## Bananas, genetics and appropriate biotechnology

Pat Heslop-Harrison www.molcyt.com









What are bananas? What is in banana DNA? What is the future for banana? What is the future for diet and farmers?

#### WINE

Luxembourg 79.5 litres (17.5 gal) By contrast, the smallest measurable wine consumer is Egypt, where the average yearly wine consumption amounts to about

two tablespoons.

#### BREAKFAST CEREAL Sweden 10.4 kg (22 lb 14 oz)

BAKED BEANS United Kingdom 5.3 kg (11 lb 10 oz)

In contrast, the USA consumes just 1.3 kg (2 lb 13 oz) of baked beans per capita.

HONEY Central African Republic 3 kg (6 lb 9.7 oz) -

#### ICE CREAM Australia 16.6 litres (3.6 gal) \*

TEA Ireland 2.6 kg (5 lb 14 oz) This equates to approximately 1,184 cups per

**DID YOU KNOW?** 

The oldest cultivated plant for

to use this plant as food.

food is the Abyssinian banana (Ensete

between 4,000 and 7,000 years ago, the

hunter-gatherers of ancient Ethiopia learned

ventricosum). Historians suggest that

person in one year. 

#### and the fit where and the fit where aggical hard product astrony Quead Sugar Loffeex and Course T Switzerland 11.5 kg (25 lb 6 oz) This is the equivalent of each person eating 230 bars weighing 50 g (1.75 oz) per year. SPIRITS

CHOCOLATE

÷

Russia 6.2 litres (1.3 gal)

Local The

Brits eat

140 million Demenar

every

The burging (Music sequentum) is the most unsumed fruit in the world. It is the 4th most important status tool world wild tool world wild and the first



### What is a banana? Monocotyledon – giant herb not a tree!







#### **Banana Evolution**

Cultivars: sterile, parthenocarpic clones

 In the wild, no fruits without a seed (and only in last decade for oranges & grapefruit)

 Introduced with farming and domesticated, along with all other major crops and animals, 8000-10000 years ago



#### Uganda

## 400 kg/person/year annual consumption

 Matoke of steamed bananas then mashed







#### Banana Plantains Musa

1-7 year plantation Vegetatively propagated (exclusively)

85% used as local staple 20-30kg fruit bunch

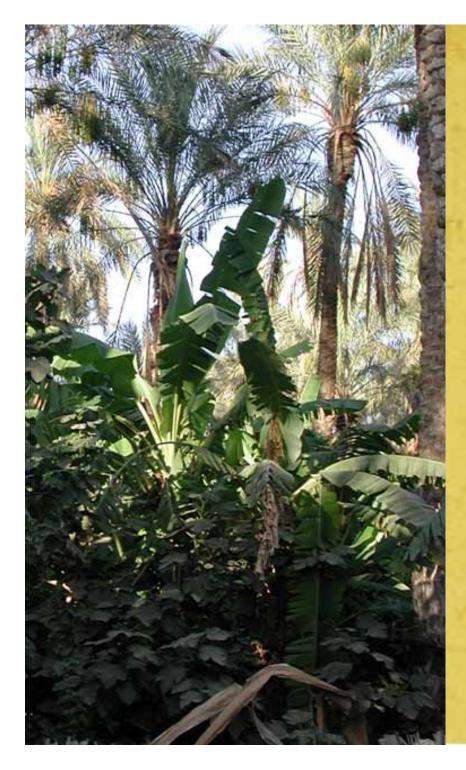
>100Mt /yr

2n=3x=33











- Subsistence agriculture
- Smallholder farms
- Cash crop
- Commercial
- Year-round production
- Eaten by all ages of people

#### Cultivated banana

- Origin from two species in Asia:
- Musa acuminata (the A genome) and Musa balbisiana (B genome)







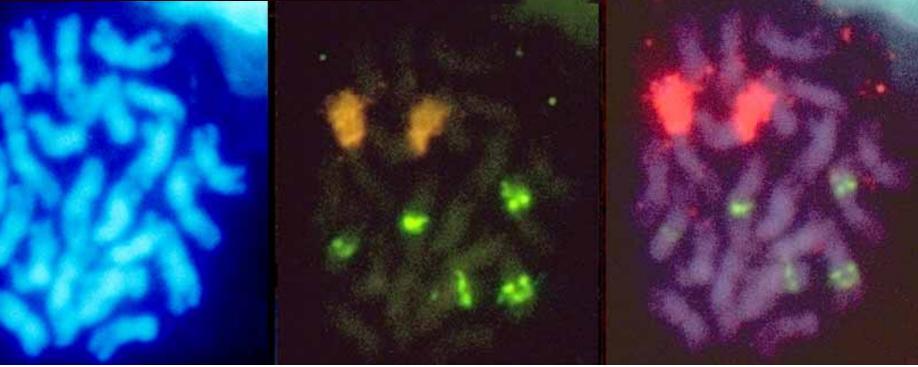
L to R: Red - AAA Palayam codan AAB (two bunch yellow, one green) Peyan ABB (green cooking banana), Njalipoovan AB (yellow) Robusta AAA (green ripe) Nendran AAB Poovan AAB (one yellow bunch) Red AAA

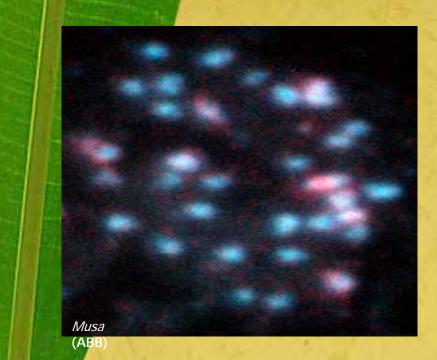
Peyan

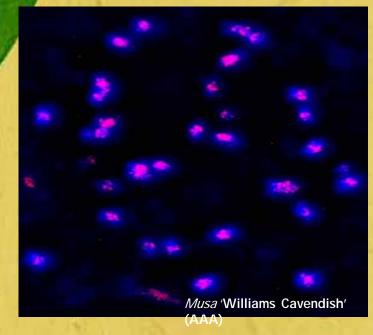
Varkala, Kerala, India

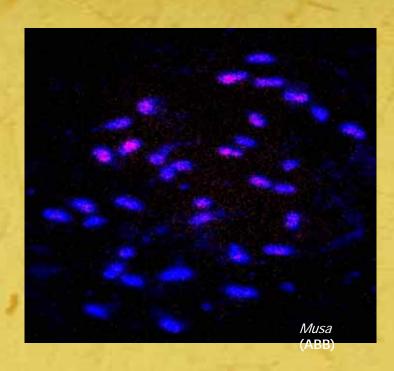


#### Wild banana *Musa acuminata* 'Calcutta 4' AA genomes, 2n=2x=22 One genome and 11 chromosomes from mother Other genome and 11 chromosomes from father







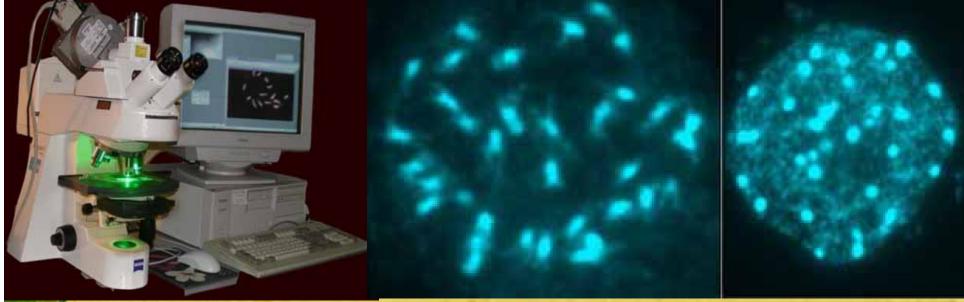


Florescent In-Situ hybridization: An A-genome specific hAT in three *Musa* hybrids (2n=3x=33) located on A-genome chromosomes.

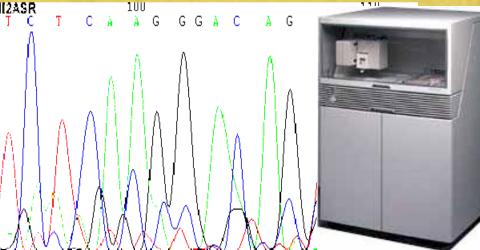
### Measuring diversity

*musa* genomics

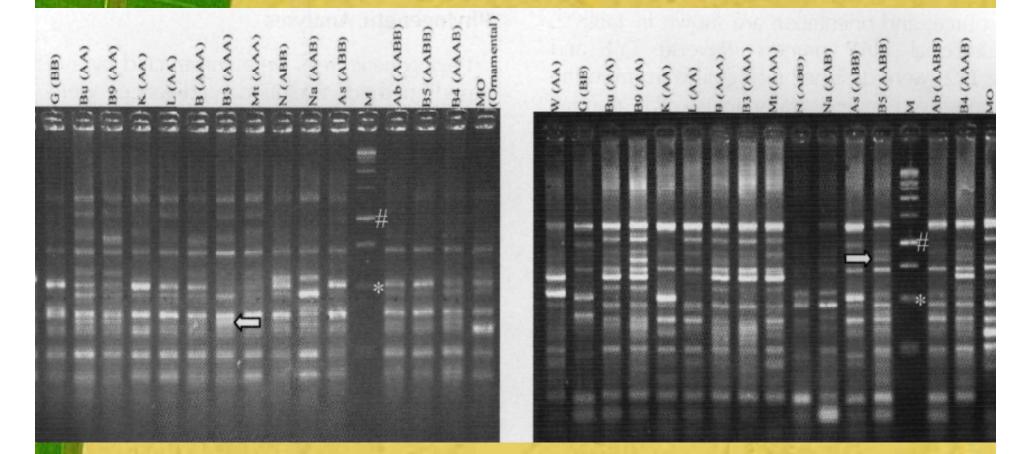
# What is a genome? In bananas and plantains, about 500 million base pairs of DNA



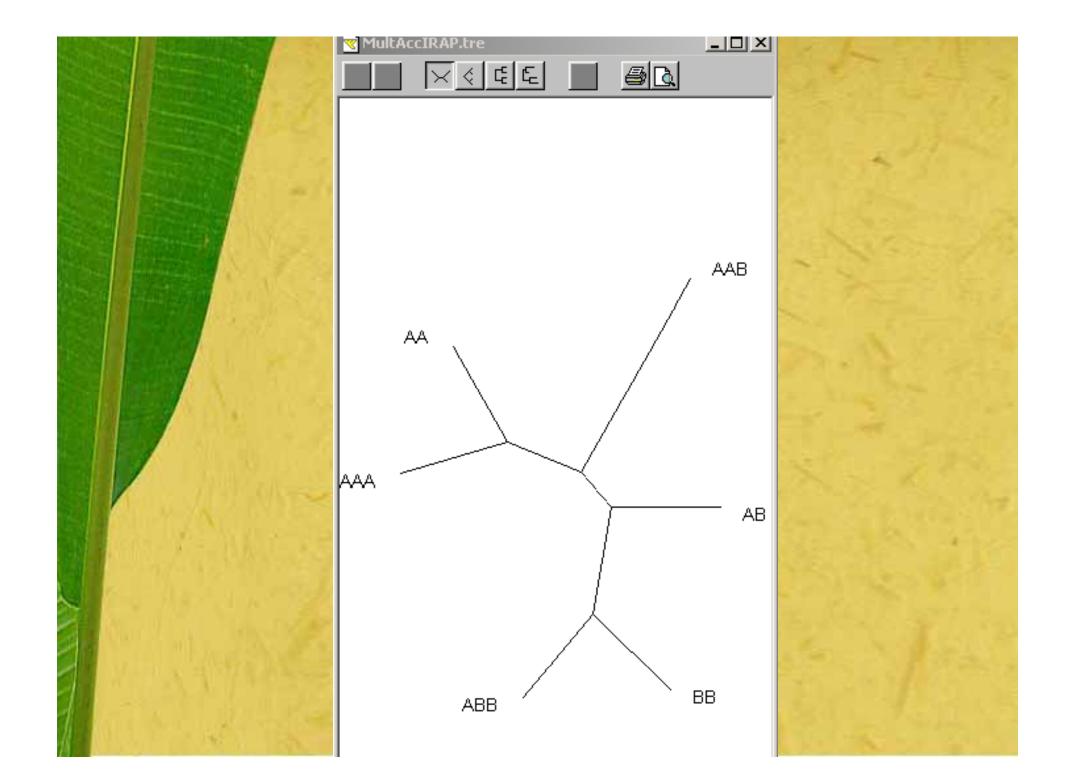
ta clone MuG9, genomic, 73268bp Cell2ASR aaatccaatcaatccagatcaatattgatcgg gacgaagcagtcaaactgatcactaaaattca gagtgctgatttcagaaacttaatcccttctg caacttacactaattagtcttaaaaactcatta ataaatgtcatattacccttccaggtcataaa atgctgaagctattggcattacacttagtctt tttaacgatatgacaatcaataatgagatagg aaatgacatttttttgaactctgcagaatta

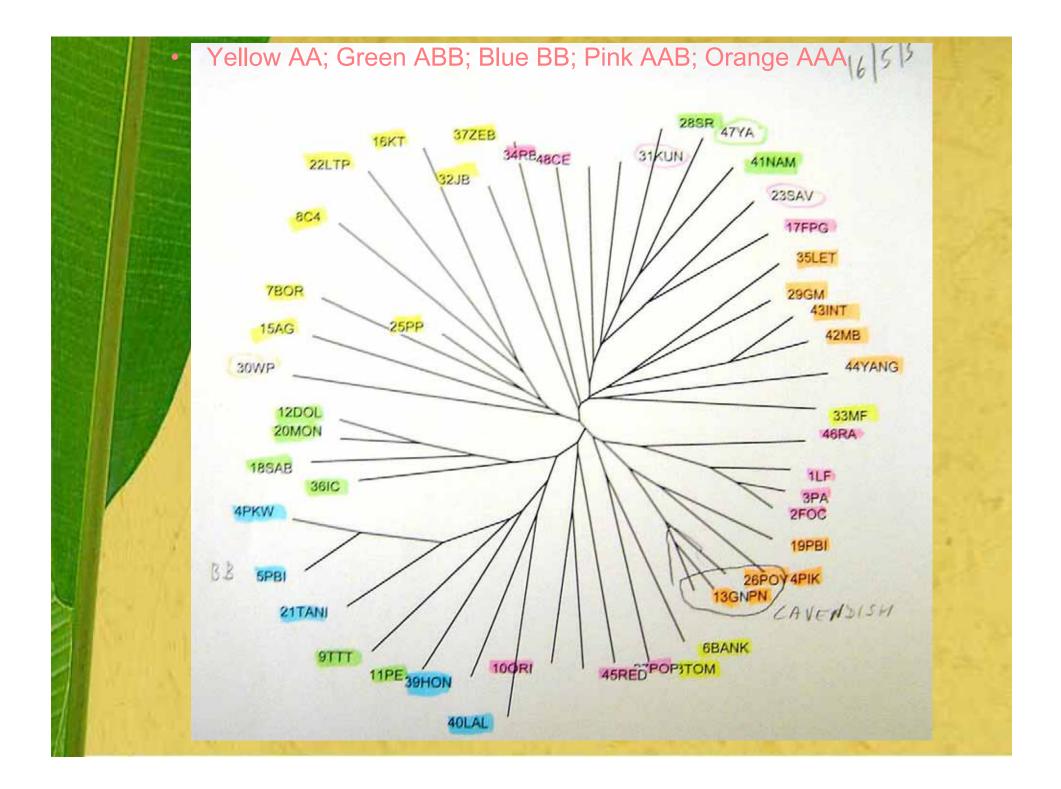


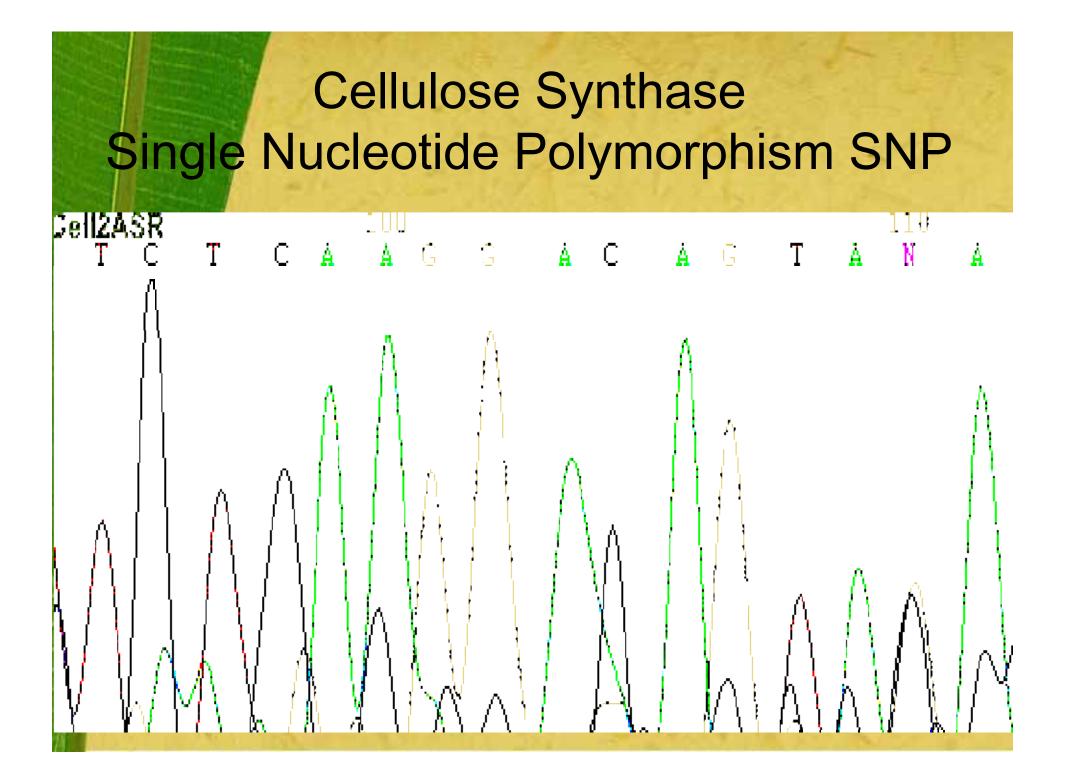
#### **IRAP** diversity in Musa



Teo, Tan, Ho, Faridah, Othman, HH, Kalendar, Schulman 2005 *J Plant Biol* Nair, Teo, Schwarzacher, HH 2006 Euphytica Desai, Maha..., HH et al. in prep.







5 ACE consensus sequence alignments with reference BAC sequence

0		
BAC Ref MA4_64C22	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACCGATTATAATTT	
Calcutta4	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACCGATTATAATTT	
Mala Allele 1	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACCGATTATAATTT	
Mala Allele 2	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACCGATTATAATTT	
Pahang Allele 1	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACC <mark>A</mark> ATTATAATTT	
Pahang Allele 2	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACCGATTATAATTT	
Pahang <u>Doub</u> Hap	CCATAGGGTTGAAGCTCCTGTTTCTAATATGAAAGTACC <mark>A</mark> ATTATAATTT	
51		
MA4_64C22	CAGCTGTACAATAATTAAAGAAGAGCCTACCAATTCCTAGACCTTTGTGT	
C410TF	CAGCTGTACAATAATTAAAGAAGAGCCTACCAATTCCTAGACCTTTGTGT	1. 20
Mala05TF	CAGCTGTACAATAATTAAAGAAGAGCCTACCAATTCCTAGACCTTTGTG <mark>G</mark>	
Mala allele2	CAGCTGTACAATAATTAAAGAAGAGCCTACCAATTCCTAGACCTTTGTGT	
Pah06TR	CAGCTGTACAATAATTAAAGAAGAGCC <mark>A</mark> ACCAATTCCTAGACCTTTGTGT	
Pah19TR	CAGCTGTACAATAATTAAAGAAGAGCCTACCAATTCCTAGACCTTTGTGT	
PDH07TF	CAGCTGTACAATAATTAAAGAAGAGCC <mark>A</mark> ACCAATTCCTAGACCTTTGTGT	1 C 1 C 1
101		
MA4_64C22	ACAGGCCCTTGTTCTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
C410TF	ACAGGCCCTTGTTCTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
Mala05TF	ACAGGCCCTTGTTCTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
Mala allele2	ACAGGCCCTTGTTCTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
PahO6TR	ACAGGCCCTTGT <mark>C</mark> CTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
Pah19TR	ACAGGCCCTTGTTCTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
PDH07TF	ACAGGCCCTTGT <mark>C</mark> CTCATGTCAATGCACAAAGGATGCACCTCAACACACA	
	51	
MA4_64C22 C410TF	CCAAACTCCAGTTGACTCATCTATACTTGGCCTAAATTGGACGGAC	
MalaO5TF	CCAAACTCCAGTTGACTCATCTATACTTGGCCTAAATTGGACGGAC	
Mala allele2	CCAAACTCCAGTTGACTCATCTATACTTGGCCTAAATTGGACGGAC	
PahO6TR	CCAAACTCCAGTTGACTCATCTATACTTGGCCTAAATTGGACGGAC	
Pah19TR	CCAAACTCCAGTTGACTCAGCTATACTTGGCCTAAATTGGACGACTTGG	
PDH07TF	CCAAACTCCAGTTGACTCATCTATACTTGGCCTAAATTGGACGGAC	
	DO	
MA4 64C22	TAGGACTTGACCTAATTTGGTCAAGCTGGGACAAATTGACCAATTCCAAC	
C410TF	TAGGACTTGACCTAATTTGGTCAAGCTGGGACAAATTGACCAATTCCAAC	
MOLOOFTE	TACCACTTCACCTAATTTCCTCAACCTCCCACACAATTCACCAATTCCAAC	
		and the second se

#### Where does diversity come from?

#### The DNA

- Single nucleotide changes
   Collulose synthese
  - Cellulose synthase
- Deletions/insertions in genes
- Duplications
  - Modifies expression
  - Important as gives something for evolution to work on
- Regulatory elements



#### Variety Cavendish

- 15% of banana production worldwide
- The vast majority of export banana to temperate countries
- Controllable ripening but very sensitive to conditions
- First collected in China in 1826 (Telfair), Sold to Duke of Devonshire, Chatsworth
- Distributed worldwide from 1836
- Became dominant variety in 1960s, replacing Gros Michel
- Has various variants: Williams, Dwarf C, Giant C, Grand Naine, Robusta, Poyo ...



 Gros Michel in Fusarium (Panama disease) trial in Malaysia

## Daily Telegraph 23 May 2006

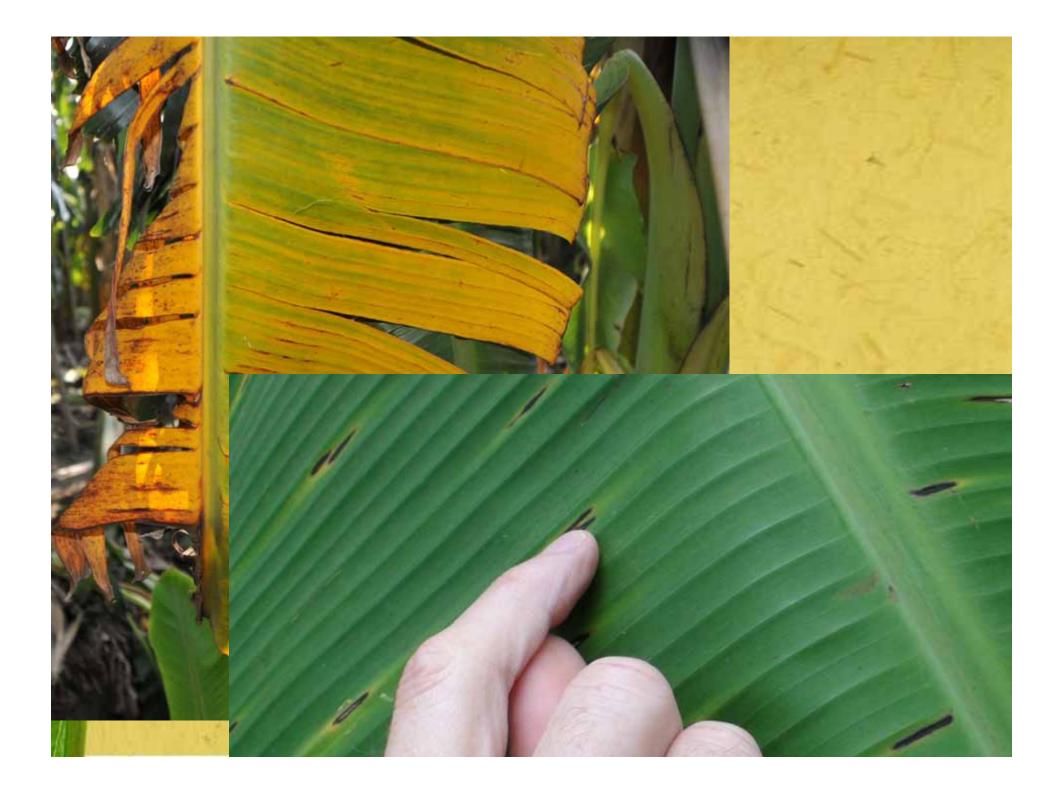
No 1 banana could face extinction By Roger Highfield, Science Editor

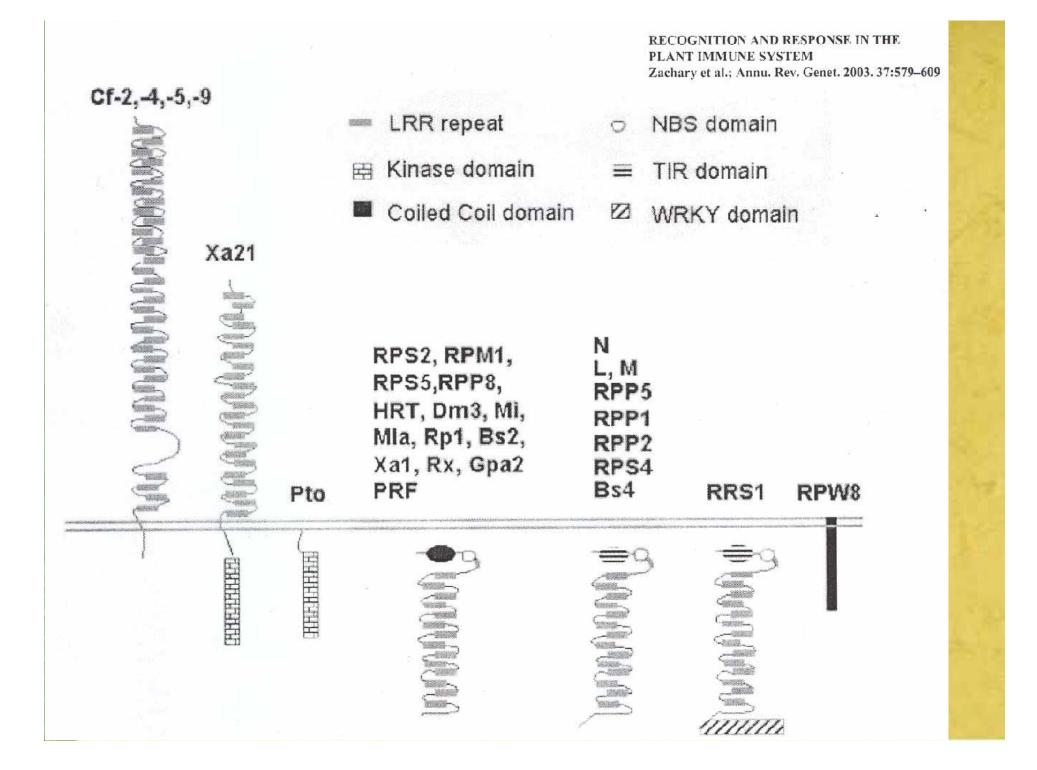
The most popular type of banana, the Cavendish, is under threat from disease. In the 1950s, Britons ate a different banana, the Gros Michel but it was wiped out by Panama disease.

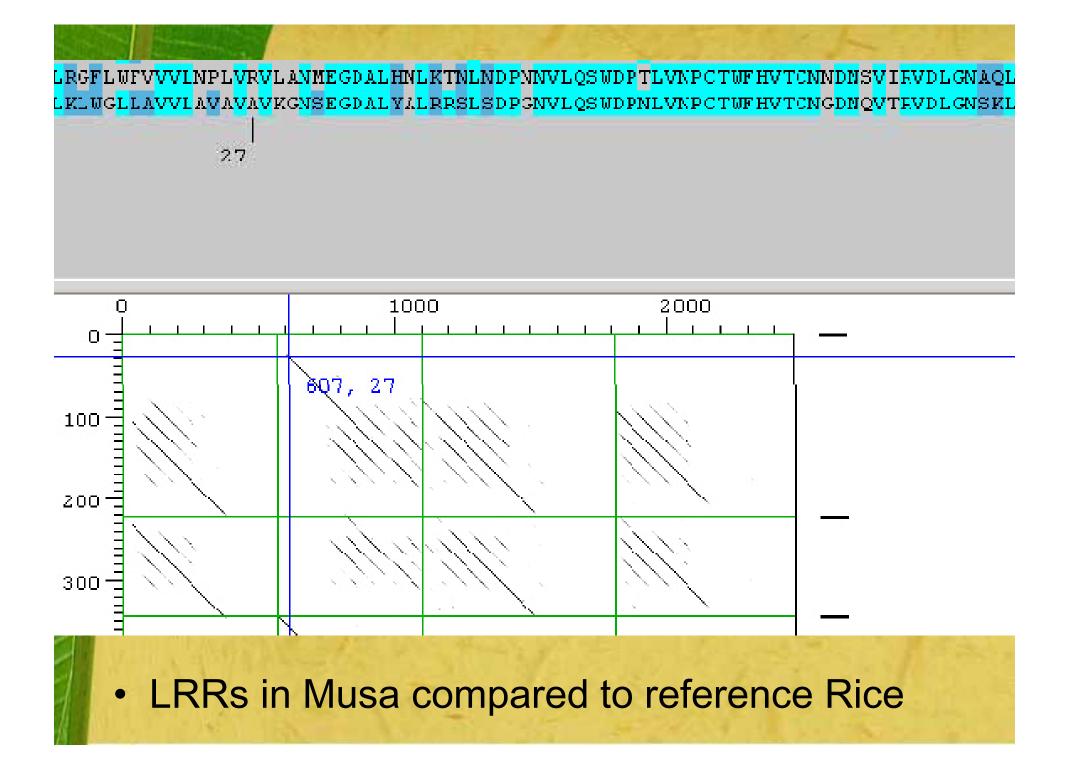
- Now the Cavendish could follow suit as a new strain of the fungus to which it was supposed to be immune has begun to attack the plants. So far, the new, more aggressive variant of Panama disease - TR4 - has not reached the main exporting countries in Latin America or Africa but it is spreading widely through Cavendish plantations in Asia - Indonesia, Taiwan, southern provinces of China and Malaysia.
- In the humid conditions of traditional banana plantations in Central America, the black Sigatoka fungus which attacks leaves, also thrives and the plants must be protected by weekly sprays of fungicides. Although the Cavendish could disappear, experts are confident that a bunch of alternative bananas could fill the void. The caveat is that the taste and texture will be changed forever and there is likely to be a rise in price.











Cytogenet Genome Res 121:59–66 (2008) DOI: <u>10.1159/000124383</u>



# Genomes, diversity and resistance gene analogues in *Musa* species

#### M. Azhar J.S. Heslop-Harrison

Abstract. Resistance genes (R genes) in plants are abundant and may represent more than 1% of all the genes. Their diversity is critical to the recognition and response to attack from diverse pathogens. Like many other crops, banana and plantain face attacks from potentially devastating fungal and bacterial diseases, increased by a combination of worldwide spread of pathogens, exploitation of a small number of varieties, new pathogen mutations, and the lack of effective, benign and cheap chemical control. The challenge for plant breeders is to identify and exploit genetic resistances to diseases, which is particularly difficult in banana and plantain where the valuable cultivars are sterile, parthenocarpic and mostly triploid so conventional genetic analysis and breeding is impossible. In this paper, we review the nature of R genes and the key motifs, particularly in the Nucleotide Binding Sites (NBS), Leucine Rich Repeat (LRR) gene class. We present data about identity, nature and evolutionary diversity of the NBS domains of Musa R genes in diploid wild

species with the Musa acuminata (A), M. balbisiana (B), M. schizocarpa (S), M. textilis (T), M. velutina and M. ornata genomes, and from various cultivated hybrid and triploid accessions, using PCR primers to isolate the domains from genomic DNA. Of 135 new sequences, 75% of the sequenced clones had uninterrupted open reading frames (ORFs), and phylogenetic UPGMA tree construction showed four clusters, one from Musa ornata, one largely from the B and T genomes, one from A and M. velutina, and the largest with A, B, T and S genomes. Only genes of the coiled-coil (non-TIR) class were found, typical of the grasses and presumably monocotyledons. The analysis of R genes in cultivated banana and plantain, and their wild relatives, has implications for identification and selection of resistance genes within the genus which may be useful for plant selection and breeding and also for defining relationships and genome evolution patterns within the genus using the multi-copy and variable resistance genes. Copyright © 2008 S. Karger AG, Basel

1	
	MT1 MT2 AW KW
-	
Annual A	
1000 bp	
800 bp	
600 bp	The second second

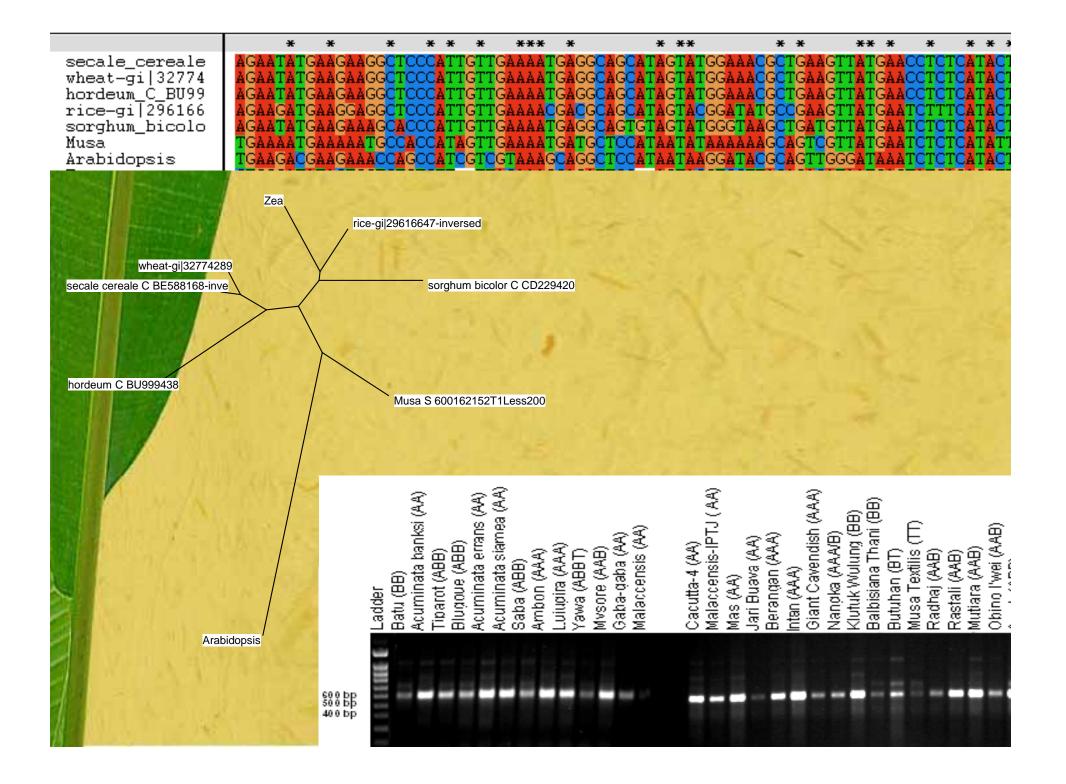
Table 9 Response of some banana cultivars to *Fusarium oxysporum* f. sp. *cubense* (FOC)

Cultivoro	Canama	Disease Reaction	
Cultivars	Genome	FOC Race 1	FOC Race 4
Pisang Mas	AA	Т	S
Pisang Lemak Manis	AA	Т	Т
Pisang Jari Buaya	AA	R	R
Pisang Berangan	AAA	S	VS
Pisang <u>Embun</u>	AAA	VS	VS
Pisang <u>Udang</u>	AAA	S	S
Grand Naine	AAA	R	S
GCTCV215-1	AAA	R	T (?)
Pisang <u>Serendah</u>	AAA	R	Т
Pisang <u>Rastali</u>	AAB	VS	VS
Mutiara (selected P. <u>Rastali</u> )	AAB	Т	Т
Pisang <u>Seribu</u>	AAB	S	S
Pisang Raja	AAB	S	S
Pisang <u>Relong</u>	AAB	S	S
Pisang <u>Nangka</u>	AAB	S	S
Pisang <u>Awak</u>	ABB	Т	S
Pisang <u>Tanduk</u>	ABB	S	S
Pisang Abu <u>Keling</u>	ABB	Т	Т
Pisang Abu <u>Nipah</u>	ABBB	S	S
Gold Finger	AAAB	R	Т

R, resistant; T, tolerant; S, susceptible; VS, very susceptible.

Primers : MLRR1-F and MLRR2-R

MT1 and MT2 – Mutiara tolerance to FOC AW - Pisang Awak KW – Klutuk Wulung



### Plant breeding Keeping up with changes

- Biotic stress
  - New disease races are continuously appearing and spreading
  - Fungi, viruses, bacteria
  - Insects, nematodes, weeds ...
- Abiotic stresses
  - Drought/flooding/salt, cold ...
- Socio-economic changes
  - More people to feed on less land
  - Urbanization of population

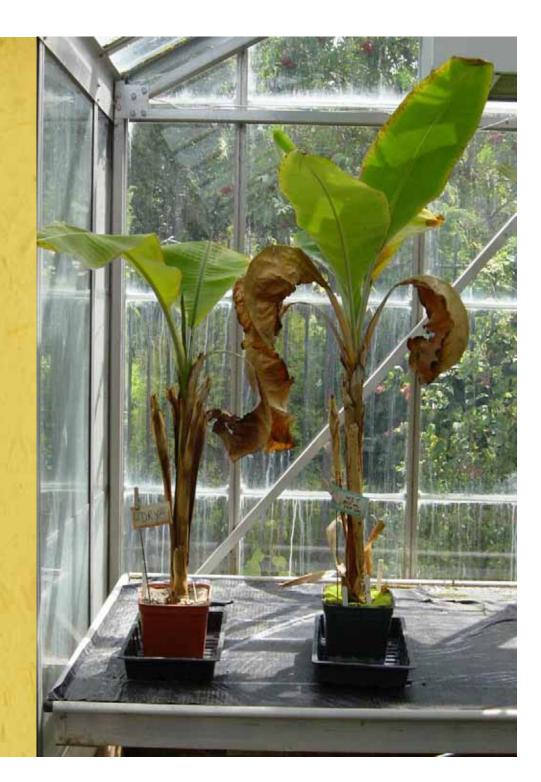




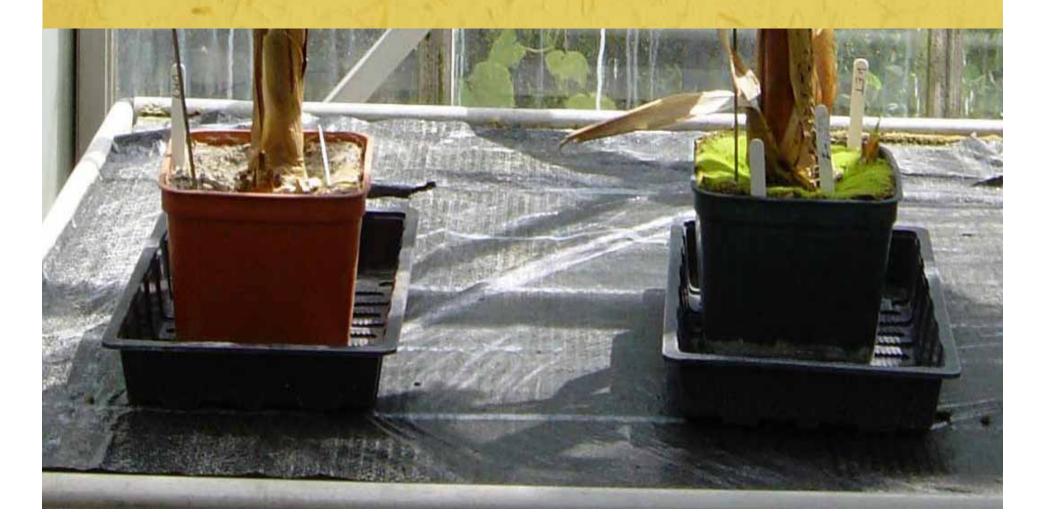


### Drought Responsive Genes

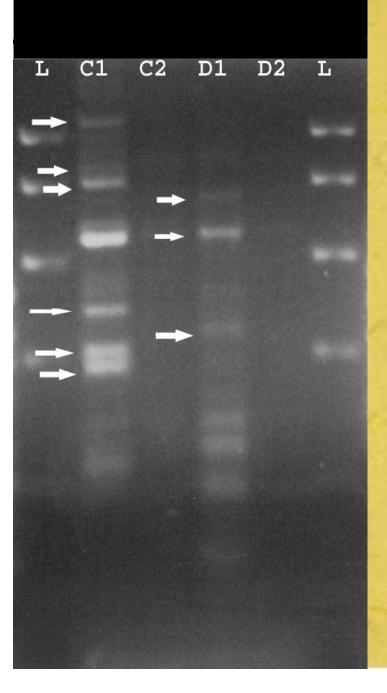
 Differential display of genes being expressed from droughted and watered *Musa* lines



### Drought Responsive Genes

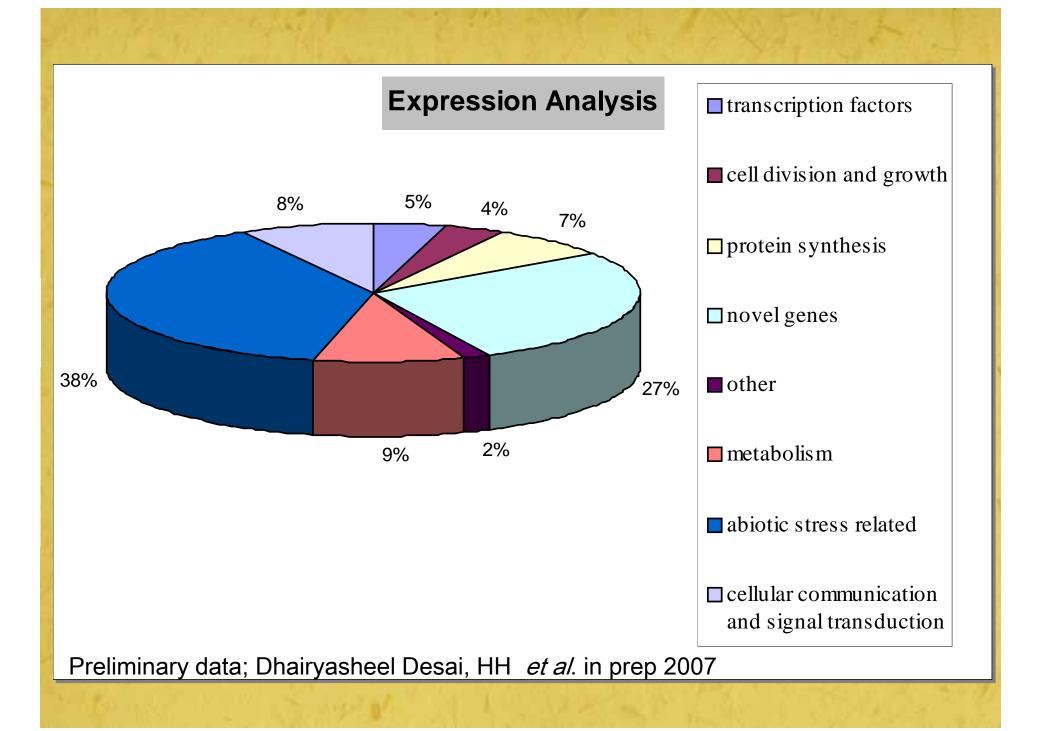






### **Differential Display**

14 DD-PCR reactions using different arbitrary and Oligo dT primer combinations, a total of 22 differentially expressed bands (MDRG)









### AICRP(TF) Collection.conservation and Evaluation of Banana Germplasm

No. OF ACCESSIONS - 256. No. OF PLANTS/ACCESSION - 5. SPACING - 2×2.5m DATE OF PLANTING - 23.10.2006.

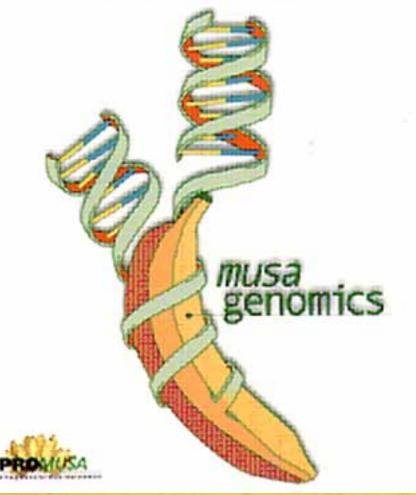






#### Strategy for the Global Musa Genomics Consortium

Report of a meeting held in Arlington, USA 17-20 July 2001 The Global Musa Genomics Consortium



### The Global Musa Genomics Consortium

To assure the sustainability of banana as a staple food crop by developing an integrated genetic and genomic understanding, allowing targeted breeding, transformation and more efficient use of Musa biodiversity







### Super-domestication: The future of banana crops

- Biotic stresses
- Abiotic stresses
- Socioeconomic factors

• ... all mean current cultivars do not meet future needs

### Super-domestication: The future of banana crops

- The genepool has the diversity there which can meet these challenges
- Breeders need to get better and faster
- Banana, has extra challenges
  - Staple food
  - Major income source in many communities
  - Sterile plant

# naturenews

Published online 1 October 2010 | Nature | doi:10.1038/news.2010.509

#### News Uganda prepares to plant transgenic bananas

Sweet pepper gene confers resistance to bacterial wilt.

#### Linda Nordling

Scientists in Uganda will next week start field trials of a banana variety genetically engineered to resist a bacterial disease that has been decimating crops across central Africa.

The new variety is part of a wider effort to improve the East African Highland banana, a fruit so important to Ugandans that its name, matooke, is synonymous with 'food' in one of the local languages. But delays to a law regulating the



Banana plants growing at Uganda's National Agricultural Research Institute. Field trials of a GM variety are set to begin next week.

Linda Nordling

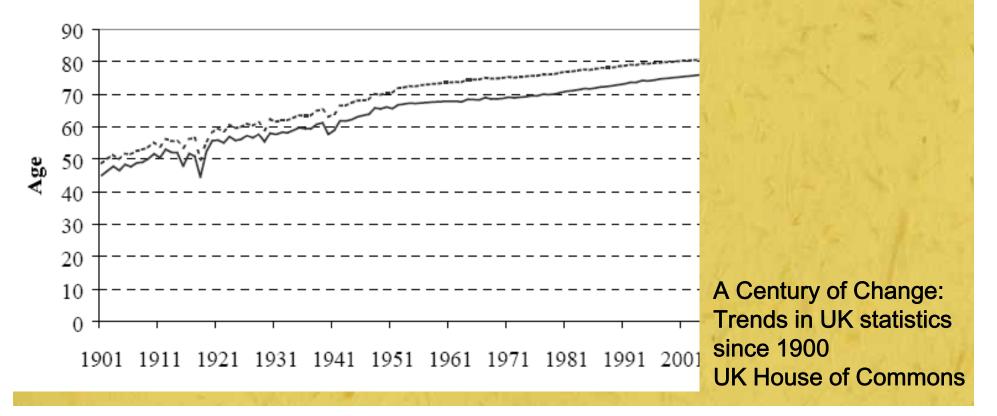
commercial growing of genetically modified (GM) food in the country means it is not clear when the improved banana could be released to farmers.

### What have farmers done?

 Increased quality and security, supporting a longer-lived, larger population

Life Expectancy at Birth

•• Women — Men



	year (millions)	
item	2007	
People	6,602	
Maize	785	
Rice, paddy	652	
Wheat	607	
Potatoes	322	
Sugar beet	248	
Cassava	228	
Soybeans	216	
Oil palm fruit	192	
Barley	136	
Sweet potatoes	126	
Tomatoes	126	
Watermelons	93	
Bananas	81	
Seed cotton	73	
Cabbages and other bra		
Grapes	66	
Sorghum	65	
Onions, dry	64	
Apples	64	
Oranges	64	
Coconuts	55	
Yams	52	
Rapeseed	49	
Cucumbers and gherkin		
Groundnuts, with shell	35	
Plantains	34	
Mangoes, mangosteens	33	
Eggplants (aubergines)		
Millet	32	

#### FAO Statistics 2007

All plant crops with >30M tons annual production

excluding sugar cane and 'other vegetables'

People: WHO

	year (mi	- 5	
item	1961	2007	
People	3,090	6,602	
Maize	205	785	
Rice, paddy	216	652	
Wheat	222	607	
Potatoes	271	322	
Sugar beet	161	248	
Cassava	71	228	
Soybeans	27	216	
Oil palm fruit	14	192	
Barley	72	136	1.1
Sweet potatoes	98	126	
Tomatoes	28	126	
Watermelons	18	93	
Bananas	21	81	
Seed cotton	27	73	
Cabbages and other br		69	
Grapes	43	66	
Sorghum	41	65	
Onions, dry	14	64	
Apples	17	64	
Oranges	16	64	
Coconuts	24	55	
Yams	8	52	
Rapeseed	4	49	
Cucumbers and gherkin		45	
Groundnuts, with shell	14	35	
Plantains	13	34	
Mangoes, mangosteens		33	
Eggplants (aubergines)		32	
Millet	26	32	

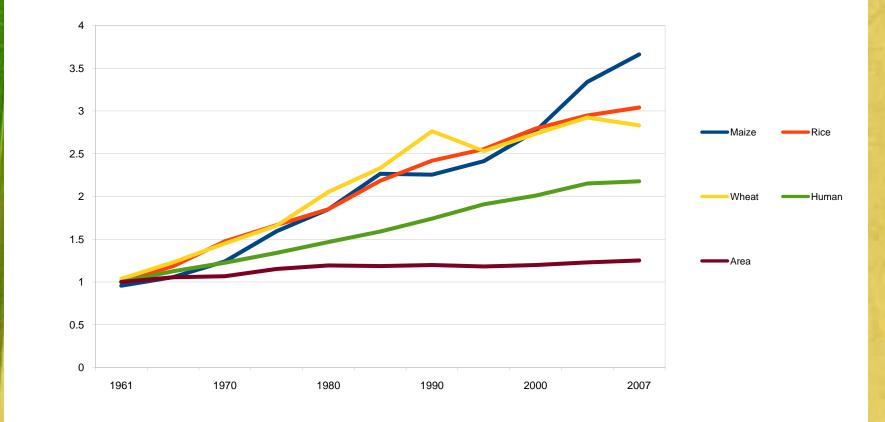


	year (millions)		
item	1961	2007	2007/1961
People	3,090	6,602	2.1
Maize	205	785	3.8
Rice, paddy	216	652	3.0
Wheat	222	607	2.7
Potatoes	271	322	1.2
Sugar beet	161	248	1.5
Cassava	71	228	3.2
Soybeans	27	216	8.0
Oil palm fruit	14	192	13.7
Barley	72	136	1.9
Sweet potatoes	98	126	1.3
Tomatoes	28	126	4.5
Watermelons	18	93	5.2
Bananas	21	81	3.9
Seed cotton	27	73	2.7
Cabbages and other bra	23	69	3.0
Grapes	43	66	1.5
Sorghum	41	65	1.6
Onions, dry	14	64	4.6
Apples	17	64	3.8
Oranges	16	64	4.0
Coconuts	24	55	2.3
Yams	8	52	6.5
Rapeseed	4	49	12.3
Cucumbers and gherkin	10	45	4.5
Groundnuts, with shell	14	35	
Plantains	13	34	
Mangoes, mangosteens	11	33	3.0
Eggplants (aubergines)	7	32	4.6
Millet	26	32	1.2

	year (mil	lions)		
item	1961	2007	2007/1961	
Oil palm fruit	14	192	13.7	
Rapeseed	4	49	12.3	
Soybeans	27	216	8.0	
Yams	8	52	6.5	
Watermelons	18	93	5.2	
Onions, dry	14	64	4.6	
Eggplants (aubergines)	7	32	4.6	
Tomatoes	28	126	4.5	
Cucumbers and gherkin	10	45	4.5	
Oranges	16	64	4.0	
Bananas	21	81	3.9	
Maize	205	785	3.8	
Apples	17	64	3.8	
Cassava	71	228	3.2	
Rice, paddy	216	652	3.0	
Cabbages and other bra	23	69	3.0	
Mangoes, mangosteens	11	33	3.0	
Wheat	222	607	2.7	
Seed cotton	27	73	2.7	
Plantains	13	34	2.6	
Groundnuts, with shell	14	35	2.5	
Coconuts	24	55	2.3	
People	3,090	6,602	2.1	
Barley	72	136	1.9	
Sorghum	41	65	1.6	
Sugar beet	161	248	1.5	5.0X (* 143
Grapes	43	66	1.5	
Sweet potatoes	98	126	1.3	
Millet	26	32	1.2	
Potatoes	271	322	1.2	



# 50 years of plant breeding progress



### UK Wheat 1948-2007 52,909 data points, 308 varieties

### Linear trends in yield

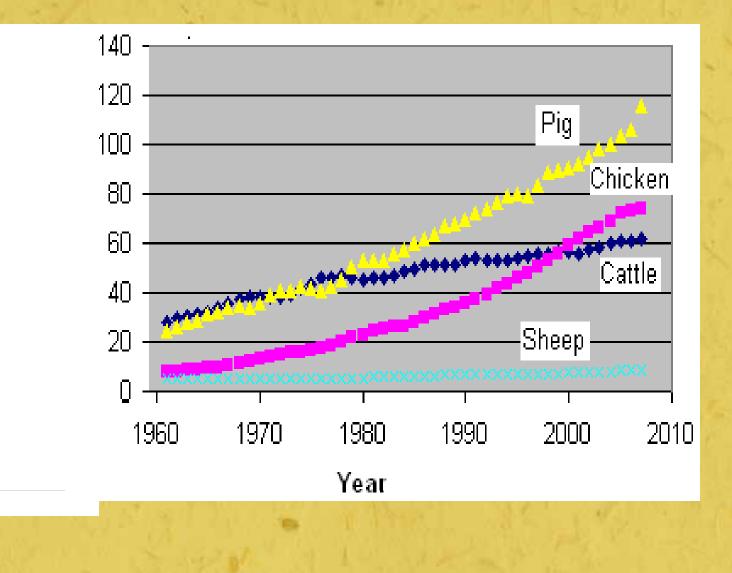
	G	E
1948 - 2007	0.071	0.069
1948 - 1981	0.061	0.041
1982 - 2007	0.074	0.010

Improvements in both agronomy and genetics brought about big improvements in yield for ~ 30 years from 1948.

However, for the last 25 years, yields have increased primarily from plant breeding alone.

From Ian Mackay, NIAB, UK. 2009. Re-analyses of historical series of variety trials: lessons from the past and opportunities for the future. SCRI website.

### **Meat Production**





#### 📼 OPEN BBC News in video and audio

Last Updated: Tuesday, 15 August 2006, 09:06 GMT 10:06 UK

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🔒 Printable version

#### Overweight 'top world's hungry'

There are now more overweight people across the world than hungry ones, according to experts.

US professor Barry Popkin said all countries – both rich and poor had failed to address the obesity boom.



The number of people overweight has topped 1bn across the world

He told the International

Association of Agricultural Economists the number of overweight people had topped 1bn, compared with 800m undernourished.

Speaking at an Australian conference, he said changing diets and people doing less physical exercise was the cause.

Professor Popkin, from the University of North Carolina, said that the change had happened quickly as obesity was rapidly spreading, while hunger was slowly declining among the world's 6.5bn population.

 What are bananas? What is in banana DNA? What is the future for banana? What is the future for diet and farmers?



### United Nations Millennium Development Goals

- Goal 1 Eradicate extreme poverty and hunger
- Goal 2 Achieve universal primary education
- Goal 3 Promote gender equity and empower women
- Goal 4 Reduce child mortality
- Goal 5 I mprove maternal health
- Goal 6- Combat HIV/AIDS, malaria and other diseases
- Goal 7 Ensure environmental sustainability
- Goal 8 Develop a global partnership for development



### Darwin: Final paragraph of "The Origin"

 It is interesting to contemplate ... many plants of many kinds ... from so simple a beginning endless forms most beautiful and most wonderful have been, and are being evolved.



## **The Banana**



### Pat Heslop-Harrison www.molcyt.com

phh4@le.ac.uk



### **Economic growth**

Separate into increases in (resources, labour and capital) and

 90% of the growth in US output per worker is attributable to technical progress

**Robert Solow – Economist** 

# FINANCIAL TIMES

From Prof Donald Braben and others. Sir, We the undersigned scientists write to draw attention to a neglected aspect of the current economic crisis. Robert Solow won the Nobel Prize in economics in 1987 for his 1950s discovery that *technical change* was the biggest source of growth, a discovery that seems to have been forgotten. Scientific advances are not

predictable.

Pat Heslop-Harrison, University of Leicester