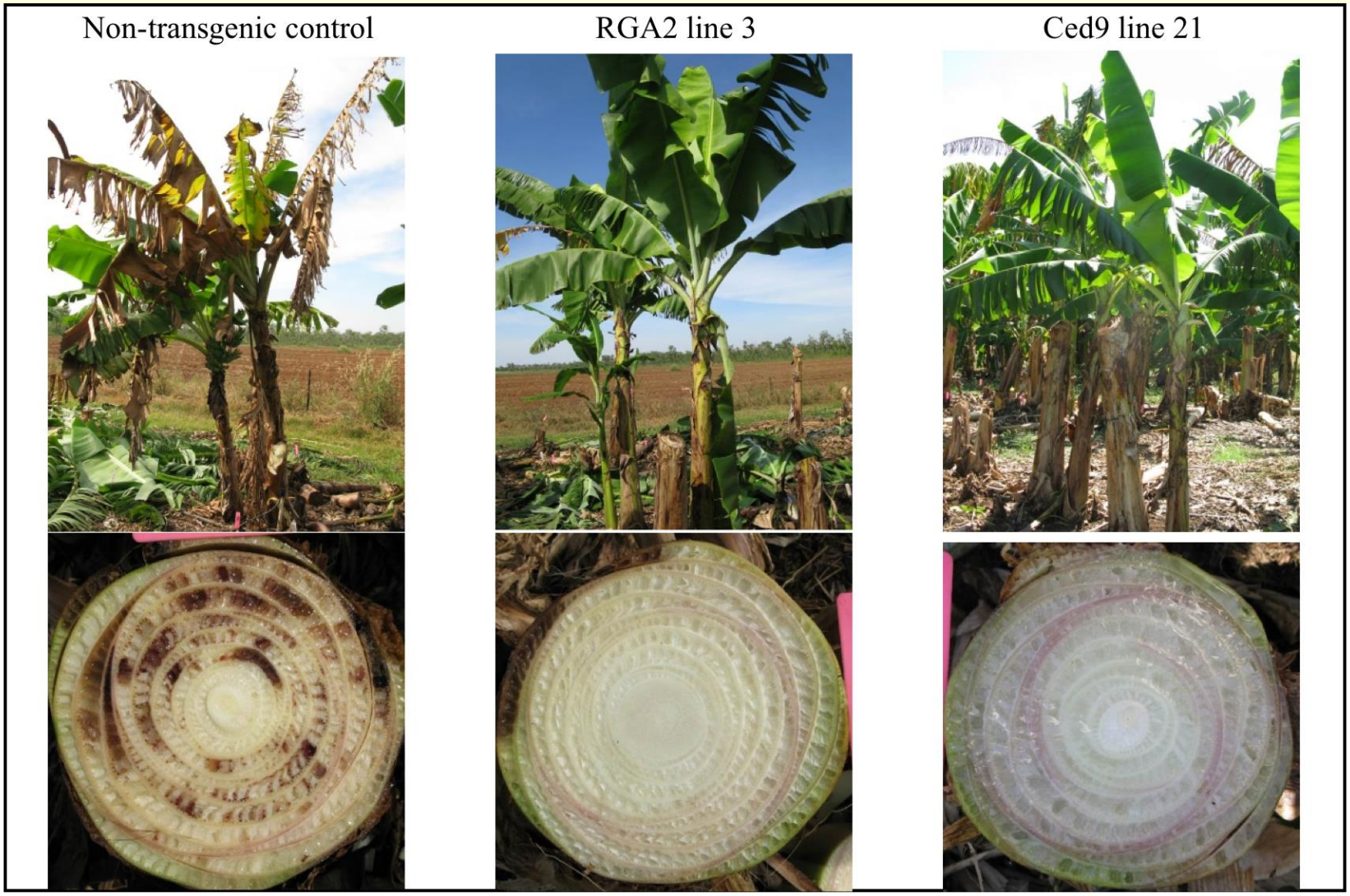
- Present in the Northern Territory for more than 20 years: 2015 only one commercial plantation remained
- March, 2015: TR4 recorded in the Tully Valley for the first time

QUT TR4 R&D Program

- Commenced 2001: hunt for resistance genes
- Identified a potential resistance gene in a resistant wild banana, *Musa acuminata malaccensis* (one of 25,000)
- Transferred the resistance gene into Cavendish by genetic modification
- Also Cavendish modified with Ced9, an anti-apoptosis gene from *C. elegans*
- Planted the first field trial near Darwin January, 2012

Resistant & Susceptible Lines in Darwin

2 GM lines with no disease after 3 years


Non-transgenic control
RGA2 line 3
Ced9 line 21

QUT's banana R&D program

- Panama Disease TR4 resistant bananas for Asia, Africa and Australia
- Bunchy top (virus) resistant bananas for Asia, Africa and Australia
- Biofortified banana for Uganda and East Africa

Bananas : sustainability : GM

Panama Disease

Bunchy top disease

Cavendish **Gros Michel**

And many others

Lakatan **Sukali Ndizi**

Bananas : sustainability : GM

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Gros Michel^{R}

Cavendish^{R}

Lakatan^{R}

Sukali Ndizi^{R}

Local traits for local crops

- Bananas: no interest to big agbiotech
- Plant genes or plant virus sequences
- Bringing back or conserving genetic diversity

Our Researchers and Supporters

QUT
James Dale
Rob Harding
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J-Y Paul
Anthony James
Bulu Mlalazi
Jen Kleidon
Phuong Huang
Benard Mwari
Maiko Kato

NARO (Biofortification)
Wilberforce Tushemereirwe
Stephen Buah
Priver Namanya
Betty Magambo
Ruth Mbabazi
Moses Matovo

Virus Resistance
Leena Tripathi (IITA Nairobi)
Misheck Soko (DARS Malawi)

Field Trails –

Jeff Daniels

Mark Smith

Financial Support

Bill and Melinda Gates Foundation

UK Department for International Development

Australian Research Council

Queensland University of Technology

Horticulture Australia Limited

LaManna Bananas