

## Evolusi Teknik Molekuler untuk Keperluan Pemuliaan Tanaman

Rudy Lukman

9 Oktober 2012



### The Emergence of Agriculture



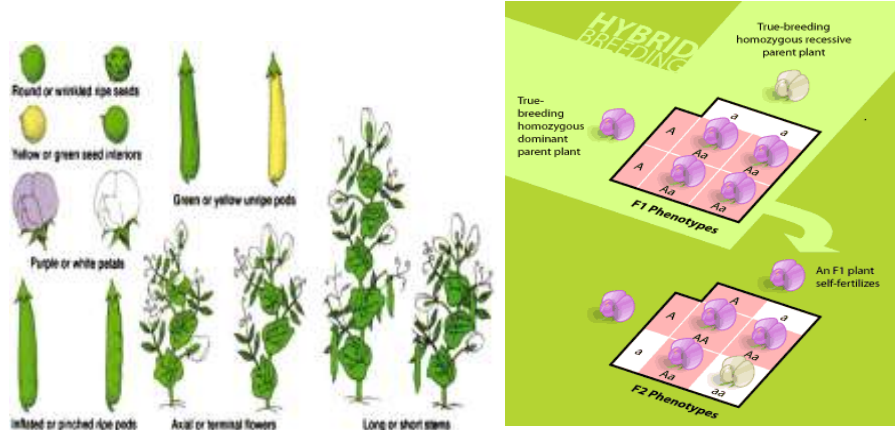
■10.000-4500 BC, hunter gatherer societies domesticated selected species of plants and animals

•All crops we grow today have undergone extensive genetic change from their wild ancestors.

•Crops, strains and genes have moved around the globe.



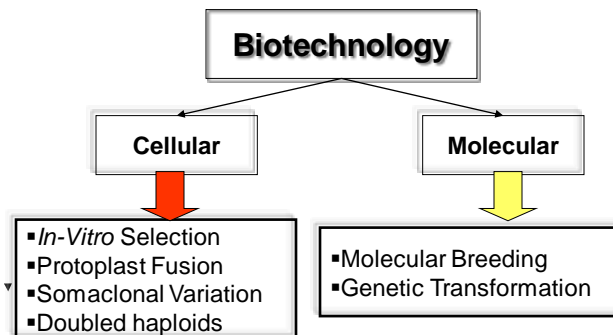
## The Seven Character Differences Studied by Mendel



After years of working with peas (*Pisum sativum*), Mendel moved on to other crops to verify his findings. In 1866, he published his results: "Experiments on plant hybrids" in the transactions of the Natural History Society of Brunn.



## Biotechnology Approaches for Crop Improvement



Various new breeding tools have been developed to assist breeding in **the last 50 years**.

# From Inventions to Applications



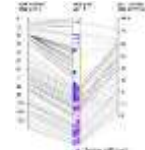
**Double helix DNA**  
1953  
James Watson & Francis Crick



**Crystallography of DNA**  
1958  
Rosalind Franklin



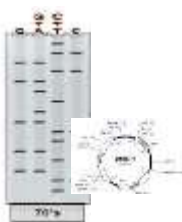
**Molecular markers**  
1985



**Genetic mapping**  
1990's



**Transgenic Tomato**  
1994



**Recombinant DNA Technology**  
1970's-1980's



**RFLP**  
1980's



**PCR**  
1983  
Kary Mullis



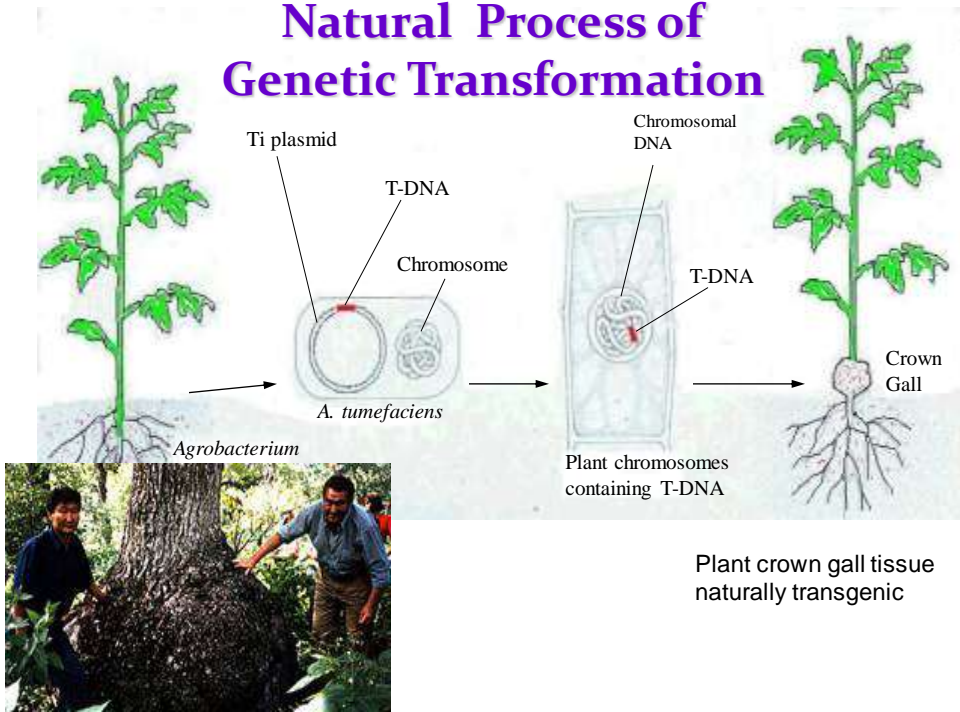
**NGS Applications**  
2005



## Genetic Engineering



## Natural Process of Genetic Transformation

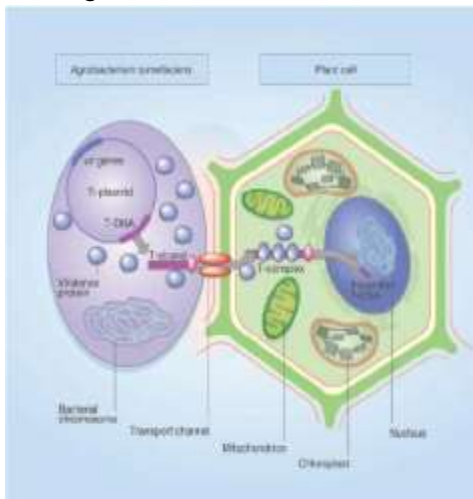


Plant crown gall tissue naturally transgenic

## How to do Gene Transformation?

Commonly Used Methods :

*Agrobacterium tumefaciens*



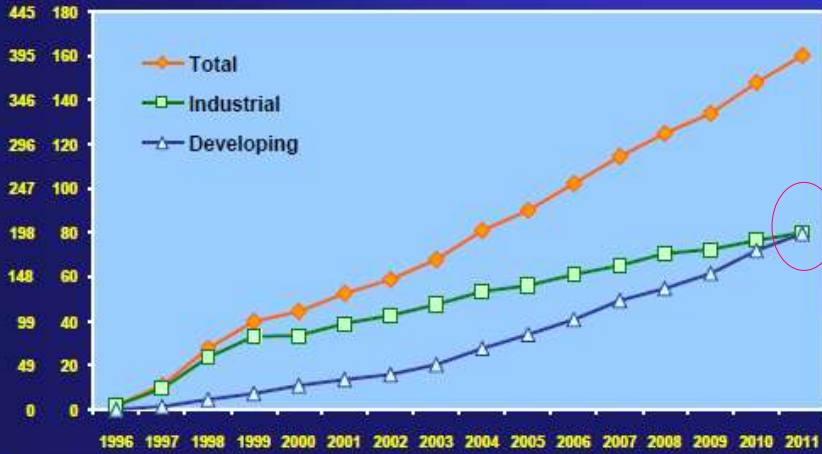
Gene gun



### Global Area of Biotech Crops, 1996 to 2011: Industrial and Developing Countries (M Has, M Acres)



M Acres

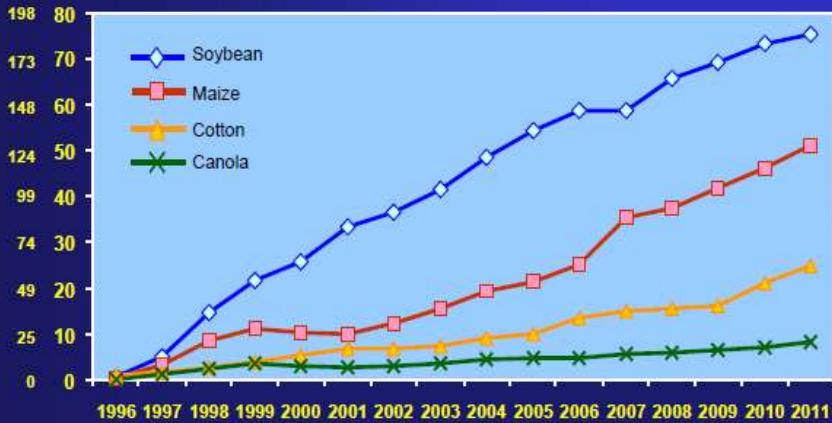


Source: Clive James, 2012

### Global Area of Biotech Crops, 1996 to 2011: By Crop (Million Hectares, Million Acres)



M Acres

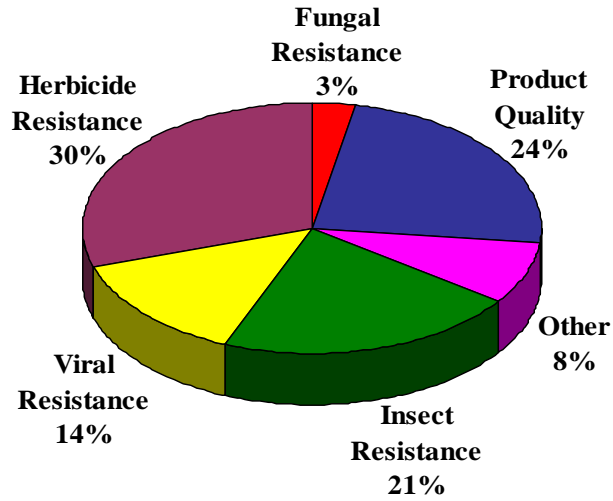


Source: Clive James, 2012



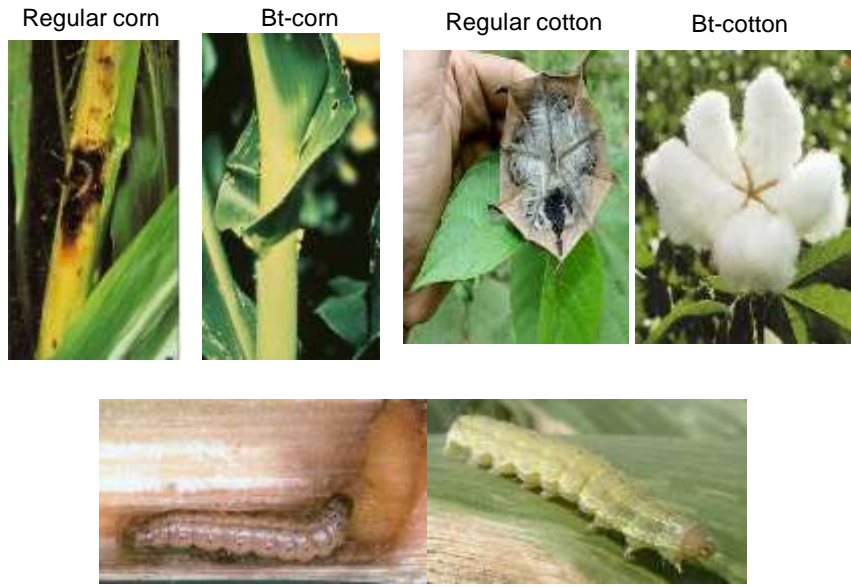
# What's Being Tested

(US field releases, cumulative since 1987)



SOURCE: US DEPARTMENT OF AGRICULTURE

## Engineered of Insect Resistance Plants



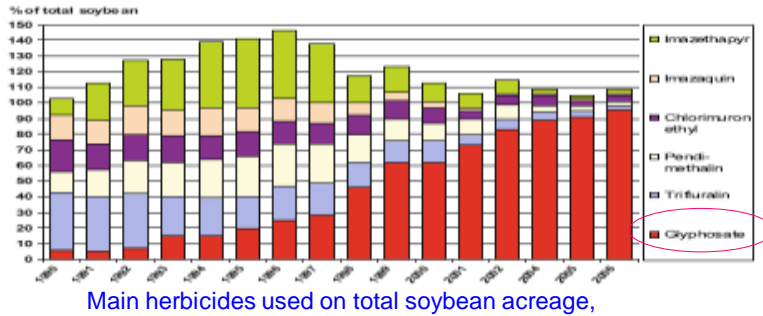
## Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review

Nyssa BOSNY\*

INRA, UMRI 1082, Systèmes d'élevage, BP 10447, Caspary de Clignancourt, 78000 VERSAILLES, France

(Accepted 10 September 2011)

**Abstract** – Transgenic crops are the subject of lively debate and controversy. Despite such controversy, transgenic soybean has undergone a rapid expansion, surpassing various types of transgenic crops, herbicide-tolerant crops appear to be of limited interest, especially in Europe. Nevertheless, herbicide-tolerant crops are the most widely spread in the world. Indeed, glyphosate-tolerant soybean was initially the most cultured transgenic plant in the world in 2006. In the USA, 94% of soybean was transgenic in 2007. Here we use this particularly significant diffusion in the USA to explain and what are its impacts? Such issues are addressed in this article, using surveys, studies of numerous national data and literature analysis. A first section underlines the importance of soybean in the current development of transgenic crops in the world, and the favorable context for their expansion in the USA. Then follows an analysis of the advantages and drawbacks of transgenic soybean for American farmers. Factors explaining the rapid diffusion of transgenic soybean are also analyzed. A comparison of transgenic versus conventional soybean reveals that transgenic glyphosate-tolerant soybean allows both the simplification of weed control and greater



## Early-Flowering Plum Tree



This FasTrack plum tree carries the early-flowering gene, meaning that it produces fruit in less than a year after being planted from seed, rather than the 3-10 years normally required for a seedling plum to produce fruit.

# Improvement of Nutritional Qualities

## Golden rice

ProVitamin A and Phytase



In 2000  
1.6 mg/g of carotenoid

In 2005  
3.7 mg/g of carotenoid

## GM Products for Energy



Cassava



Corn

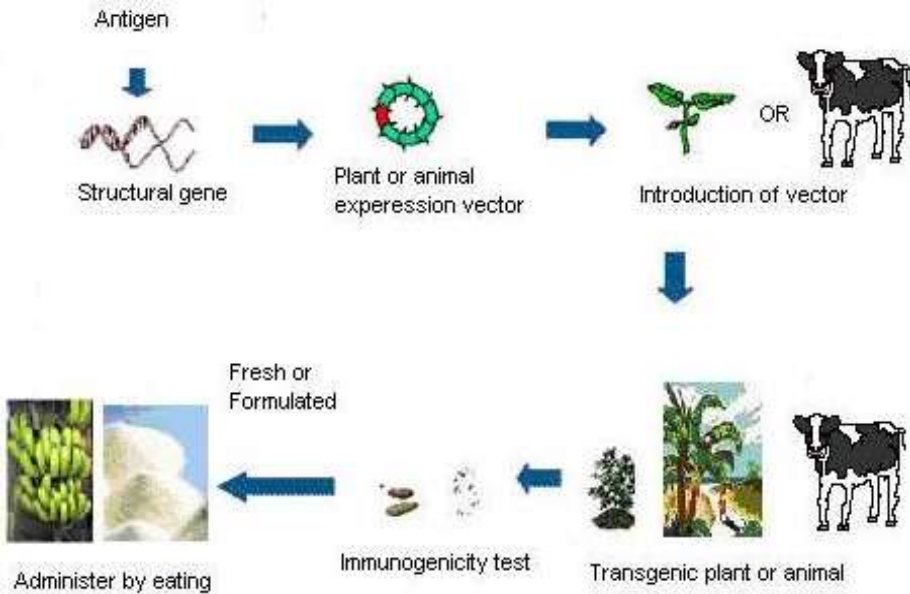


Bio-ethanol

Reduce: Tailpipe carbon monoxide emissions by 30%  
VOC (volatile organic compounds) emissions by 12%  
Particulate matter (PM) emissions by more than 25%



## Edible Vaccines



## Some Issues of GM Products

### Assessments of GM Product

Environment



Food & Feed safety



### The Biosafety Clearing House

The BCH is a mechanism set up under the Cartagena Protocol on Biosafety to facilitate the exchange of information on LMOs and assist countries that are Parties to the Protocol to better comply with their obligations.



# Molecular Breeding Technology

## GENETIC MARKERS



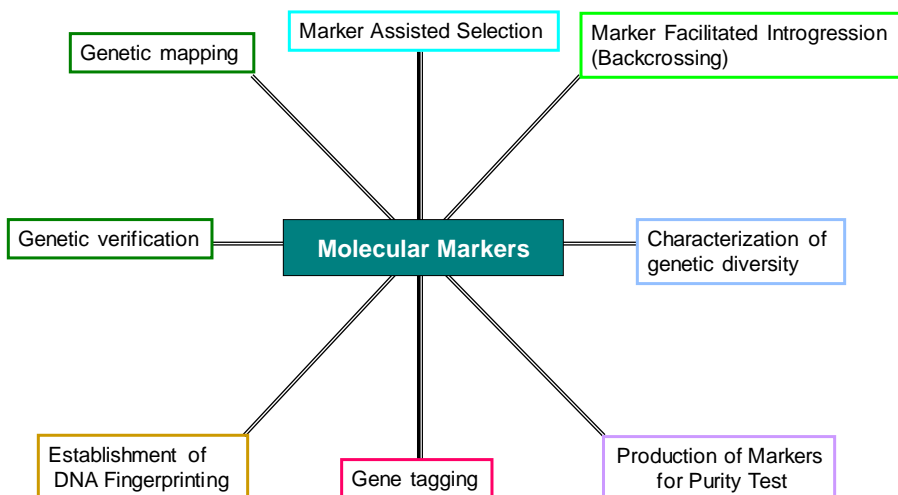
"The green section indicates the presence of a desirable gene in an organisms' genetic code that is associated with two genetic markers (red flags)."

## Molecular Markers:

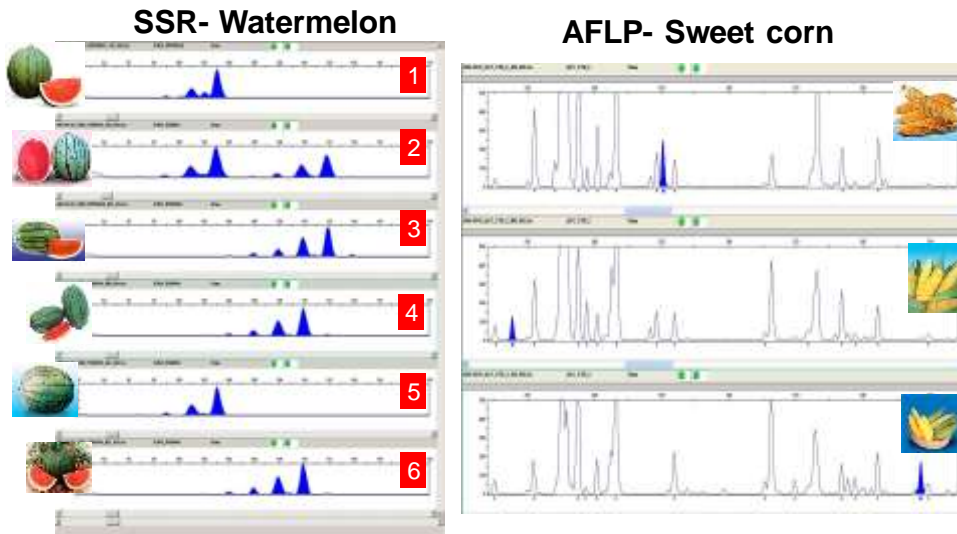
- They are identifiable DNA sequences, found at specific location of the genome, and transmitted by the standard locus of inheritance from one generation to the next
- Should not be considered as normal genes, as they usually do not have any biological effect
- "Constant landmarks in the genome"

Source: <http://usda-ars-beaumont.tamu.edu/dblhelix.jpg>

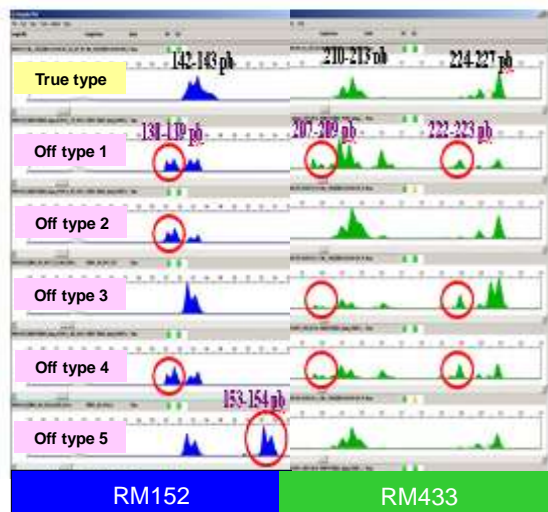
## Applications



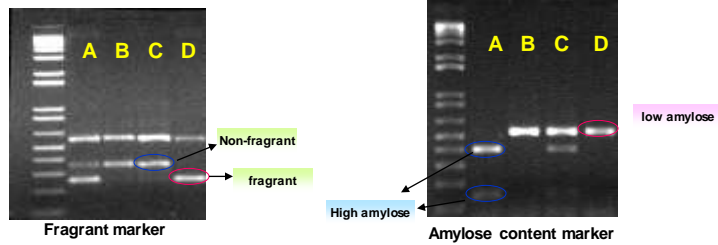
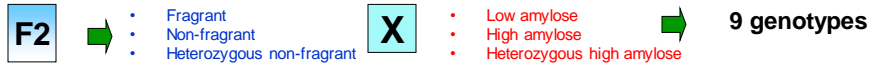
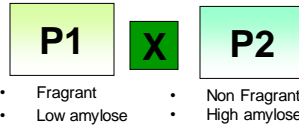
## Establishment of DNA fingerprinting



## Genetic Purity Test Assessment



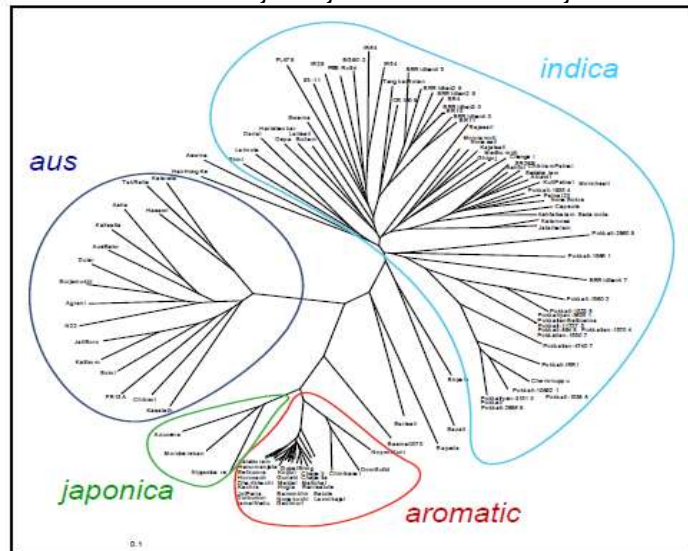
# Identification of Fragrance and Amylose Content in Rice Population



- A** : heterozygous non-fragrant, high amylose
- B** : non-fragrant, low amylose
- C** : non-fragrant, heterozygous high amylose
- D** : fragrant, low amylose

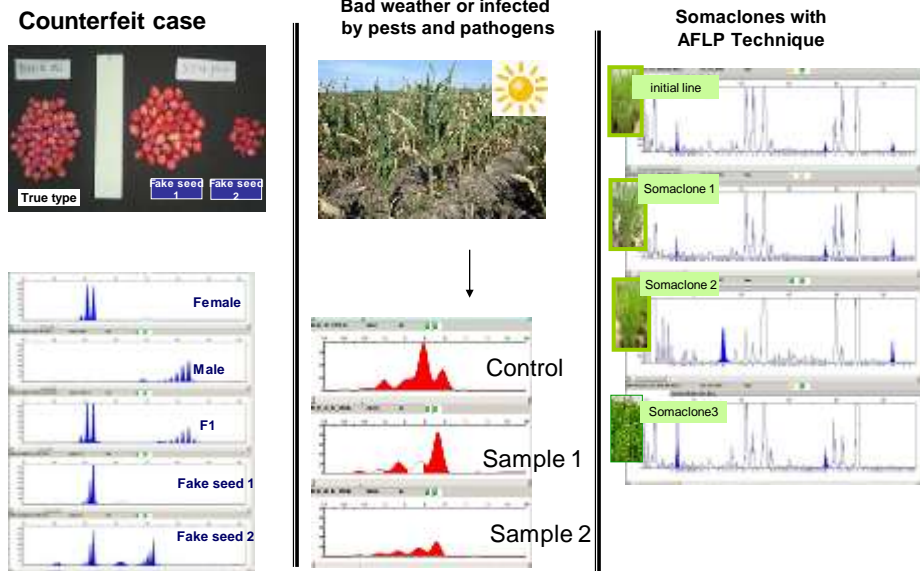
## Study of Genetic Diversity

Diversity analysis of rice for salinity tolerance

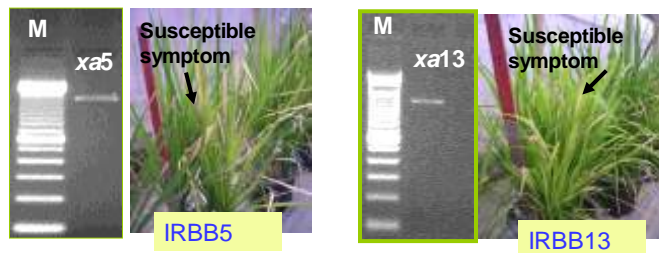


Michael.J. Thompson, 2010

## Verification of Genetic Identity



## Gene Tagging of R Genes

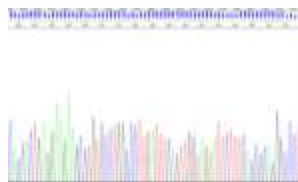


Line	R genes	Race			
		1	4	9	10
IRBB4	<i>Xa4</i>	R	MS	S	R
IRBB5	<i>xa5</i>	R	S	R	R
IRBB13	<i>xa13</i>	S	S	S	S
IRBB21	<i>Xa 21</i>	R	R	MR	R

# Reaction of IRRI Lines to *Xanthomonas oryzae* pv. *Oryzae* Races

NILs	Xa-gene(s)	Race 1 PXO61	Race 2 PXO86	Race 3B PXO79	Race 3C PXO340	Race 4 PXO71	Race 5 PXO112	Race 6 PXO99	Race 7 PXO145	Race 8 PXO280	Race 9a PXO339	Race 10 PXO341
IRBB1	Xa1	S	S	S	S	S	S	S	S	S	S	S
IRBB2	Xa2	S	S	S	S	S	S	S	S	S	S	S
IRBB3	Xa3	S	S	S	S	S	S	S	S	S	S	S
IRBB4	Xa4	R	S	S	S	MR-MS	R	S	R	R	S	R
IRBB5	xa5	R	R	R	R	S	R	S	R	R	R	R
IRBB7	Xa7	MS	R	R	R	S	R	S	R	R	S	R
IRBB8	xa8	S	S	S	S	S	MR	S	S	S	S	S
IRBB10	Xa10	S	R	S	S	S	R	S	R	S	S	S
IRBB11	Xa11	S	S	S	S	S	S	S	S	S	S	S
IRBB13	xa13	S	S	S	S	S	S	R	S	S	S	S
IRBB14	Xa14	S	S	S	S	S	R	S	S	R	S	S
IRBB21	Xa21	R	R	R	MR	R	R	MR	MR	MR	MR	S
IRBB50	Xa4xa5	R	R	R	R	R	R	S	R	R	R	R
IRBB51	Xa4xa13	R	S	S	S	MS	R	R	R	MR	S	R
IRBB52	Xa4Xa21	R	R	R	MR	MR	R	MR	R	R	MR	R
IRBB55	xa13Xa21	R	R	R	MR	R	R	R	MR	MR	MR	S
IRBB56	Xa4xa5xa13	R	R	R	R	R	R	MR	R	R	R	R
<b>IRBB60</b>	<b>Xa4xa5xa13Xa21</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>
IRBB61	Xa4xa5Xa7	R	R	R	R	MR	R	S	R	R	R	R
IRBB62	Xa4Xa7Xa21	R	R	R	R	R	R	MR	R	R	MS	R
IRBB65	Xa4Xa7xa13Xa21	R	R	R	R	R	R	R	R	R	MR	R

## Detection of Pathogens

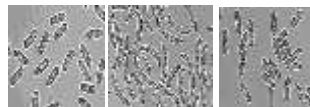


16S r-DNA

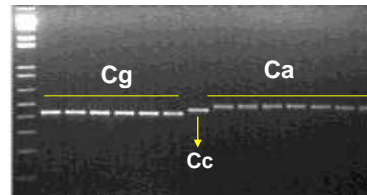
Sequences producing significant alignments:

Accession	Description	Max	Total	Query
		62/69	60/69	100%
<a href="#">F61221.1</a>	<i>Colletotrichum nicotianae</i> subsp. <i>nicotianae</i> strain UC-3679 6S	61	60	100%
<a href="#">F61221.1</a>	<i>Colletotrichum nicotianae</i> subsp. <i>nicotianae</i> strain UC-3679 6S	61	60	100%
<a href="#">F61221.1</a>	<i>Colletotrichum nicotianae</i> subsp. <i>epiphyticum</i> partial 16S rDNA gene	61	60	100%

BLAST Result



**C. Gloeosporoides**  
**C. Capsici**  
**C.acutatum**

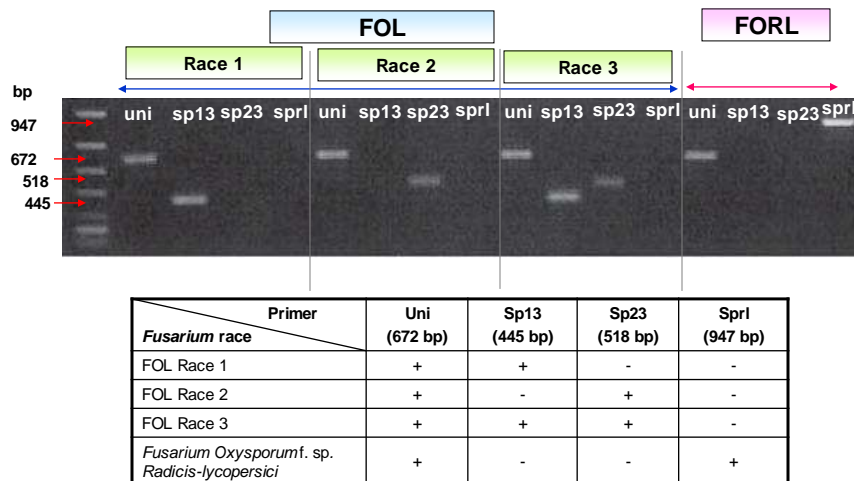


Three different *Colletotrichum* species:

Ca: *Colletotrichum acutatum*  
Cg: *C. gloeosporoides*  
Cc: *C. capsici*



## Identification of *Fusarium oxysporum* Races in Tomato with Specific Primer sets



## Determination of *Fusarium* Races based on SNaPshot™ Minisequencing procedure

Sequence alignment of endo polygalacturonase (pg1) gene and exo polygalacturonase (pgx4) gene revealed several point mutations. These markers were further employed as SNaPshot primers to simultaneously amplify SNPs associated with different races





## Future Perspectives



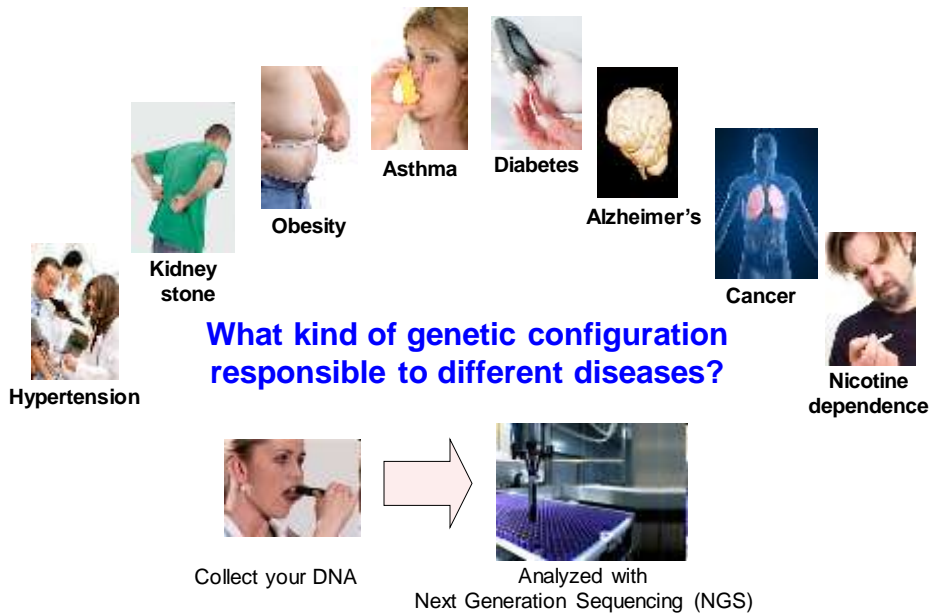
Sequencing the human genome may be the greatest accomplishment of all time in the biological sciences



Genetic Analyzer  
Read length= 1000bp/run



**3.3 billion base pairs= 3300 such books** would be needed in order to store the complete genome.



## Applications of NGS for Crop Improvement

De novo sequencing & Re-sequencing

Genomic Selection

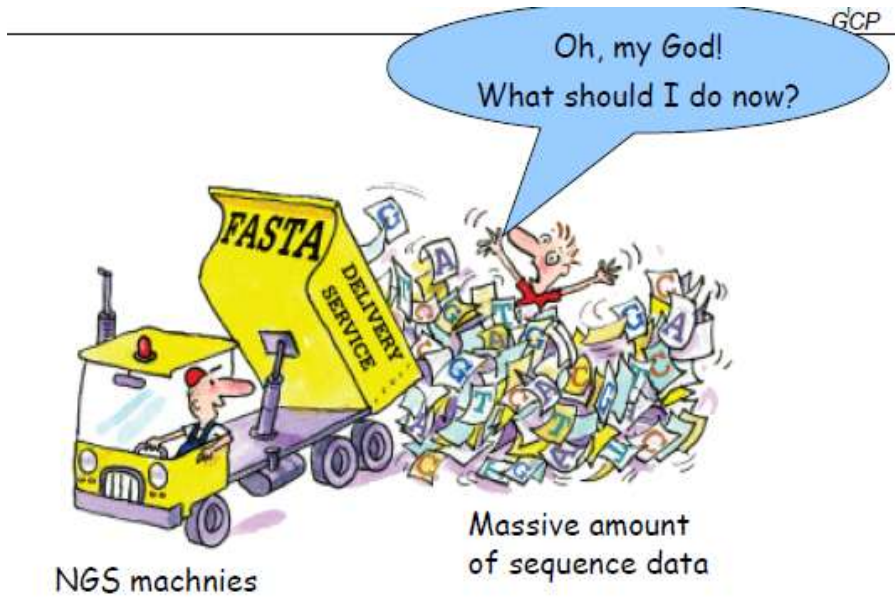
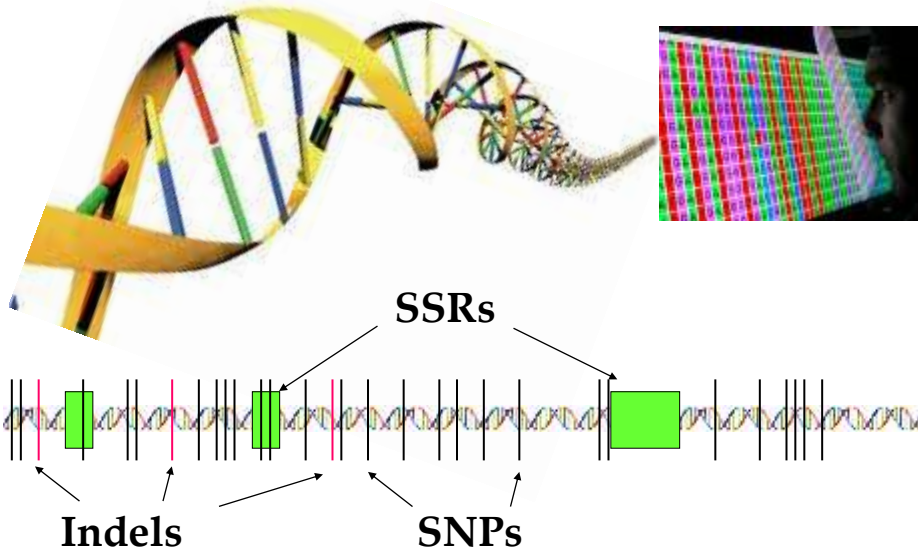


Transcriptome analysis

Epigenetic modification

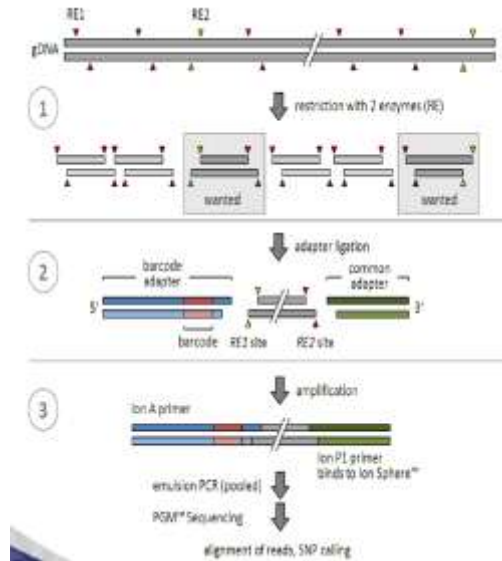
# Whole Genome Sequencing

Molecular marker & Copy Number Variation Discovery



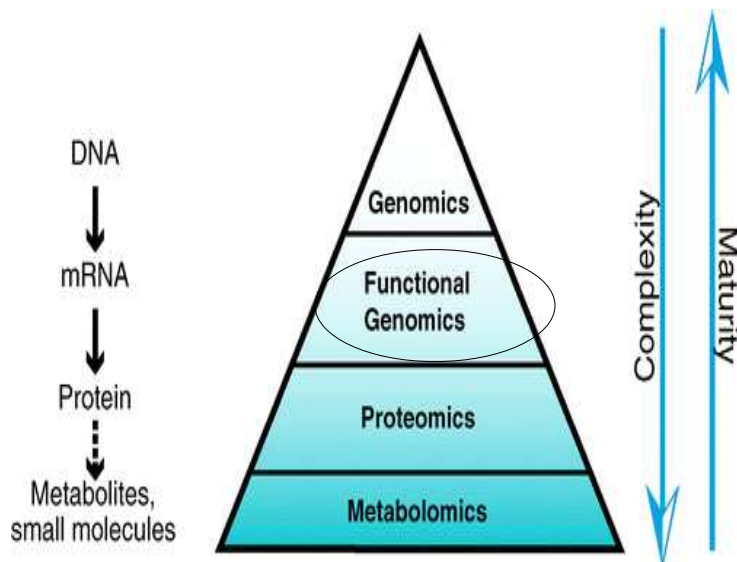
## Genotyping by Sequencing (GBS)

- GBS has been developed as a tool for association studies and genomics-assisted breeding in a range of species including those with complex genomes.
- GBS uses restriction enzymes for targeted complexity reduction followed by multiplex sequencing to produce high-quality polymorphism data at a relatively low per sample cost

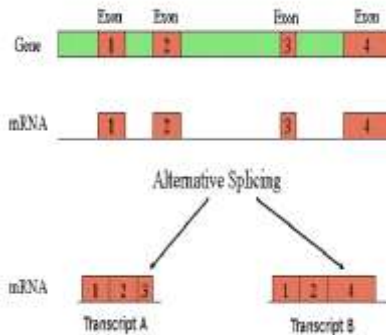


Courtesy: Life technologies, 2012

## Levels of “omics” analyses



## Transcriptome Sequencing (RNA seq)



- Transcriptomes are typically 10-100 times smaller than genomes and are therefore 10-100 times cheaper to sequence
- The identified SNPs are within known expressed genes

Parchman et al. *BMC Genomics* 2010, **11**:180  
<http://www.biomedcentral.com/1471-2164/11/180>



RESEARCH ARTICLE

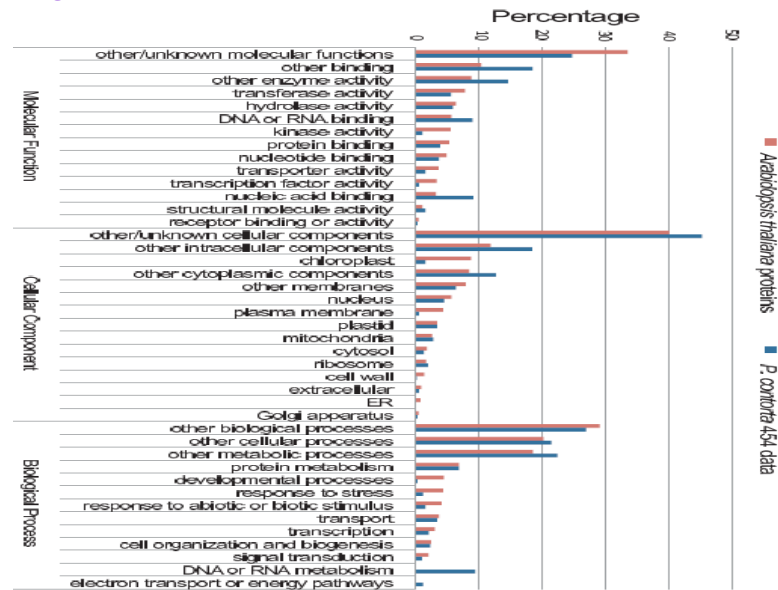
Open Access

### Transcriptome sequencing in an ecologically important tree species: assembly, annotation, and marker discovery

Thomas L Parchman<sup>1\*</sup>, Katherine S Geist<sup>2</sup>, Johan A Grahnen<sup>3</sup>, Craig W Benkman<sup>4</sup>, C Alex Buerkle<sup>1</sup>

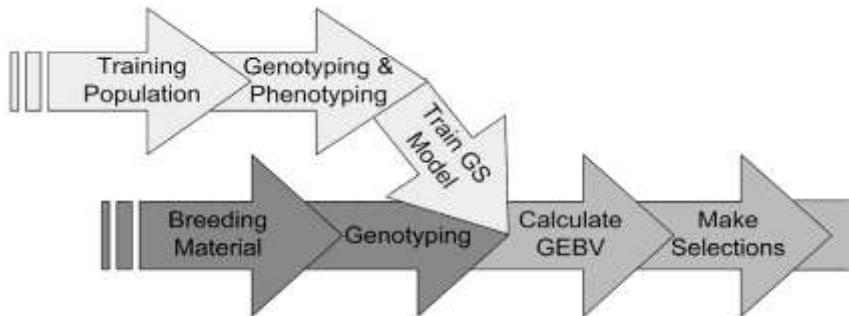


## Gene ontology assignments for Lodgepole pine (*P.contorta*) & *Arabidopsis thaliana*



Organism	# of ESTs	# of ESTs matching RTs	% Total
<i>Arabidopsis thaliana</i>	1,527,298	920	0.0602
<i>Triticum aestivum</i>	1,066,854	1,123	0.1053
<i>Vitis vinifera</i>	353,941	217	0.0613
<i>Populus nigra</i>	51,361	84	0.1635
<i>Populus tremula</i>	37,313	15	0.0402
<i>Populus trichocarpa</i>	89,943	94	0.1045
<i>Picea glauca</i>	297,913	210	0.0705
<i>Picea sitchensis</i>	168,675	54	0.0320
<i>Pinus pinaster</i>	27,847	15	0.0539
<i>Pinus taeda</i>	328,628	217	0.0660
<b><i>Pinus contorta</i></b>	<b>586,372</b>	<b>22,862</b>	<b>3.8989</b>

## Genomic Selection for Breeding Value Estimation



The population of individuals with both phenotypic and genotypic data is known as the “training population” as it is used to estimate model parameters that will subsequently be used to calculate GEBVs of selection candidates (e.g., breeding lines) having only genotypic data.

These GEBVs are then used to select the individuals for advancement in the breeding cycle. Therefore, selection of an individual without phenotypic data can be performed by using a model to predict the individual’s breeding value

## In Depth Study of Genetic Configuration that Compose Superior Phenotype

Which Wheat Line Is The Best Yielding?

—Wheat Yield Trial – Very Expensive-Land Intensive Way to Determine Best Wheat Lines—



- Predict more winners through genomic selection
- Increase likelihood & frequency of success

Which Durian is better without opening it

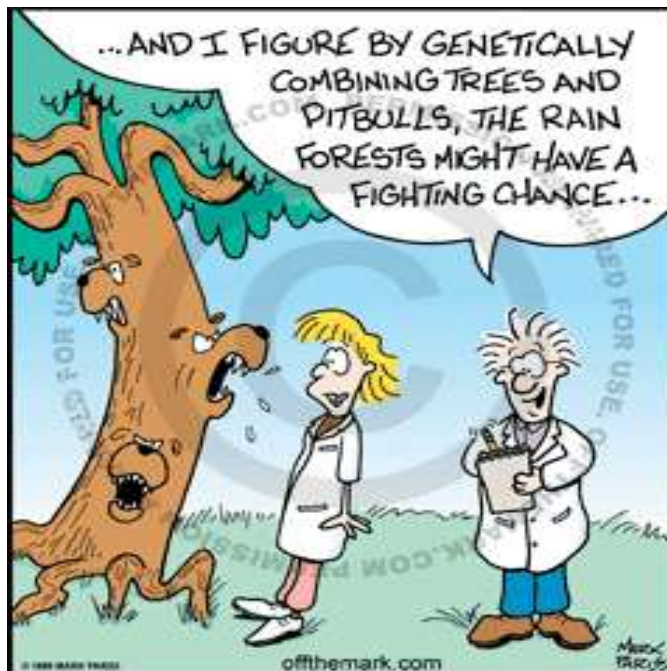


Increase quality through genomic selection

Courtesy: Illumina, 2010

## Kesimpulan

- Tersedianya dua teknik molekuler yaitu rekayasa genetika dan *molecular breeding* memberikan pilihan bagi pemulia tanaman dalam menentukan metode yang sesuai untuk mempercepat proses pemuliaan tanaman dan perakitan varietas baru.
- Mempertimbangkan berbagai issue, teknologi transgenik sebaiknya diaplikasikan untuk karakter yang tidak mudah diimprove secara genetik melalui pendekatan konvensional karena gen yang diinginkan tidak tersedia dalam gene pools.
- Pemanfaatan teknologi NGS akan memfasilitasi pendalaman studi mengenai genome dan transcriptome tanaman, penemuan marka molekuler dan gen-gen baru serta memprediksi morfologi dari individu berdasarkan genotipenya.





Norman Borlaug, Agricultural scientist and 1970 Nobel Peace Laureate at the launch of the UN Millennium Development Goals, 2000

*“I **challenge** the **next generation** to use these **new scientific tools and techniques** to address the problems that plague the world’s poor.”*



**THANK  
YOU**