Biotechnology Applications for Plant Breeding and Genetics

---------Summary

Guo-qing Song

January 2010

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Outline

- Why use biotechnology for plant improvement

- Steps in application of biotechnology

What is biotechnology?

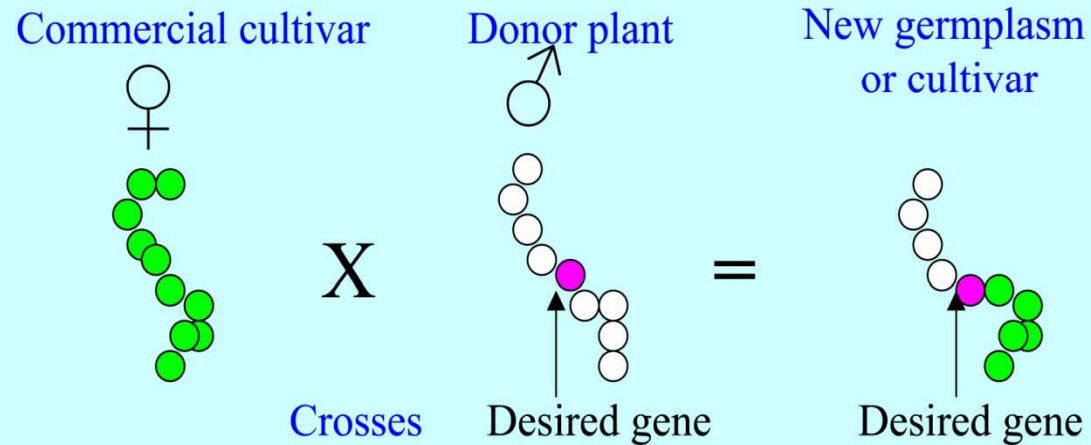Definition: *Bio* = life and *technology* = applying science to solve a problem

Bio-tech-nol-o-gy, noun (1941): *A collective term for a variety of scientific techniques that use living cells or components of cells to improve crops, animals, or microorganisms.*

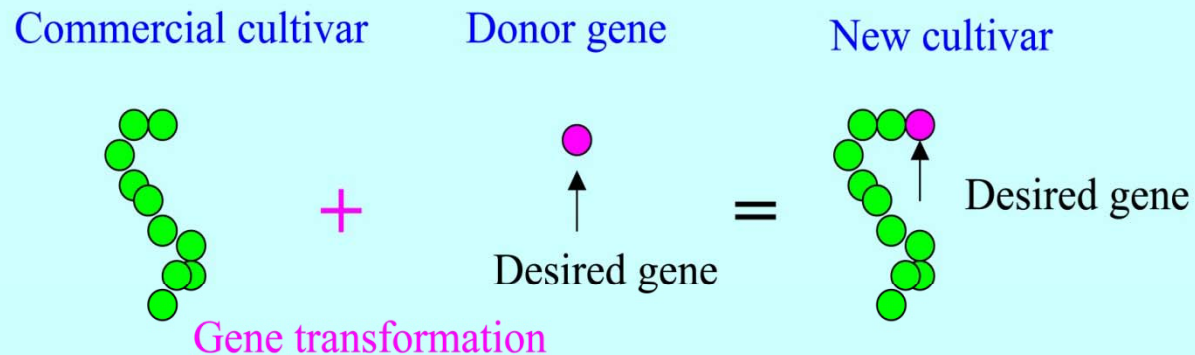A definition of biotechnology from the U.S. Office of Technology Assessments reads,

"*Any technique that uses living organisms to make or modify products, to improve plants or animals, or to develop microorganisms for specific purposes.*"

Most people connect the word biotechnology with the idea of moving genes from one plant or animal or microbe to another, because genetic engineering is an important tool for a biotechnologist.

Traditional Plant Breeding

Commercial cultivar   Donor plant   New germplasm or cultivar

X   =

Crosses   Desired gene   Desired gene

Plant Breeding via genetic transformation

Commercial cultivar   Donor gene   New cultivar

+   =   Desired gene

Desired gene

Gene transformation

Traditional vs. Transgenic Breeding

| Traditional Breeding	Transgenic Plants
Novel proteins may be introduced from closely-related plant species. Highly heterozygous nature; Lengthy intra- and inter-specific crosses; Limitations of the available germplasms.	Novel proteins may be introduced from ANY species. Vegetative propagation nature is a unique advantage for woody plants
LITTLE control over how or where a gene is expressed.	PRECISE control over how or where a gene is expressed.
Many genes exchange.	Only one gene added or inactivated.
Some unsafe traits can be bred out.	Increased number of ways to make foods safer.

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Branches:

Biotechnology branches	Genetic engineering
	Diagnostic techniques
	Cell/tissue techniques

Applications:

Biotechnology applications	Agriculture
	Medicine
	Food processing
	Bioremediation
	Energy production

Why alter plants?

Growing populations
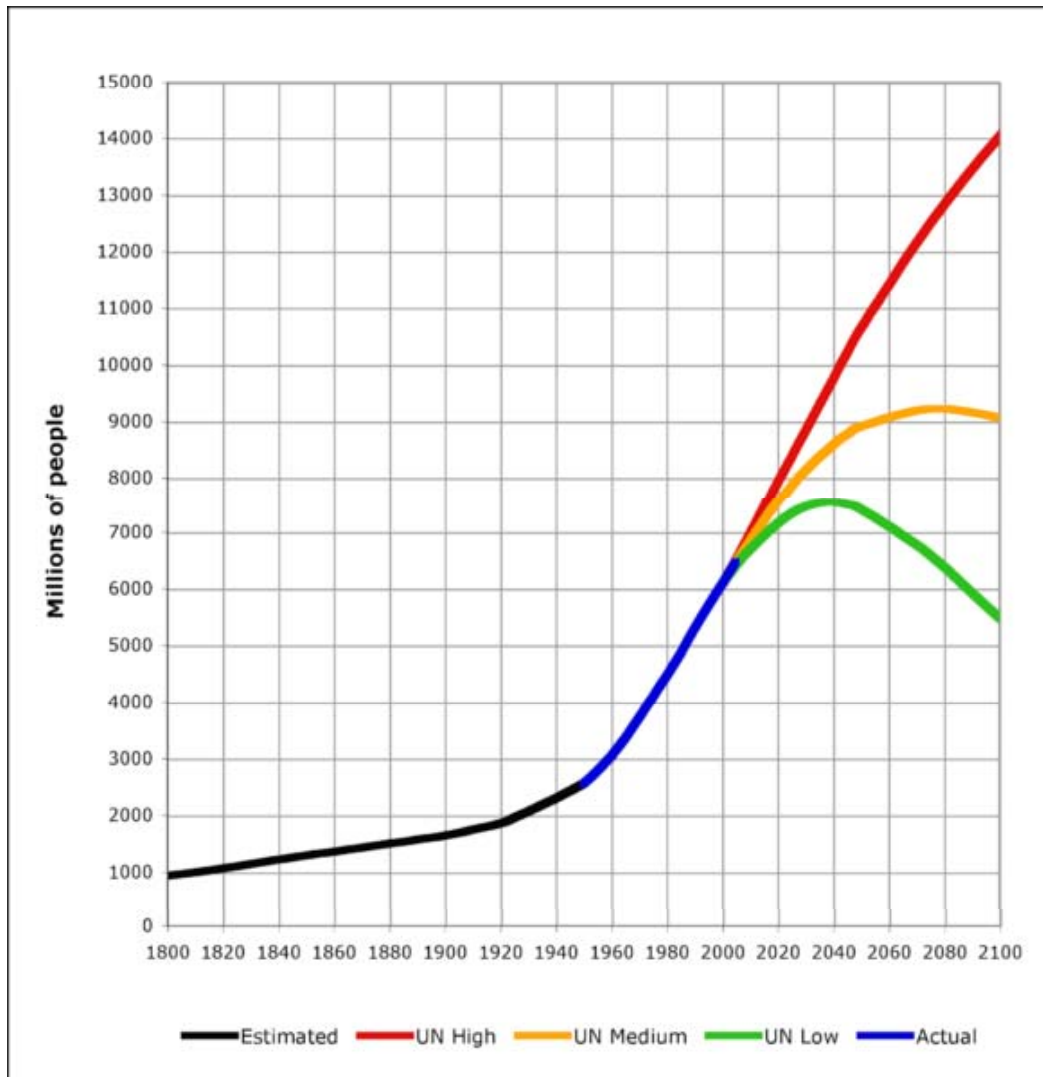
World population from 1800 to 2100, based on UN 2004 projections (red, orange, green) and US Census Bureau historical estimates (black).

Why alter plants?

Growing populations, falling energy sources and food shortages will create the **"perfect storm" by 2030**, the UK government chief scientist has warned. He said food reserves are at a 50-year low but the world requires 50% more energy, food and water by 2030 (BBC News, Mar 19, 2009).
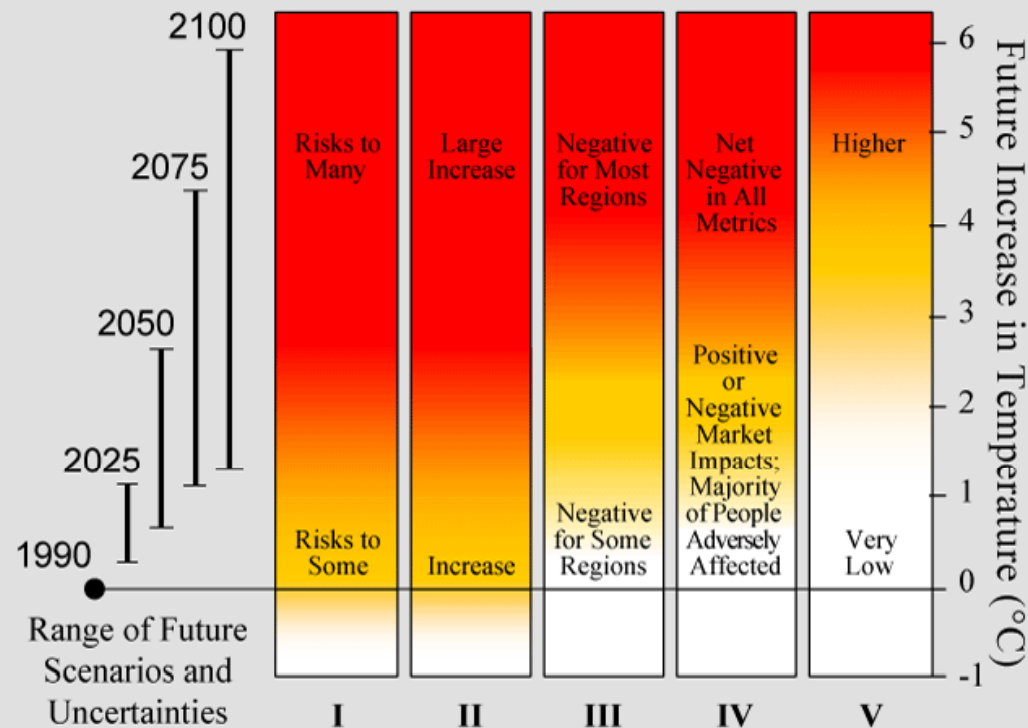
- Improving agricultural productivity globally
- At present, 30-40% of all crops are lost due to pest and disease before they are harvested.
- Professor Beddington said: "We have to address that. We need more disease-resistant and pest-resistant plants and better practices, better harvesting procedures.
- **"Genetically-modified food could also be part of the solution"**. We need plants that are resistant to drought and salinity - a mixture of genetic modification and conventional plant breeding.
- Better water storage and cleaner energy supplies are also essential.
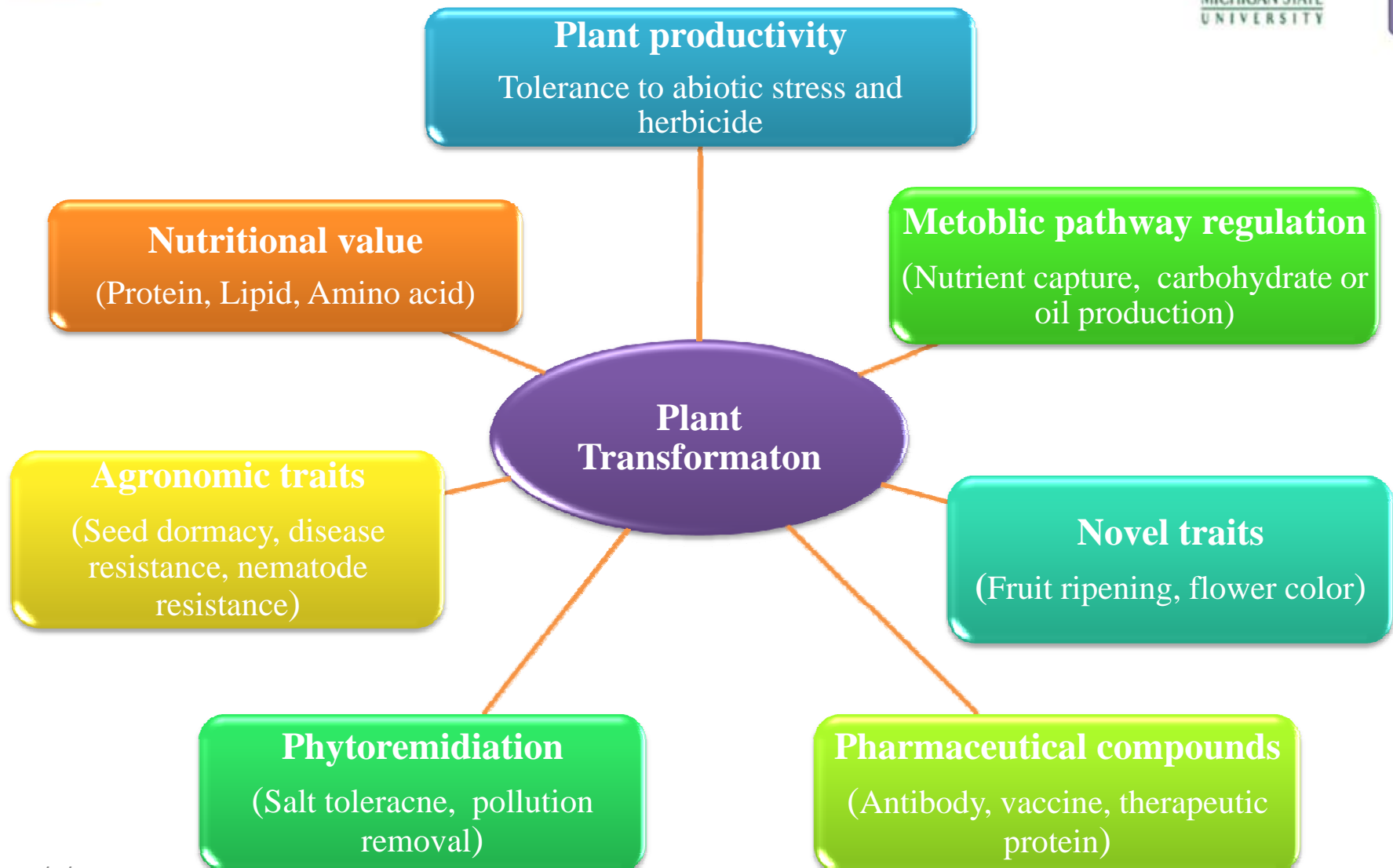
Why alter plants?

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Why alter plants?

Plant productivity

Tolerance to abiotic stress and herbicide

Nutritional value

(Protein, Lipid, Amino acid)

Metoblic pathway regulation

(Nutrient capture, carbohydrate or oil production)

Plant Transformaton

Agronomic traits

(Seed dormacy, disease resistance, nematode resistance)

Novel traits

(Fruit ripening, flower color)

Phytoremidiation

(Salt toleracne, pollution removal)

Pharmaceutical compounds

(Antibody, vaccine, therapeutic protein)

GM Plants-Milestones

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2008 — 25 biotech crop countries. biotech crop area reached over 160 million hectares

1997 — First weed- and inset-resistant biotech crops commercialized

1994 — First FDA approval for a whole food produced through biotechnology: FLAVRSAVR™ tomato

1992 — The FDA declares that biotech foods are "not inherently dangerous"

1989 — First approval for field test of insect-protected cotton.

1987 — First approval for field-test of modified food plants: virus-resistant tomatoes.

1985 — Transgenic plants resistant to insects, viruses and bacteria are field-tested for the first time.

1982 — First genetic transformation of plant cell: petunia.

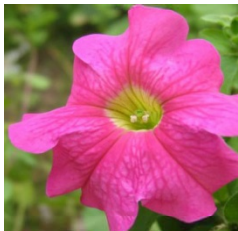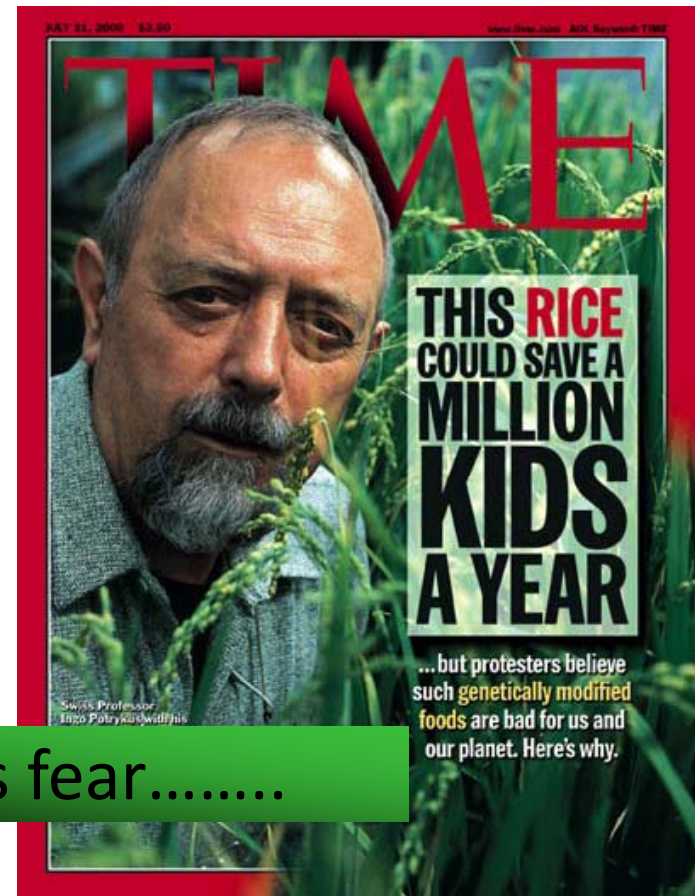# GM Plants-Biotechnology Milestones

1994

July 31, 2000

First FDA approval for a whole food produced through biotechnology: FLAVRSAVR™ tomato

1983

First genetic transformation of plant cell: petunia.

TIME

THIS RICE COULD SAVE A MILLION KIDS A YEAR

...but protesters believe such genetically modified foods are bad for us and our planet. Here's why.

Swiss Professor Ingo Potrykus with his

But Protesters fear........

Kryder, R. D., S. P. Kowalski, et al. (2002). ISAAA Briefs 20: 1-56.
Nash, J. M. (2000). Time 156(5): 38-46.

GM food crops

Wild type

Golden rice

TIME

JULY 31, 2000 $3.50

THIS RICE COULD SAVE A MILLION KIDS A YEAR

...but protesters believe such genetically modified foods are bad for us and our planet. Here's why.

Swiss Professor Ingo Potrykus with his beta-carotene-enriched rice

November 21, 2000

Golden Rice accumulates provitamin A (β-carotene) in the grain (http://www.goldenrice.org/)

Biofuel plants

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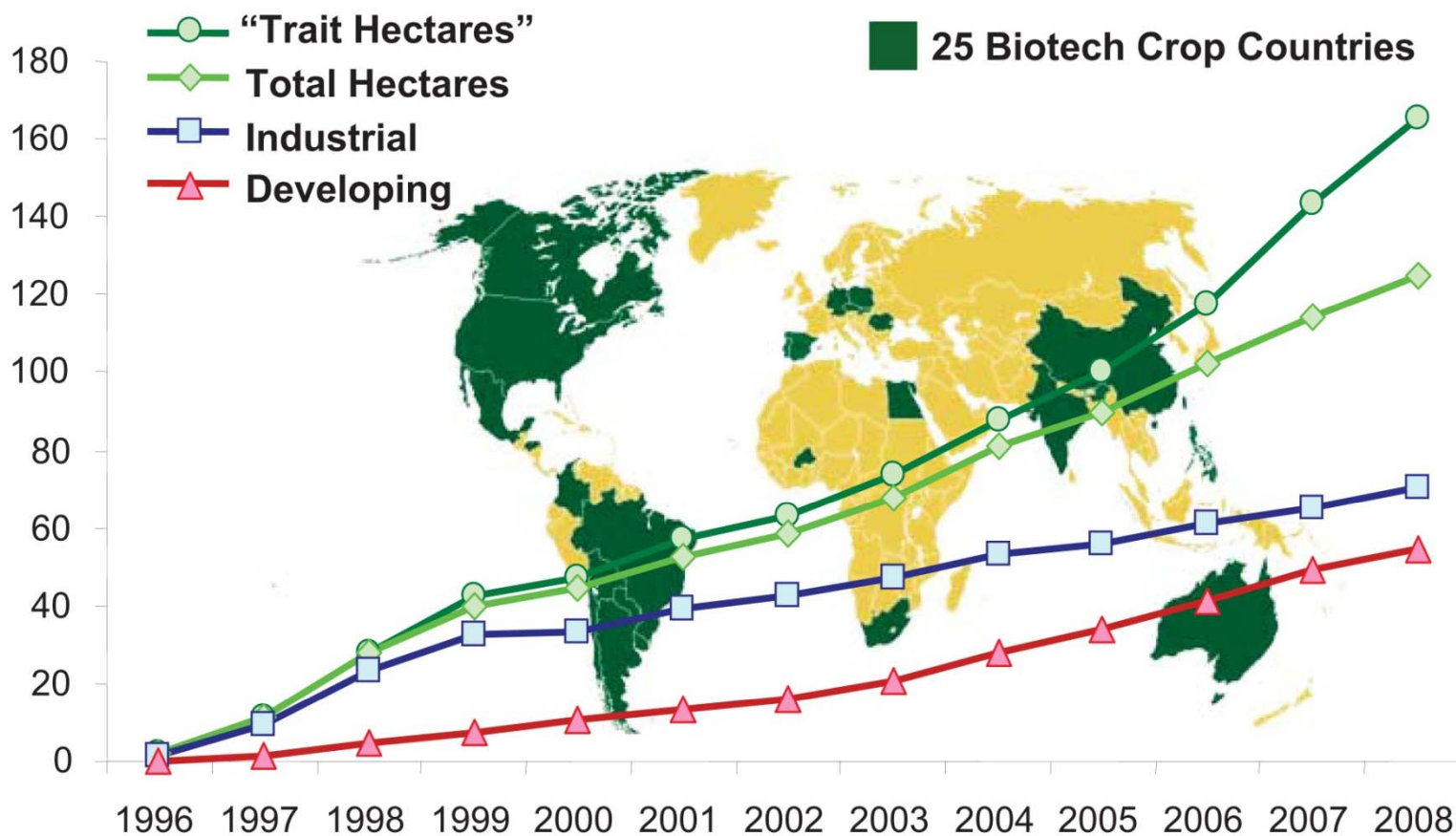Biofuels are transportation fuels produced from biomass.

First-generation biofuels are produced in two ways. One way is through the fermentation of either starch-based food products — such as corn kernels — or sugar-based food products — such as sugar cane — into ethanol. Another way is by processing vegetable oils, such as soy, rapeseed and palm, into biodiesel.

Second-generation biofuels are made from a wider variety of nonfood sources, such as cellulose, algae and recovered waste products. These fuels have the potential to be created from renewable resources such as switchgrass, forest and agricultural residues, municipal solid waste, and new energy crops.

In the United States, the Energy Independence and Security Act of 2007 set a mandatory Renewable Fuel Standard requiring fuel producers to use at least 36 billion gallons of biofuels by 2022. This increase in renewable fuels is projected to represent roughly 5 percent of the total U.S. gasoline consumption. Most of this increase is expected to be ethanol.

April 7, 2008

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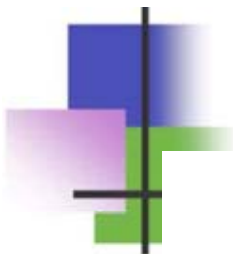
GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996 to 2008)

Legend:
- "Trait Hectares"
- Total Hectares
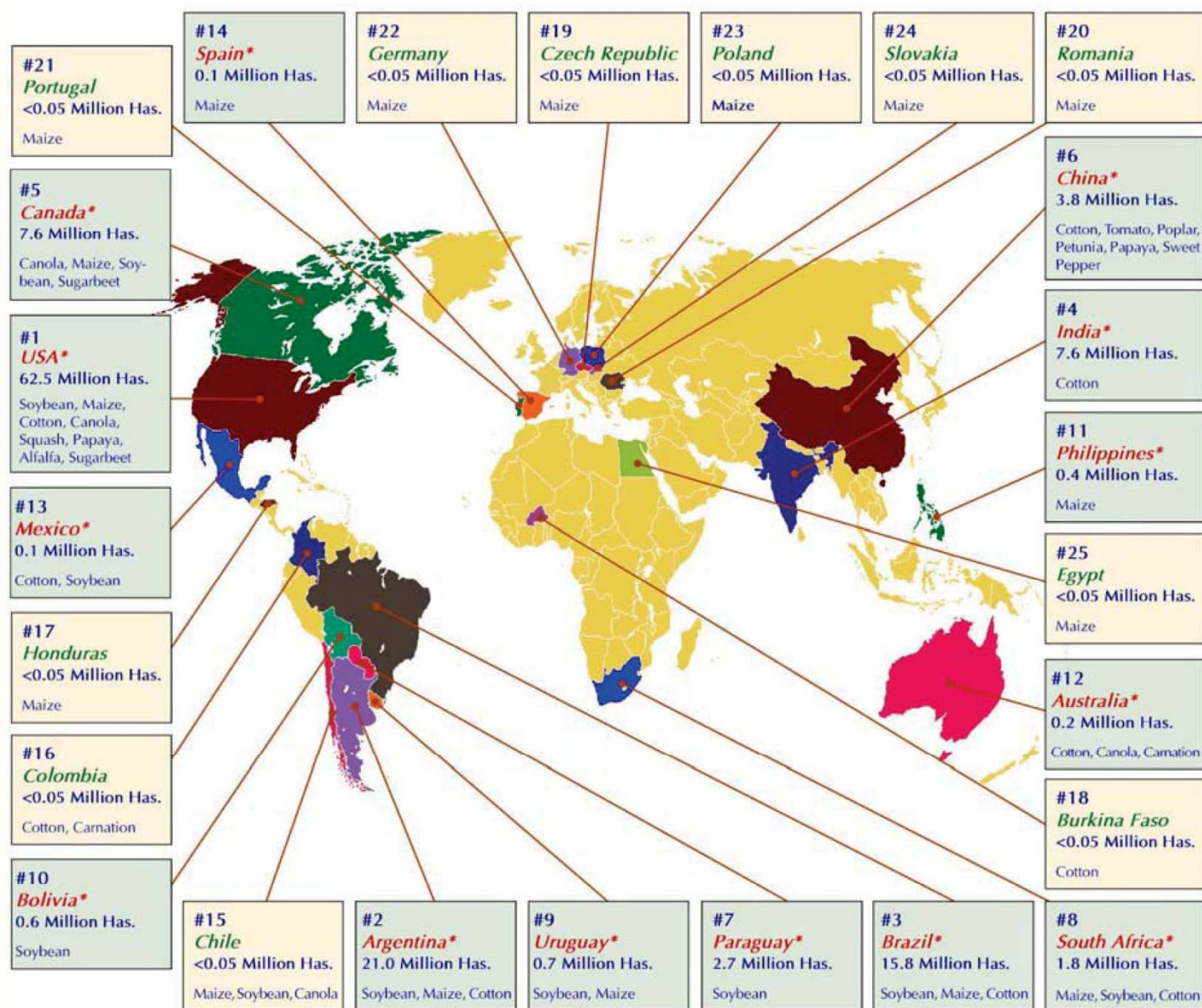- Industrial
- Developing
- 25 Biotech Crop Countries

An "apparent" increase of 9.4% or 10.7 million hectares between 2007 and 2008, equivalent to a "real" increase of 15% or 22 million "trait hectares"

Source: Clive James, 2009.

http://www.isaaa.org/

Biotech Crop Countries and Mega-Countries, 2008

#21 Portugal
<0.05 Million Has.
Maize

#14 Spain*
0.1 Million Has.
Maize

#22 Germany
<0.05 Million Has.
Maize

#19 Czech Republic
<0.05 Million Has.
Maize

#23 Poland
<0.05 Million Has.
Maize

#24 Slovakia
<0.05 Million Has.
Maize

#20 Romania
<0.05 Million Has.
Maize

#5 Canada*
7.6 Million Has.
Canola, Maize, Soybean, Sugarbeet

#1 USA*
62.5 Million Has.
Soybean, Maize, Cotton, Canola, Squash, Papaya, Alfalfa, Sugarbeet

#13 Mexico*
0.1 Million Has.
Cotton, Soybean

#17 Honduras
<0.05 Million Has.
Maize

#16 Colombia
<0.05 Million Has.
Cotton, Carnation

#10 Bolivia*
0.6 Million Has.
Soybean

#6 China*
3.8 Million Has.
Cotton, Tomato, Poplar, Petunia, Papaya, Sweet Pepper

#4 India*
7.6 Million Has.
Cotton

#11 Philippines*
0.4 Million Has.
Maize

#25 Egypt
<0.05 Million Has.
Maize

#12 Australia*
0.2 Million Has.
Cotton, Canola, Carnation

#18 Burkina Faso
<0.05 Million Has.
Cotton

#15 Chile
<0.05 Million Has.
Maize, Soybean, Canola

#2 Argentina*
21.0 Million Has.
Soybean, Maize, Cotton

#9 Uruguay*
0.7 Million Has.
Soybean, Maize

#7 Paraguay*
2.7 Million Has.
Soybean

#3 Brazil*
15.8 Million Has.
Soybean, Maize, Cotton

#8 South Africa*
1.8 Million Has.
Maize, Soybean, Cotton

* 14 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.

Source: Clive James, 2008.

http://www.isaaa.org/

Global Adoption Rates (%) for Principal Biotech Crops (Million Hectares, Million Acres), 2008
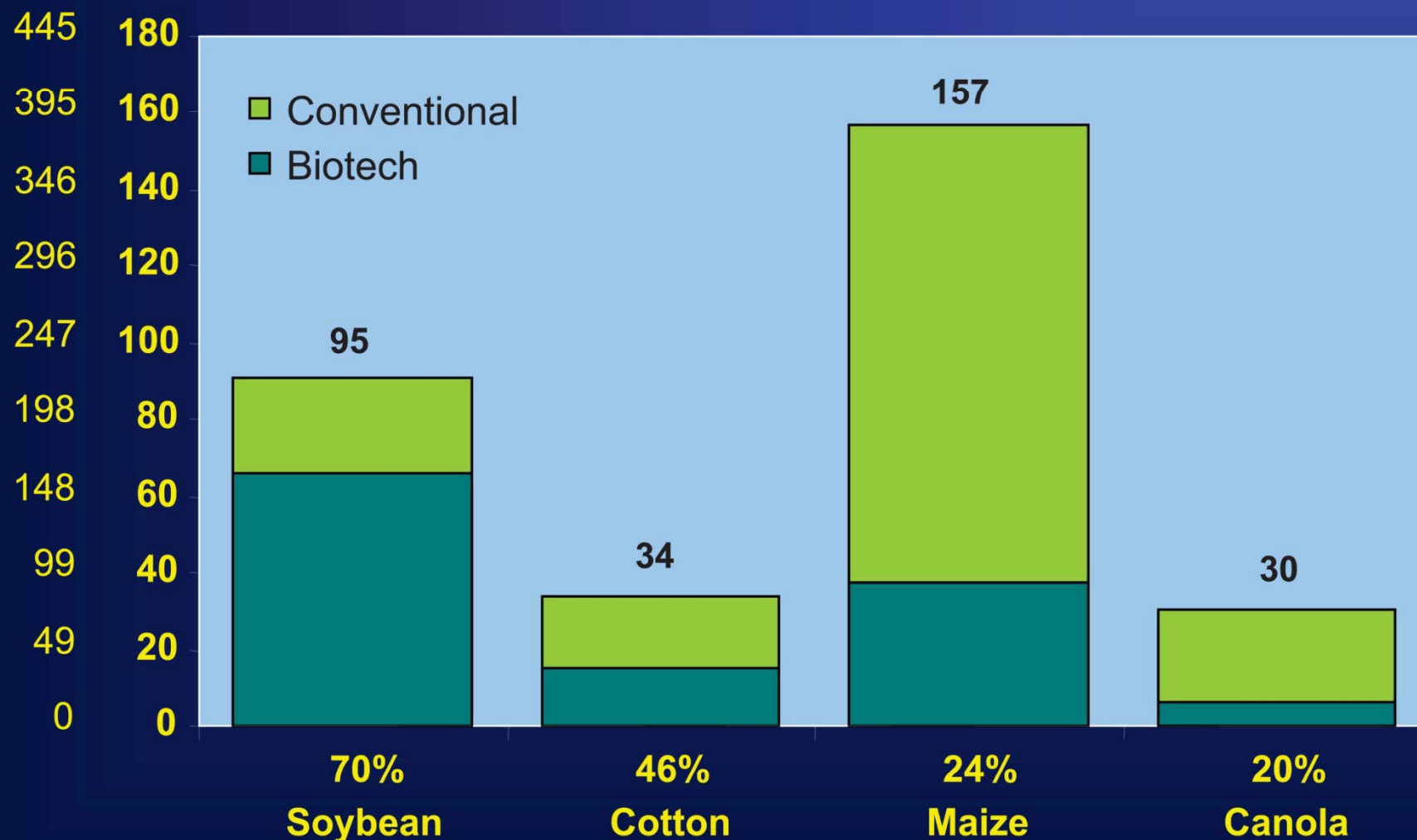
Source: Clive James, 2009

http://www.isaaa.org/

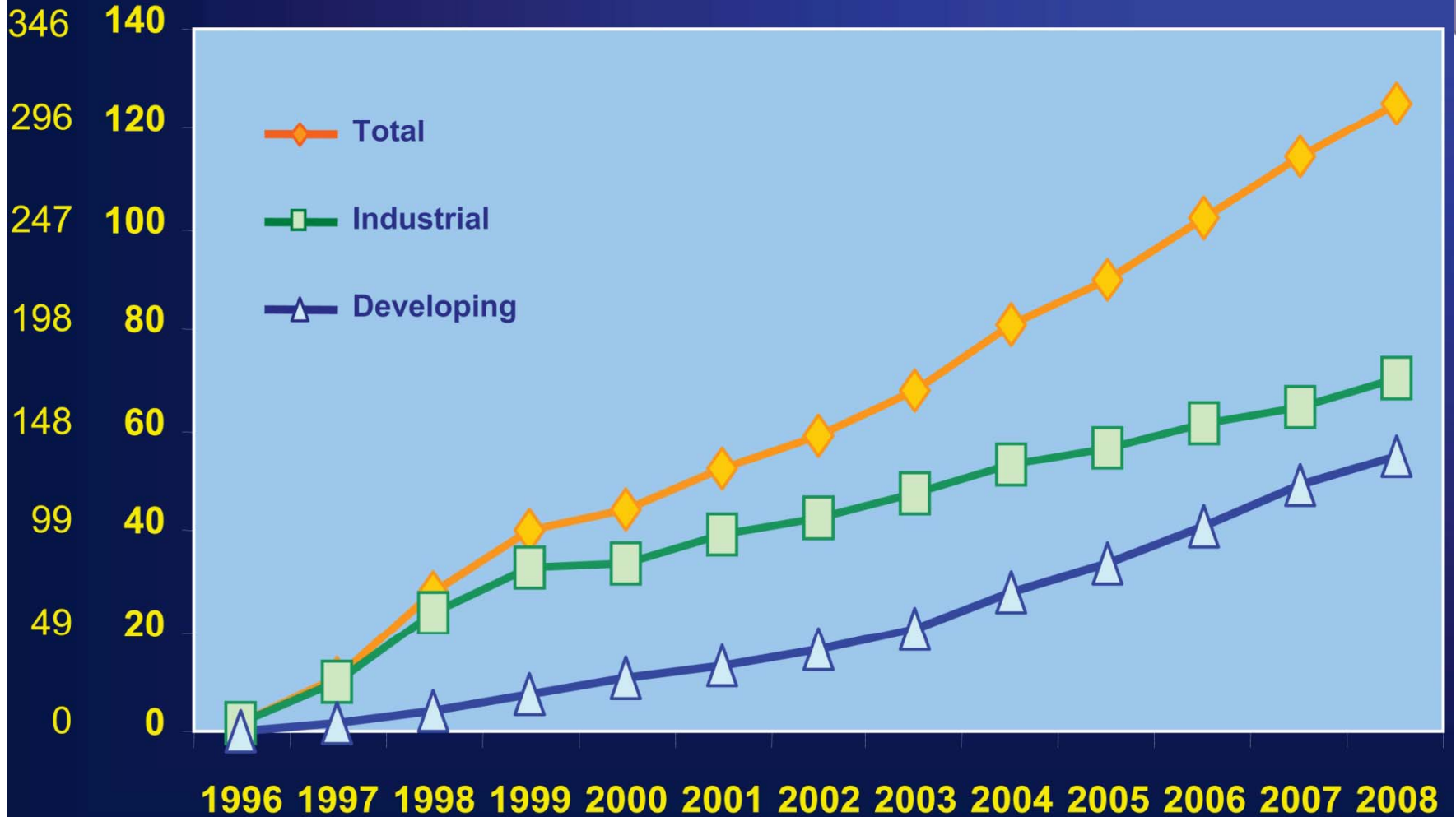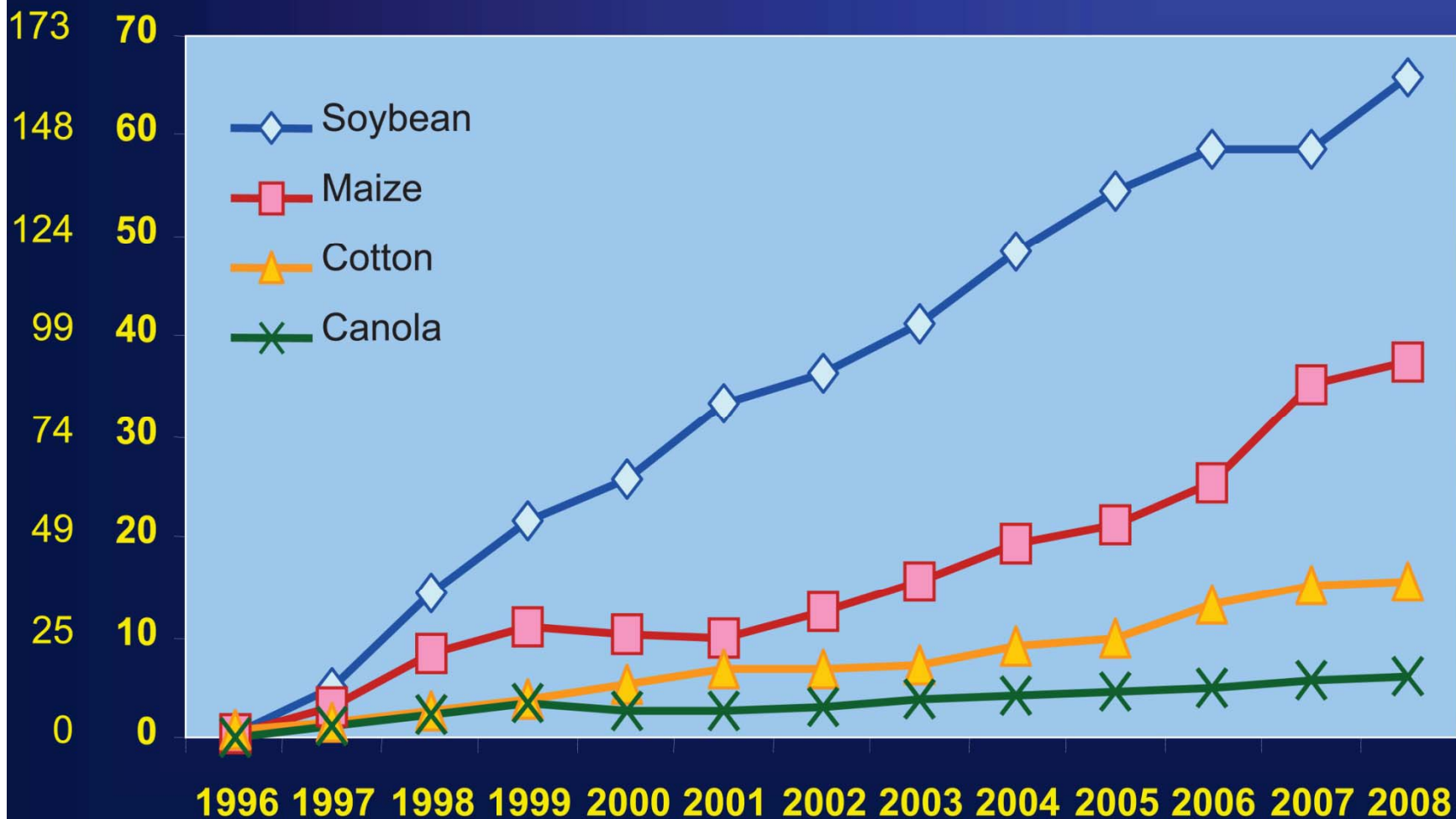**Global Area of Biotech Crops, 1996 to 2008: By Crop (Million Hectares, Million Acres)**

Source: Clive James, 2009

18

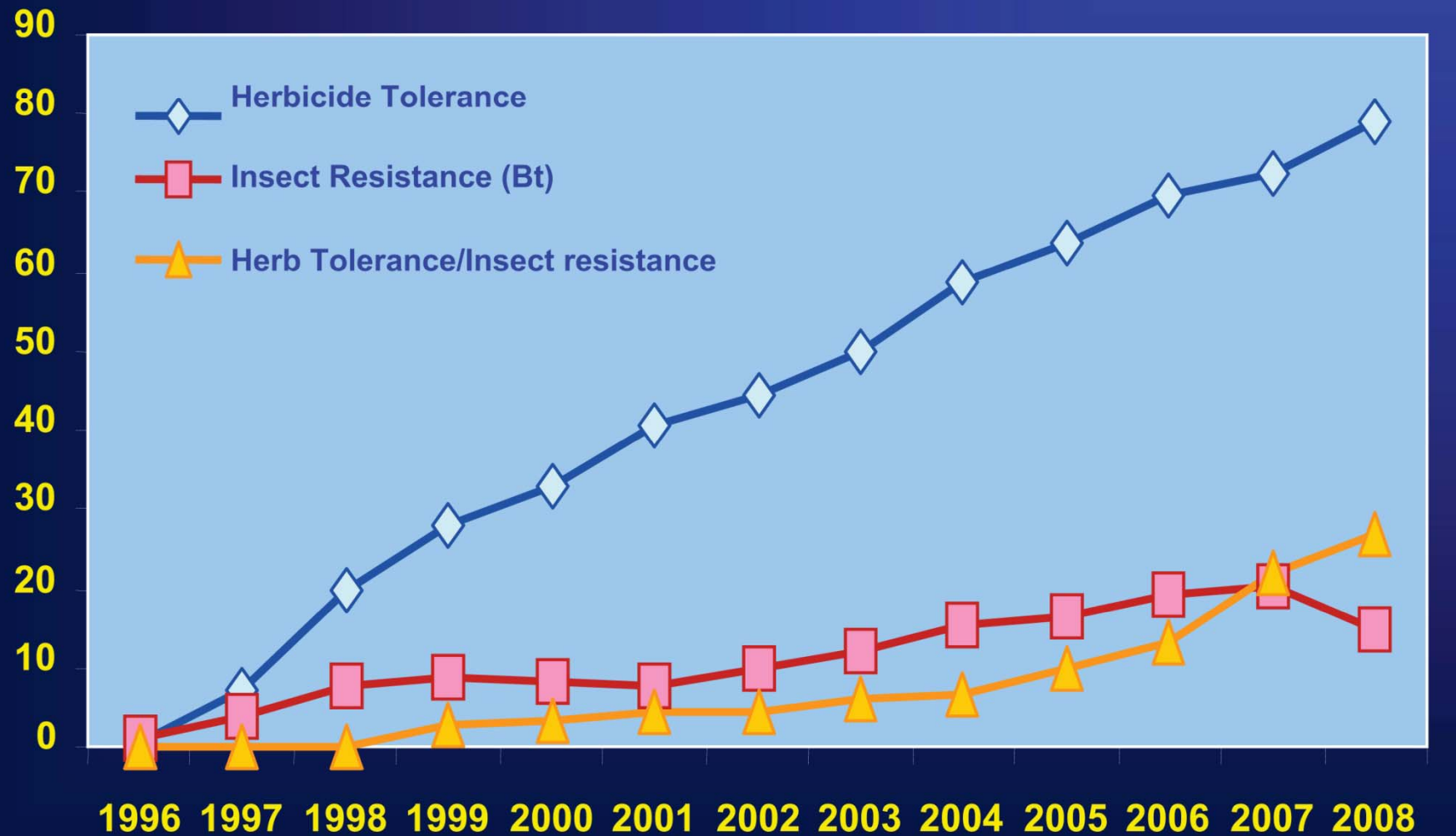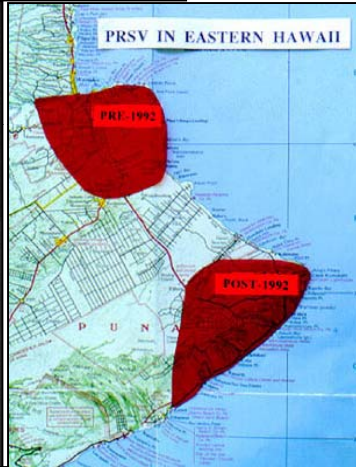**Global Area of Biotech Crops, 1996 to 2008: By Crop (Million Hectares, Million Acres)**

M Acres

Soybean
Maize
Cotton
Canola

1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008

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Global Area of Biotech Crops, 1996 to 2008: By Trait (Million Hectares, Million Acres)

M Hectares

Source: Clive James, 2009

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Story of Virus Resistant Papaya

Threat from Papaya Ring Spot Virus (PRSV) in Hawaii

1940 - PRSV discovered

1950s –

1. Eliminated large productions from Oahu Island

2. Papaya Industry relocated to Puna District (free of PRSV)

1980s –

Research started on resistance through transgenic approach

1992 –

PRSV spread to the Papaya fields in Puna
Small scale field trial with the transgenic lines

1998 –

GM papaya commercialized

Sequential Steps for Plant Engineering

Gene identification	Gene cloning	Transformation	Greenhouse & Field Test

- <u>Four major steps</u> are needed for successful engineering of plants.

Gene identification
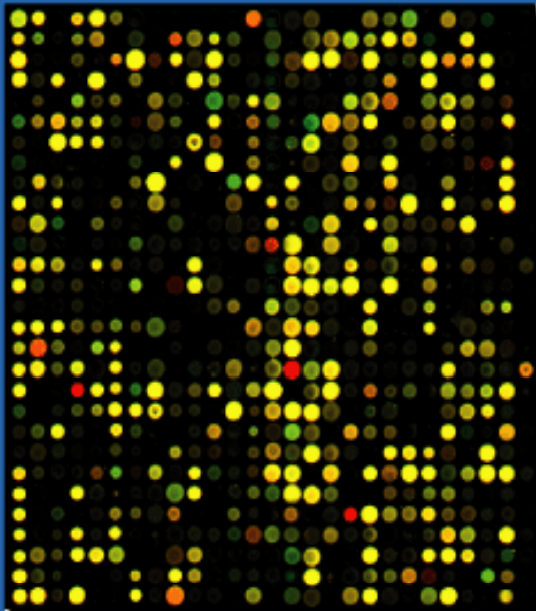
1. Search GenBank Database

2. Gene identification through EST sequence

3. Gene identification by microarray genomic analysis
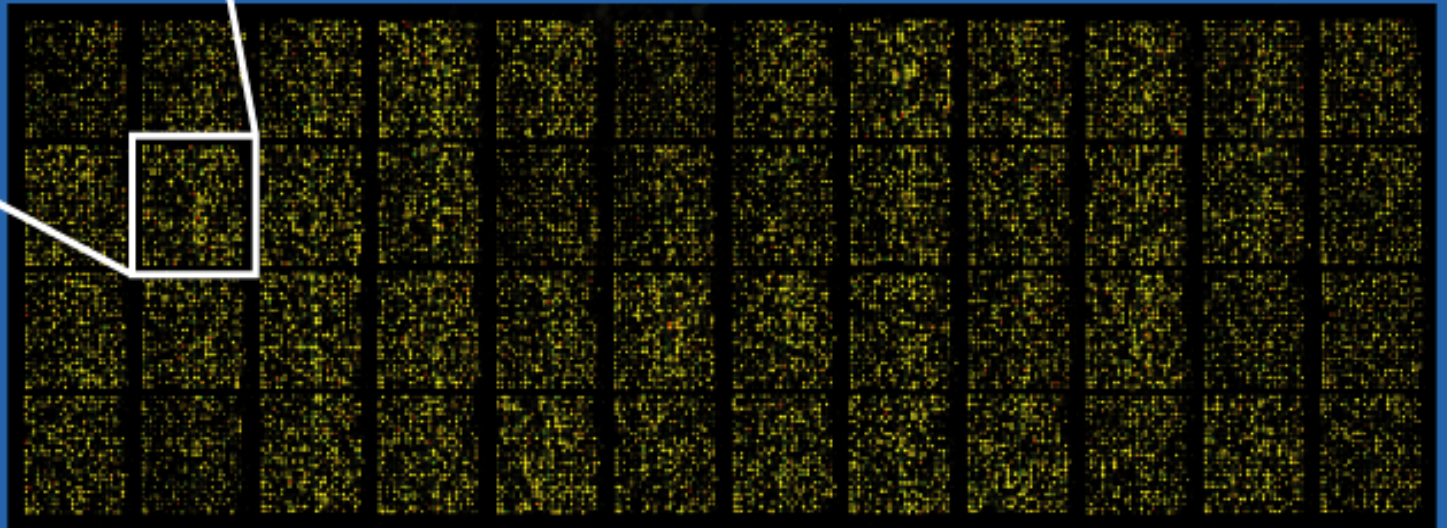
Search GenBank Database

The sequences for thousands of genes, and the proteins they encode, are known.

BLAST: The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences.

Gene identification through EST sequence

cDNAs: DNA copies of mRNAs, called cDNAs. A cDNA "library", containing clones for thousands of genes, can be made using mRNA from almost any tissue from any species.

Expressed Sequence Tag (EST): A unique stretch of DNA within a coding region of a gene that is useful for identifying full-length genes and serves as a landmark for mapping. An EST is a sequence tagged site (STS) derived from cDNA.

Gene identification by microarray genomic analysis

Microarray: A microarray is a 2D array on a solid substrate (usually a glass slide or silicon thin-film cell) that assays large amounts of biological material using high-throughput screening methods.

Chapter 12 ---Studying Genomes (P252-274)

Concepts:

Genomics, Post-genomics (or Functional genomics), Bioinformatics, Transcriptome, Proteome, Genetic map, Physical map, SSR (Single sequence repeat or short tandem repeats), RFLPs (Restriction fragment leagth polymorphisms), SNPs (Single nucleotide polymorphisms), Open reading frame (ORF),

Strategies for sequence assembly: Shotgun approach and Clone contig approach

- How to sequence a genome?
- **Trying to understand a genome sequence**

An analogy to the human genome stored on DNA is that of instructions stored in a library:

1. The library would contain 46 books (chromosomes)
2. The books range in size from 400 to 3340 pages (genes), which is 48 to 250 million letters (A,C,G,T) per book.
3. Hence the library contains over six billion letters total;
4. The library fits into a cell nucleus the size of a pinpoint;
5. A copy of the library (all 46 books) is contained in almost every cell of our body.

Application of **next generation sequencing technologies** will greatly enhance generation of an unlimited set of sequence resources for more species and/or genotypes.
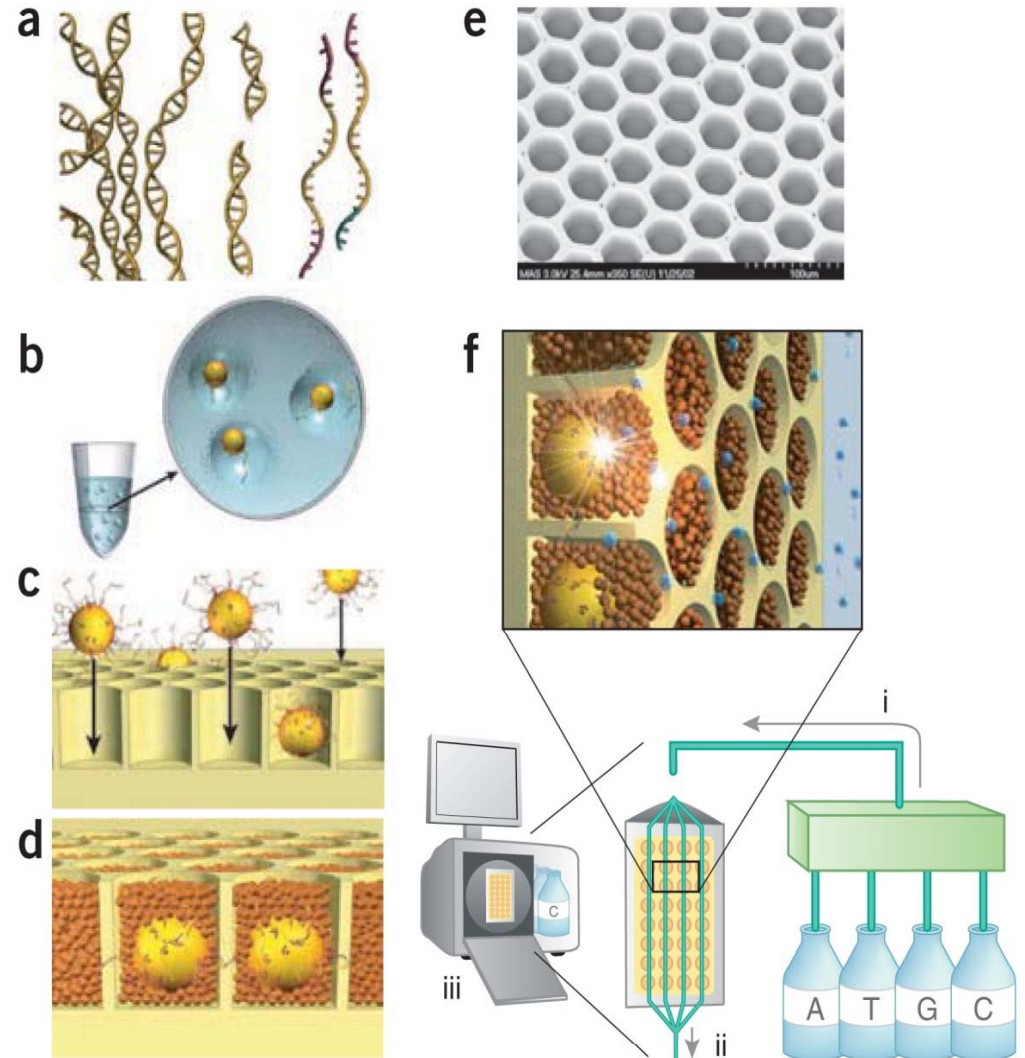
Overview of the 454 sequencing technology

An example of Poaceae species
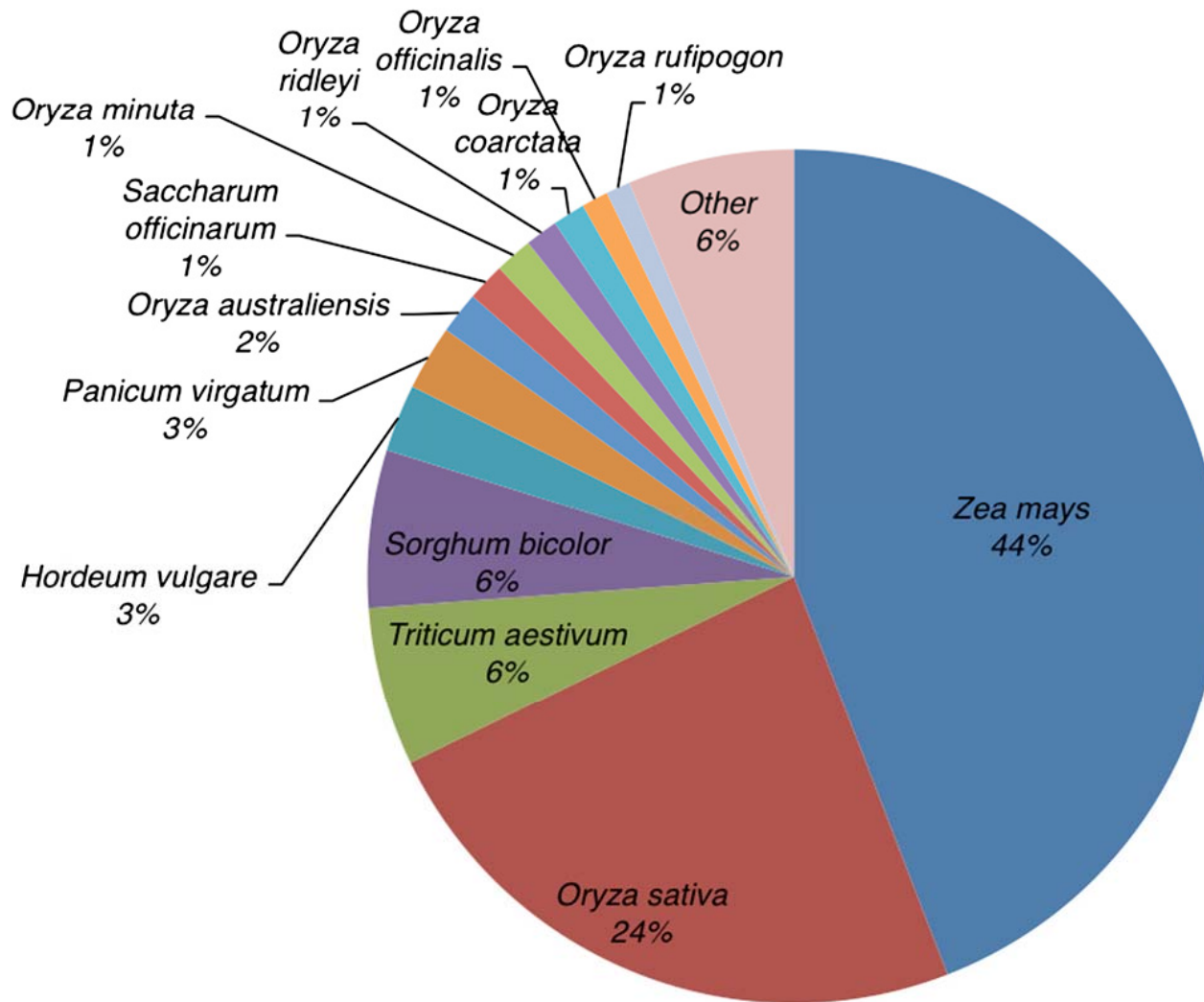
Genome sequence availability for 47 Poaceae species with genome projects. Sequence was downloaded for all 47 species from GenBank (October, 2008) and summed for all divisions. Thirteen species are represented individually in the pie chart; sequence for 34 species with less than 100 Mb of total sequence in GenBank were grouped into Other.

Buell CR (2009) Plant Physiology 149:111-116.

1. **Bioinformaticists** will develop reliable genomic resources, database, and analysis tools for further use of these genome datasets.

2. **Biotechnologists** will be able to identify and test more genes of interest.

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Gene cloning

A well-designed vector is key to success in gene transformation and expression

A well-designed vector is key to success in gene transformation and expression

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Gene Cloning & DNA Analysis
----- T.A. Brown

1. **Part 1: The Basic Principles of Gene Cloning and DNA Analysis**

2. **Part 2: The Application of Gene Cloning and DNA Analysis in Research**

3. **Part 3: The Application of Gene Cloning and DNA Analysis in Biotechnology**

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A well-designed vector is key to success in gene transformation and expression

Chapter 12 ---Studying Genomes (P252-274)

Concepts:
Genomics, Post-genomics (or Functional genomics), Bioinformatics, Transcriptome, Proteome, Genetic map, Physical map, SSR (Single sequence repeat or short tandem repeats), RFLPs (Restriction fragment leagth polymorphisms), SNPs (Single nucleotide polymorphisms), Open reading frame (ORF),

Strategies for sequence assembly: Shotgun approach and Clone contig approach

- How to sequence a genome?
- **Trying to understand a genome sequence**

A well-designed vector is key to success in expression of the transgenes

Promoter (Constitutive promoters vs inducible promoters)? Terminator?

Constitutive promoters: CaMV35S

Inducible prompters: cab

35S   cab   Wild type

33

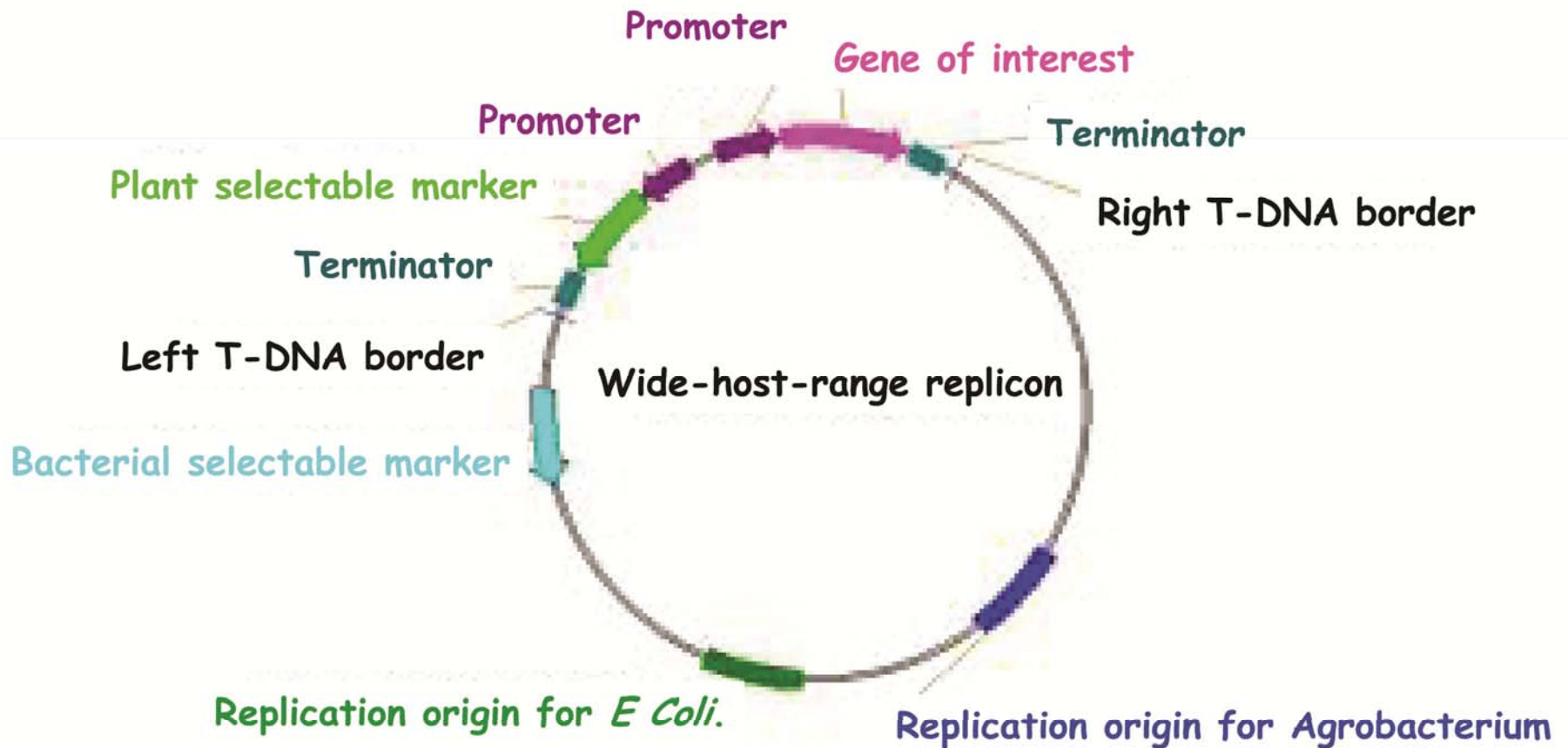# A well-designed vector is key to success in gene transformation

1. Binary vector vs. cloning vector
2. Size (usually <50 kb)
3. Selectable marker gene (*NPT*II, *hpt*, *bar*, and etc)? Screenable marker gene (*Lac*Z, *gus*A, *GFP*, and etc)? Or Maker-gene free (co-transformation, Cre/Lox, and etc)?

*Lac*Z
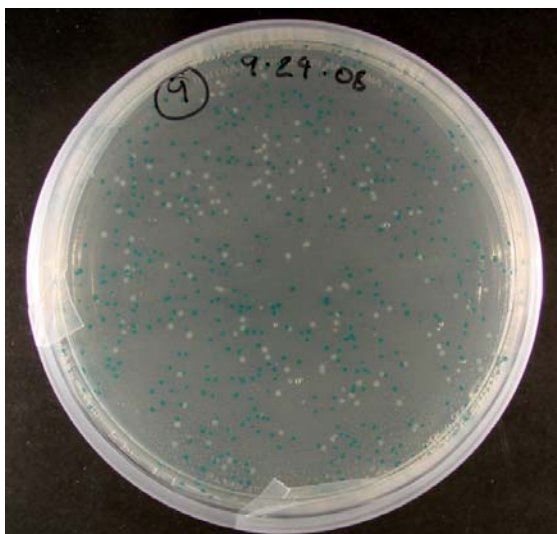
GFP
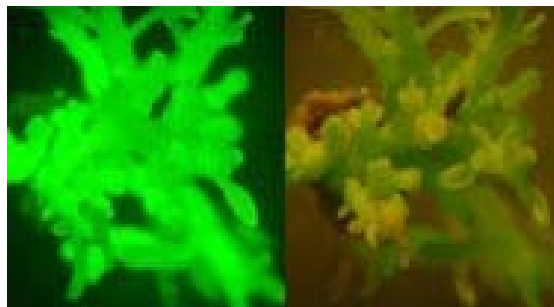
*gus*A
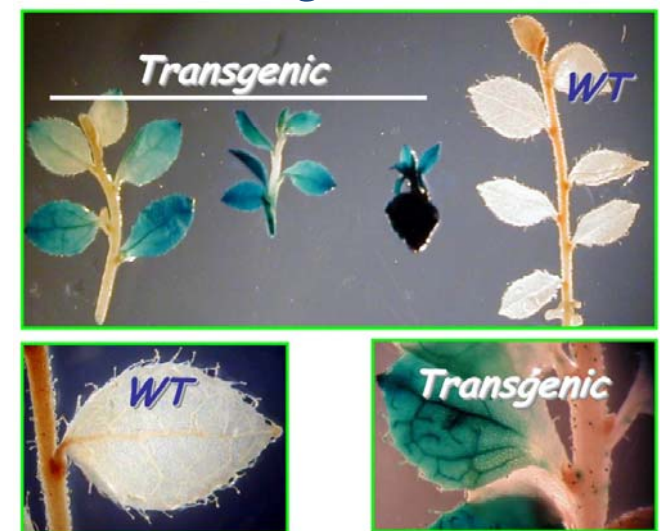
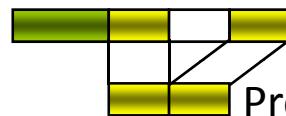# A well-designed vector is key to success in gene transformation

A binary vector map

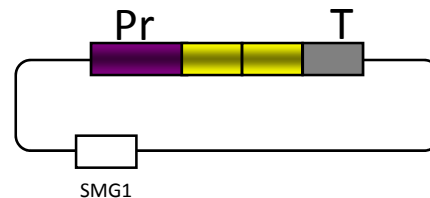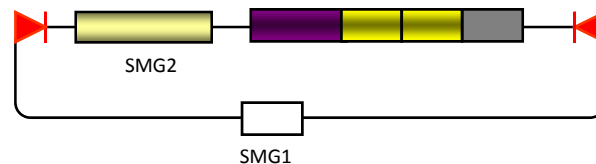# A well-designed vector is key to success in gene transformation
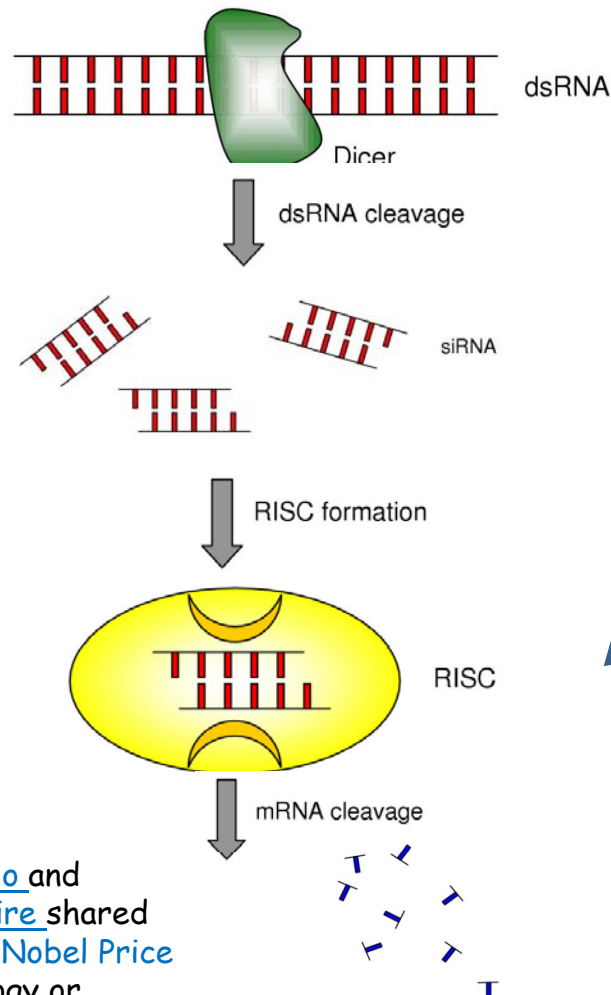

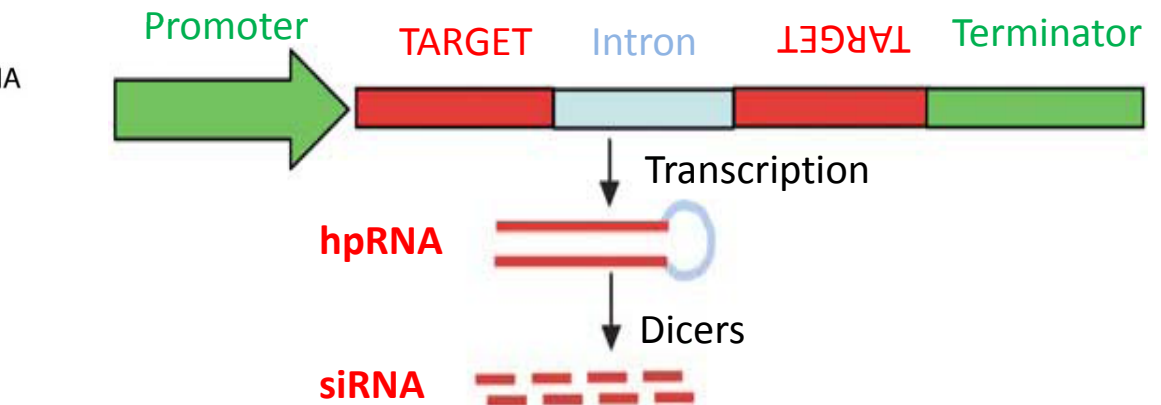Promoter

cDNA

Protein coding region

Cloning vector

Pr   T

SMG1

Binary vector

SMG2

SMG1

The interference RNA (RNAi) strategy

RNAi Mechanism

Hairpin RNAs (hpRNAs)-Mediated RNAi

dsRNA

Dicer

dsRNA cleavage

siRNA

RISC formation

RISC

mRNA cleavage

Promoter   TARGET   Intron   TARGET   Terminator

Transcription

hpRNA

Dicers

siRNA

RISC formation

Craig Mello and Andrew Fire shared the 2006 Nobel Price in Physiology or Medicine

hpRNA-mediated RNAi in plants operates through the viral defence pathway. This enables RNAi as a new strategy for engineering plants with virus resistance.

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Mocellin and Provenzano, *Journal of Translational Medicine* 2004 **2**:39
Li et al. Transgenic Research 2008 **17**: 679-694

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The interference RNA (RNAi) strategy

Selectable (or screenable) marker gene (SMG)-free strategies

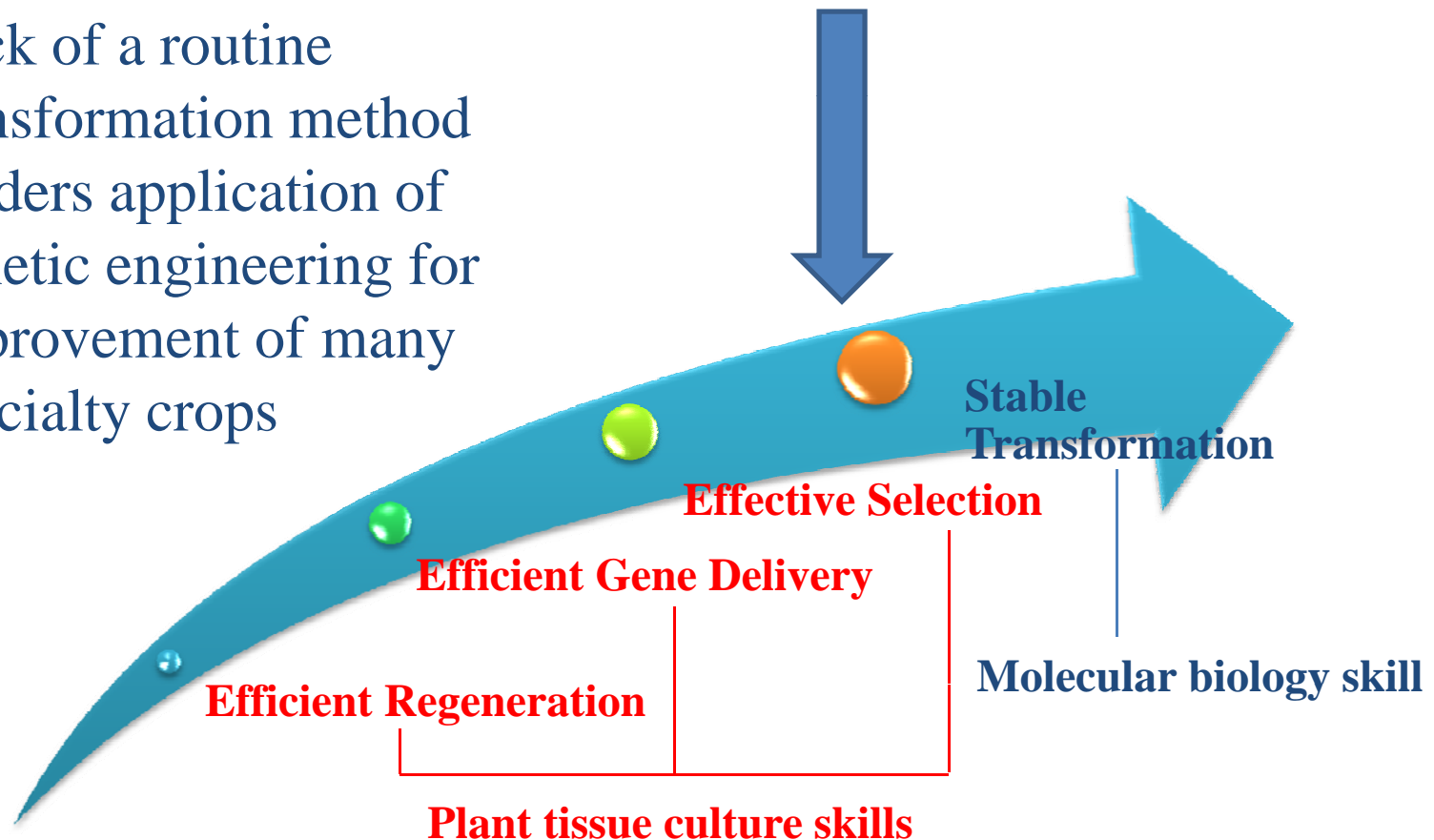**Engineered minichromosomes in plants?**
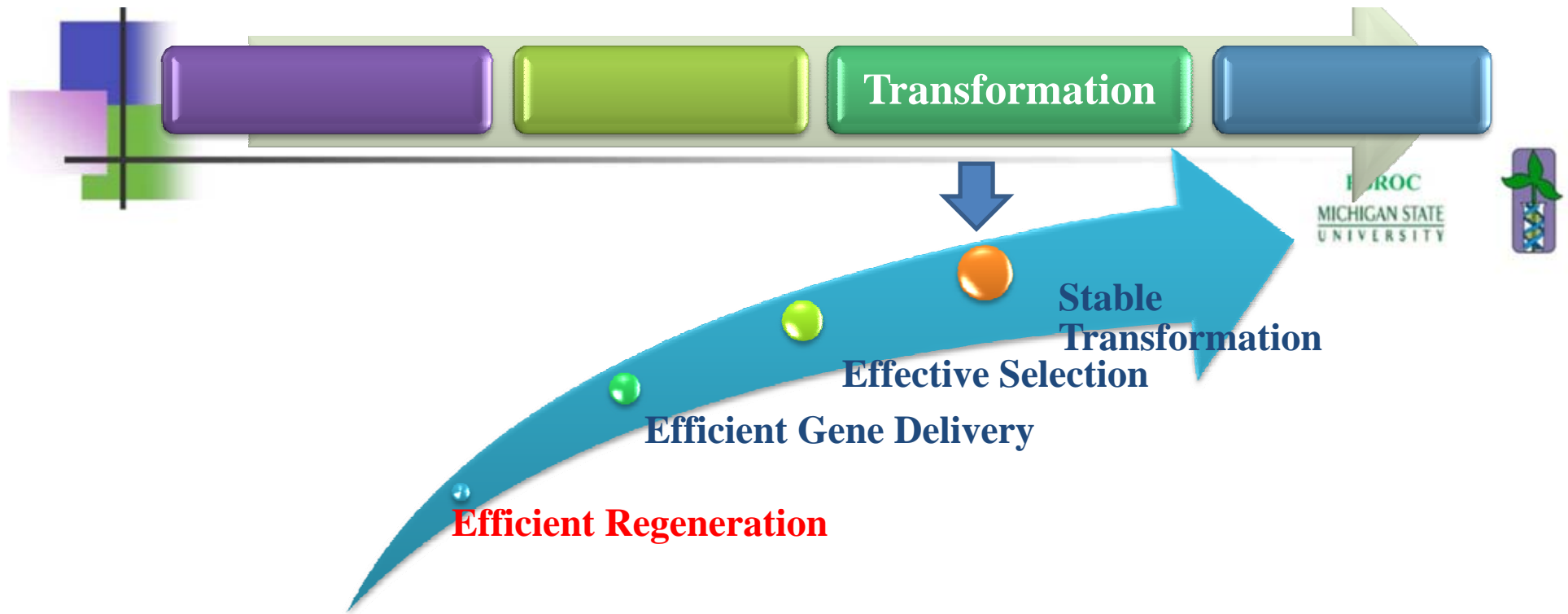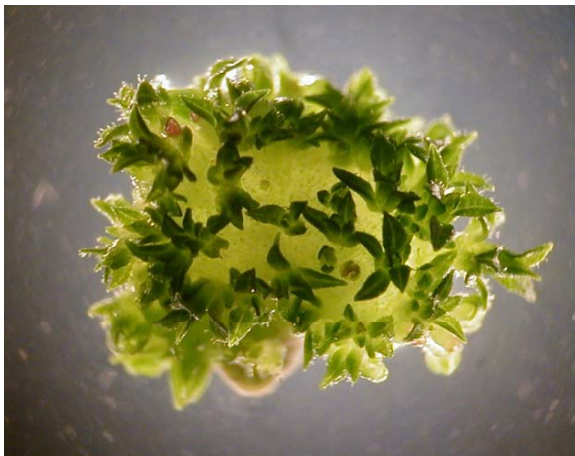
Transformation

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Transformation

Lack of a routine transformation method hinders application of genetic engineering for improvement of many specialty crops
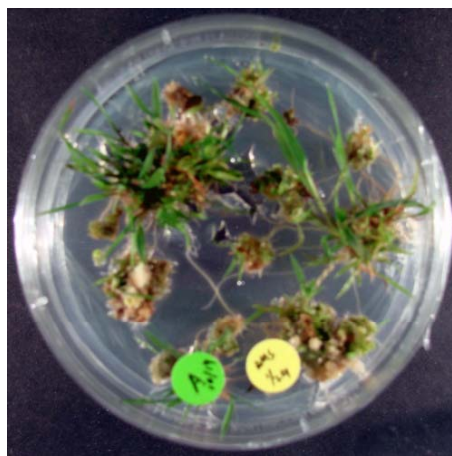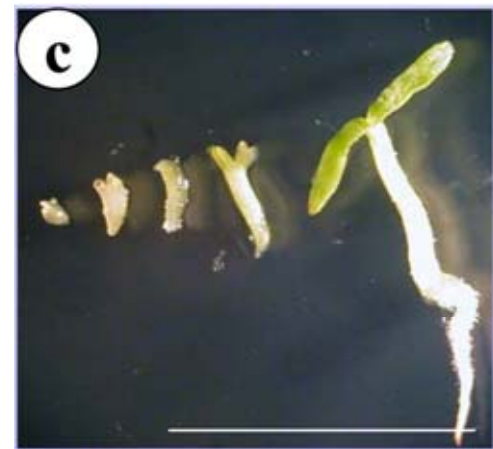
Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration

Plant tissue culture skills

Molecular biology skill

Transformation

Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration

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Blueberry

Switchgrass

Celery

40

Transformation

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Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration
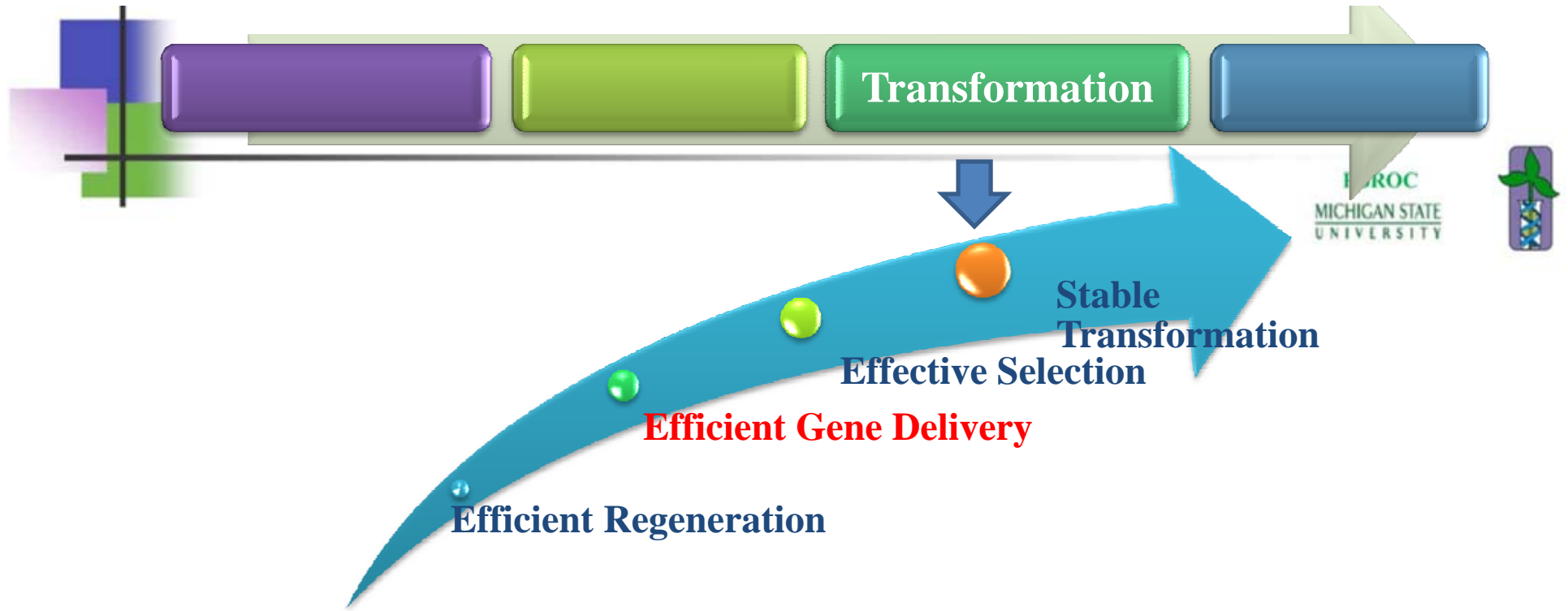
Biolistics

Agrobacterium

EHA105   LBA4404   GV3101

Transformation

Stable
Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration

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Transformation

Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration
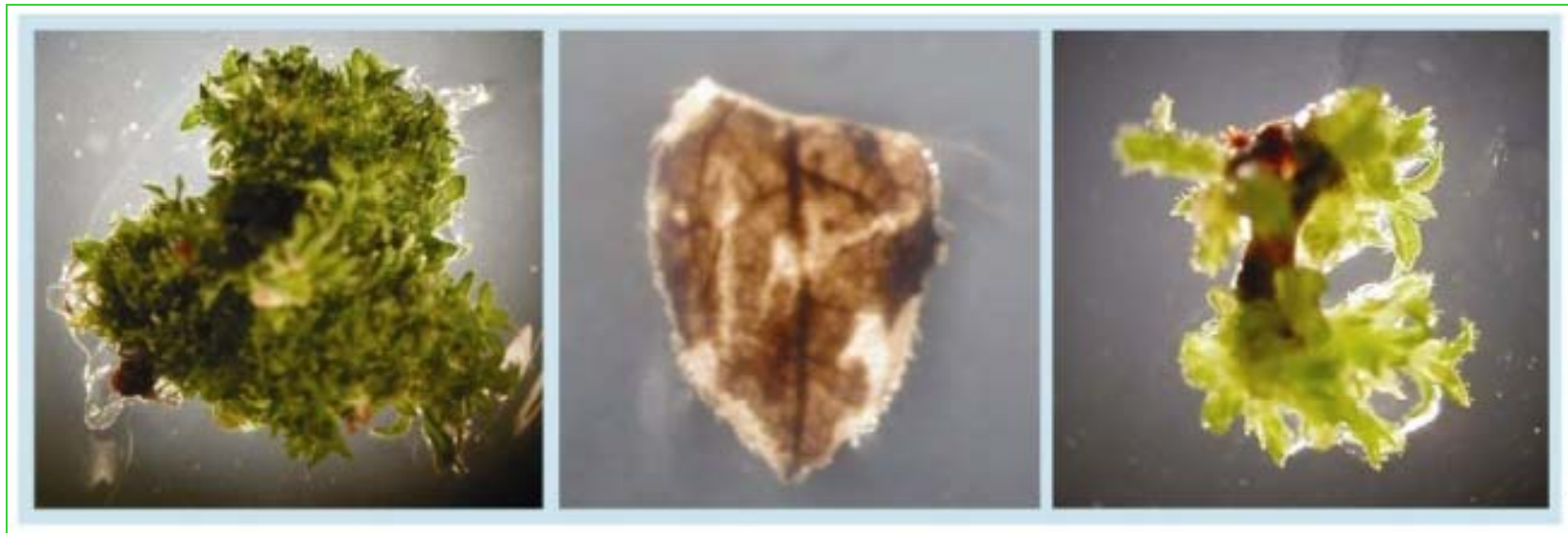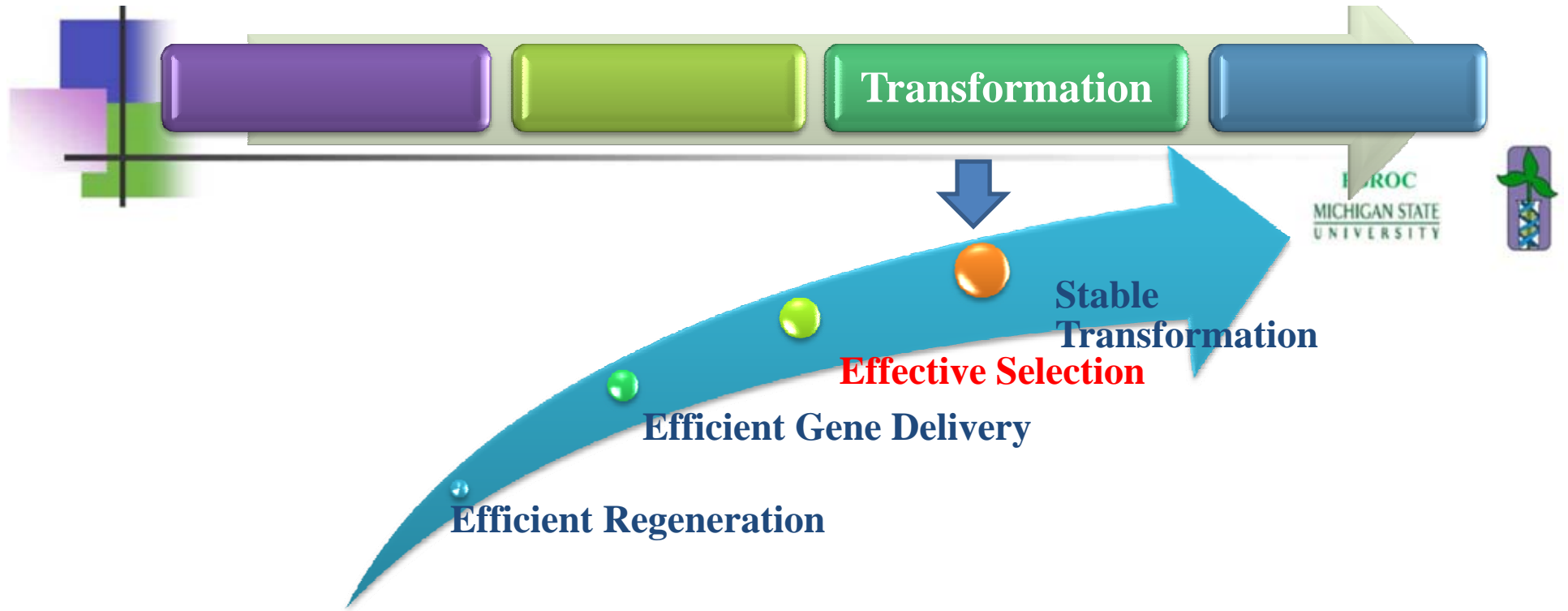
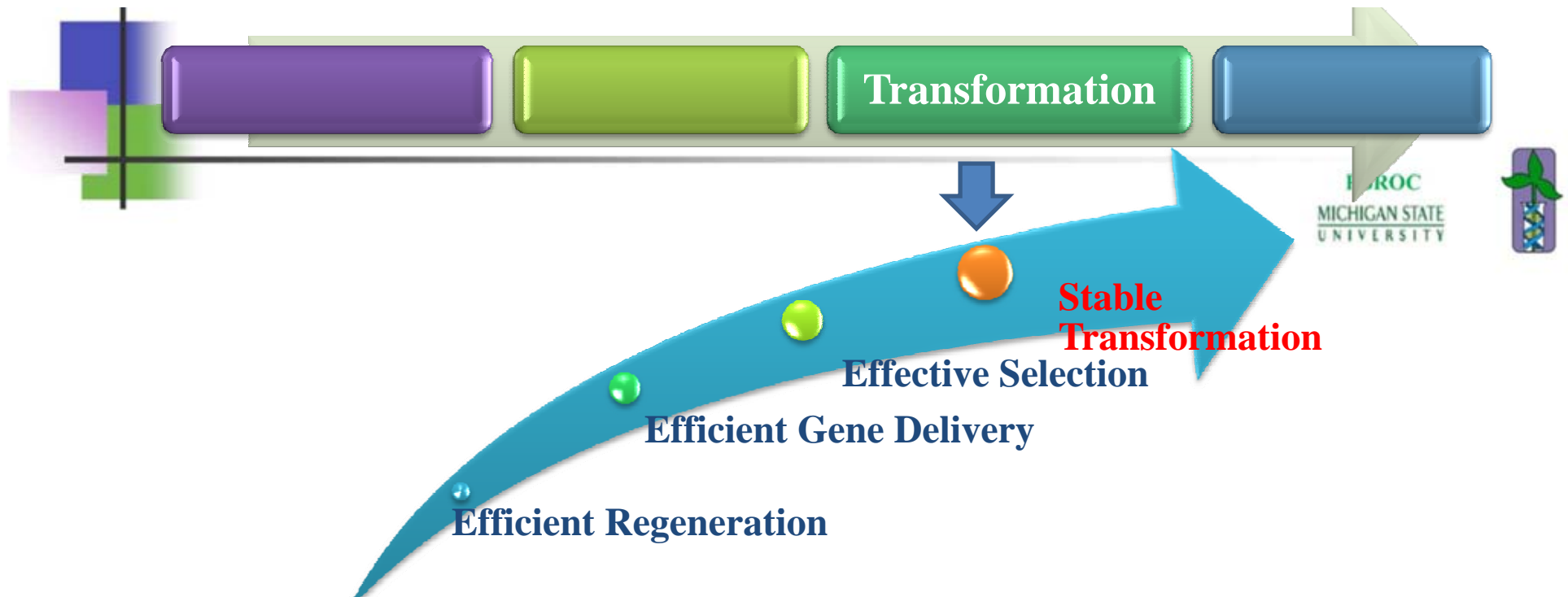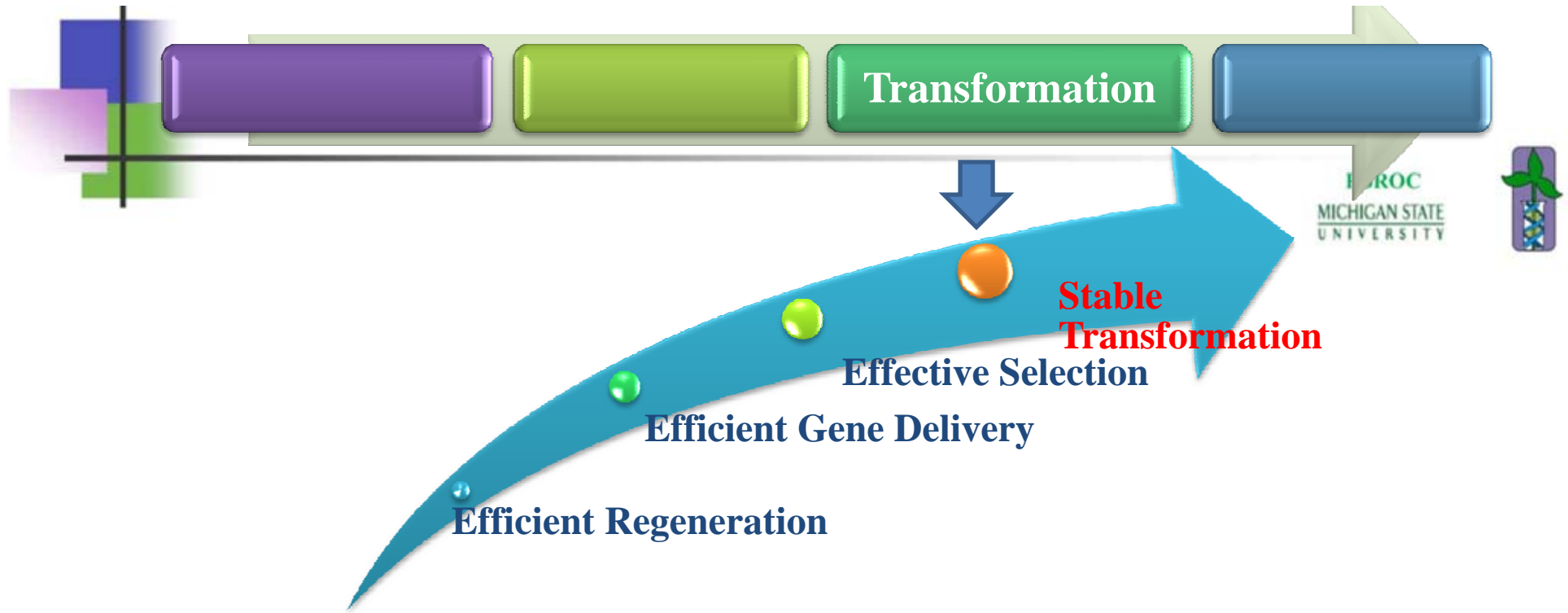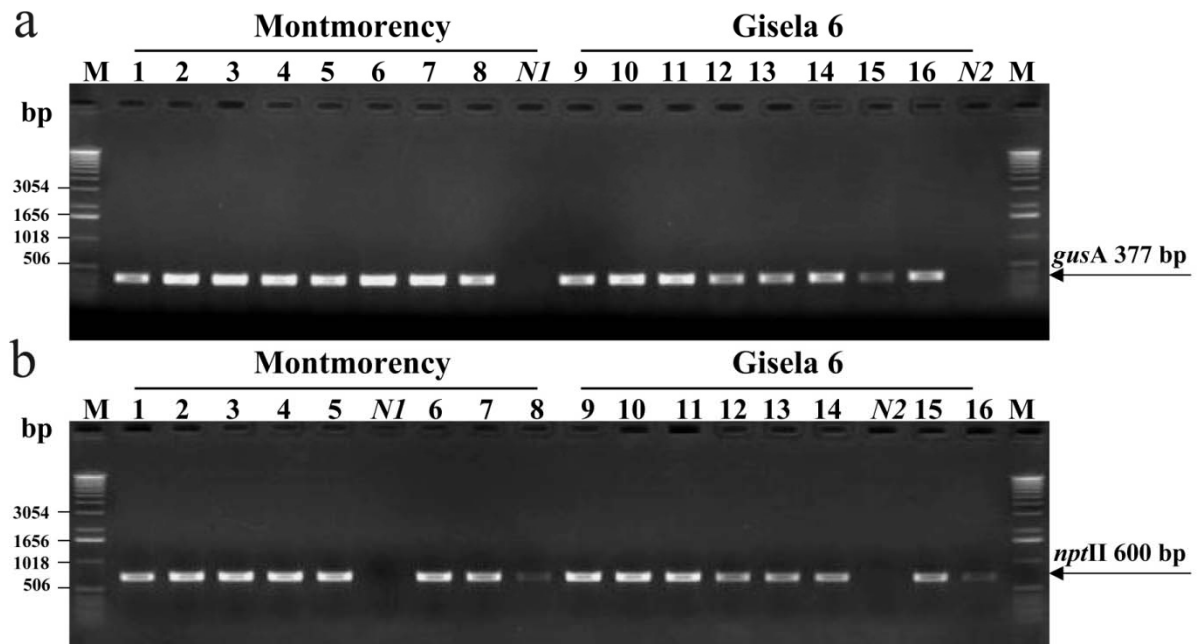|	
PCR	Primary screen of transformants (DNA level)
Southern blot	To confirm stable transformaiton and transgene copy number (DNA level)
RT-PCR (Reverse Transcriptase PCR)	To check if the transgene is transcripted or not (mRNA level)
Northern blot	To confirm the transcription of transgenes (mRNA level)
Western blot	To confirm functional transgene product (protein level)

Transformation

Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration

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PCR
Southern blot
RT-PCR (Reverse Transcriptase PCR)
Northern blot
Western blot

a

Montmorency   Gisela 6

M 1 2 3 4 5 6 7 8 *N1* 9 10 11 12 13 14 15 16 *N2* M

bp

3054
1656
1018
506

*gus*A 377 bp

b

Montmorency   Gisela 6

M 1 2 3 4 5 *N1* 6 7 8 9 10 11 12 13 14 *N2* 15 16 M

bp

3054
1656
1018
506

*npt*II 600 bp

Transformation

Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration

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| PCR |
| **Southern blot** |
| RT-PCR (Reverse Transcriptase PCR) |
| Northern blot |
| Western blot |

Montmorency clones | Gisela 6 clones

Kb

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | N1 | 8 | 9 | N2 | 10 | 11 | 12 |

12.2 —

6.1 —

3.1 —

Transformation

Stable Transformation

Effective Selection

Efficient Gene Delivery

Efficient Regeneration

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PCR
Southern blot
RT-PCR (Reverse Transcriptase PCR)
Northern blot
Western blot

A	WT	A 16	M 16	A 19	M 11	M 31	M 51	M 63	M 12	A 18	M 25	M 30	M 5	M 6	M 7	M 1

B	WT	A 16	M 16	A 19	M 11	M 31	M 51	M 63	M 12	A 18	M 25	M 30	M 5	M 6	M 7	M 1

Greenhouse and Field Test

Greenhouse & Field Test

- Commercialization of genetically modified (GM) crops in the US is regulated by three organizations.
  APHIS of USDA: the Animal and Plant Health Inspection Service of USDA. It regulates the import, transport, and field testing of GM crops through notification and permitting procedures. APHIS msut determine whether a GM plant is likely to have negative impact on agriculture and/or environment.
  FDA (Food and Drug Administration) determines food safty.
  EPA (Environmental Protection Agency) regulates GM plants that are engineered for pest resistance.
- Handling of recombinant DNA is regulated by Institutional Biosafety Committee (IBC) at the institutional level.

- **Why do we need these test?** Field tests are necessary not only for regulatory compliance and for evaluation of the efficacy of intentionally inserted transgenes, but also to assess unintentional interruptions of the native genome.

PPT=7,500 ppm, 1 week,
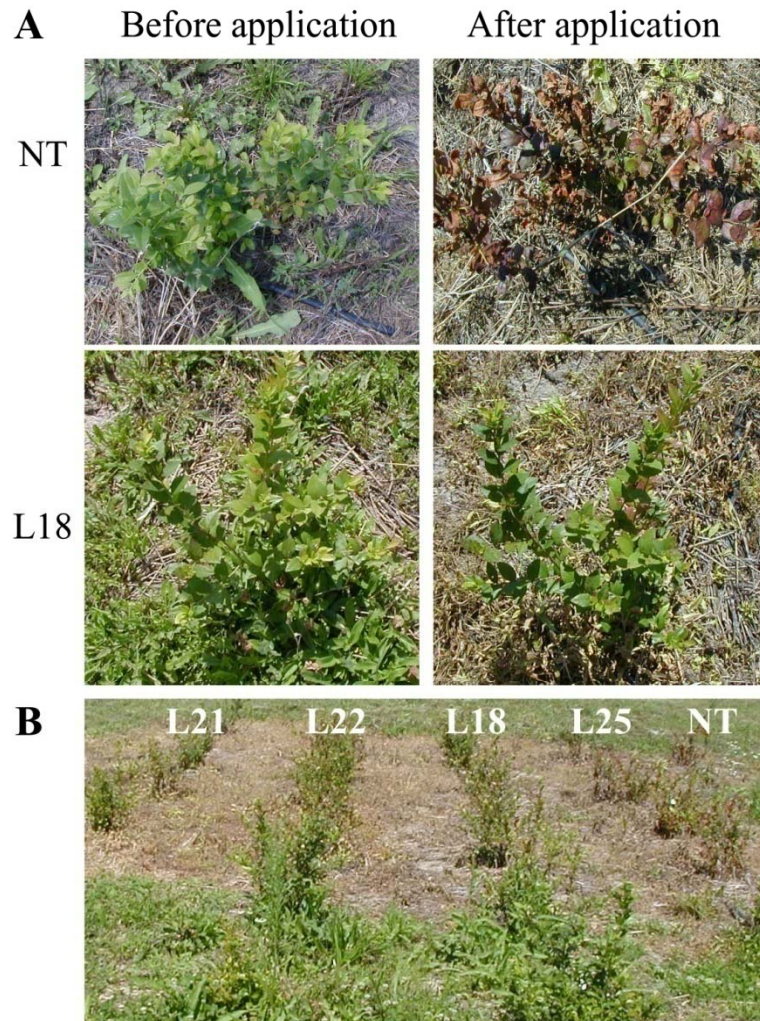under controlled environmental conditions
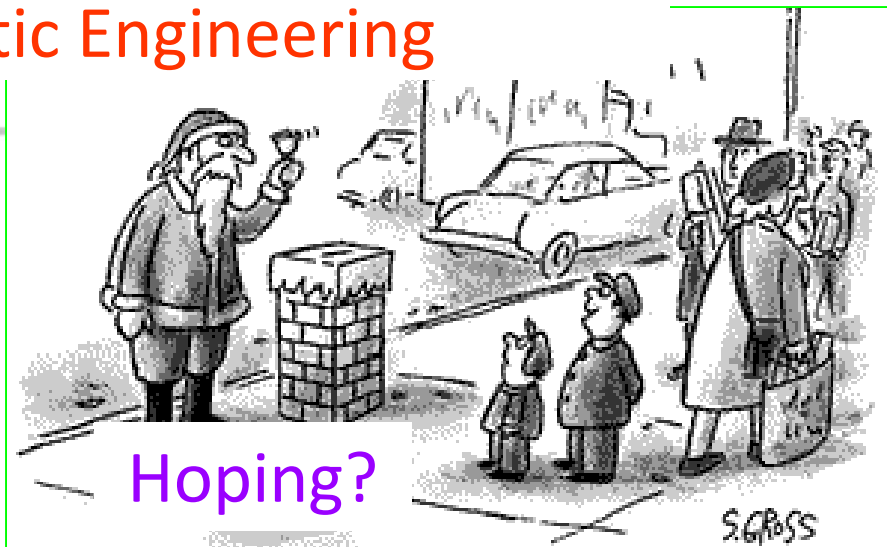
PPT=750 ppm, 2 weeks

Field trail

- **To evaluate transgenes as well as the other traits**

Song et al. Acta Horticulturae 738: 397-408 (2007)
Song et al. JASHS. 133(4): 605-611 (2008)

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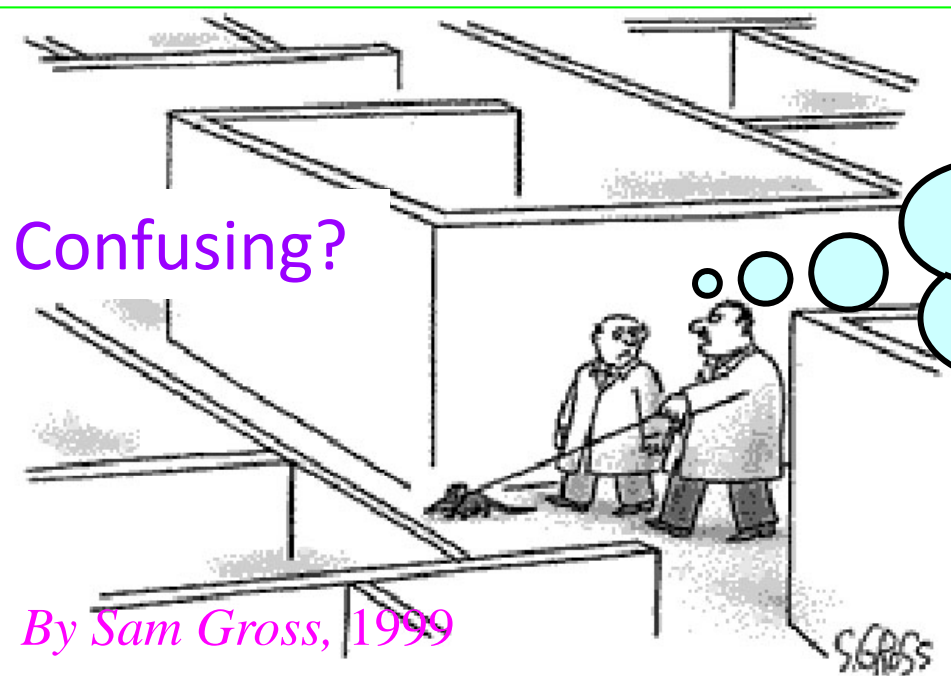# Debate on Genetic Engineering

Scaring?

By Sam Gross, 1991
"Some genetic engineers we turned out to be!"

Hoping?

By Sam Gross, 1997
"Years ago, there was only one Santa Claus. Now because of genetic engineering, there can be lots of them."

Confusing?

By Sam Gross, 1999

Genetic engineering got us into this mess, and genetic engineering will get us out of it."

Lets be prepared to engineer a guy like this!!