INNO-FOOD

Plant Breeding

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Researcher

Plant Breeding

- Conventional breeding
- Molecular Breeding
- Genetic Engineering

What is plant breeding?

- Genetic improvement through crossing plants with desired traits and selecting progeny with improved performance and/or improved combinations of traits.
- “Accelerated” and “targeted evolution”.
- Application of genetics principles to crop improvement.
- Systematic procedures used to improve trait phenotypes by crossing and selection, directed manipulation of the genotype at the DNA sequence level, and introduction of new genes.

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Do we still need to train students in plant breeding?

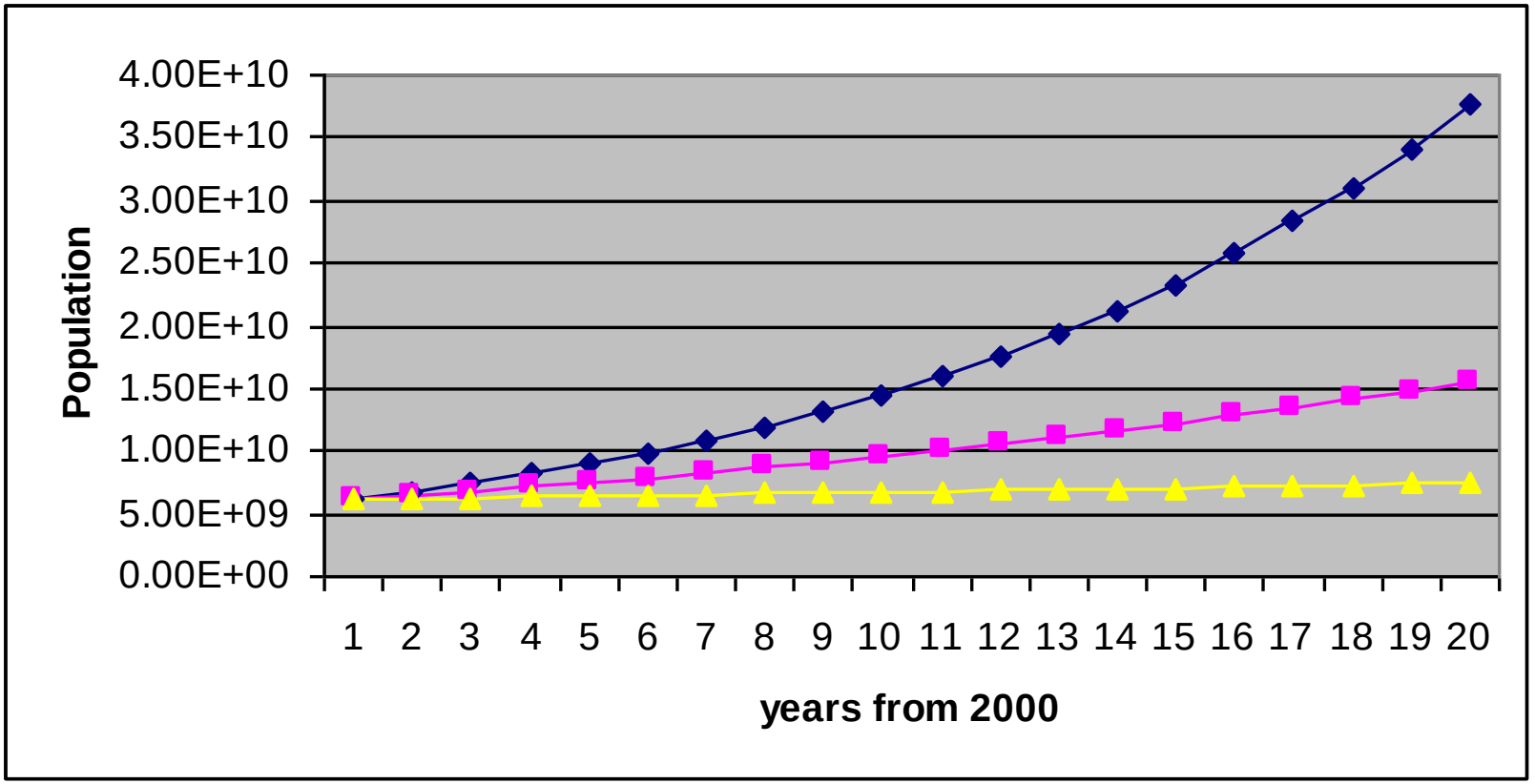
Agricultural food production

- Each year humans re-create the food supply that feeds 6.15 billion people
- Reserves of staple foods would feed the world for less than two months and were as low as 48 days in 1995
- 800 million people go to bed hungry every night

Food and Human nutrition

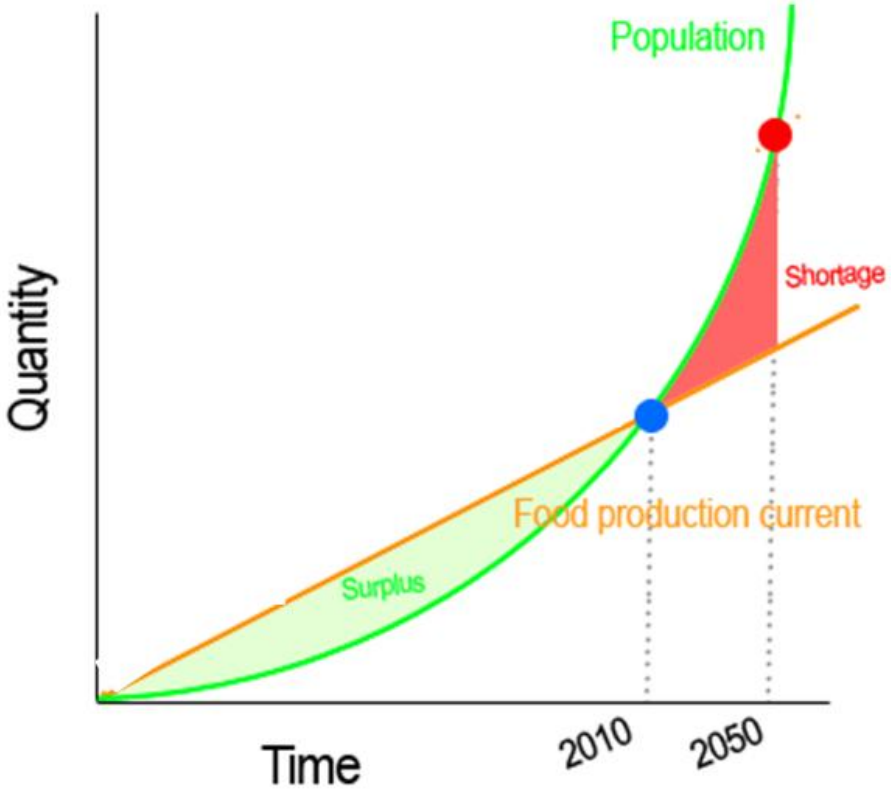
- http://www.harvestplus.org/
- Vitamin A deficiency affects 750 million people

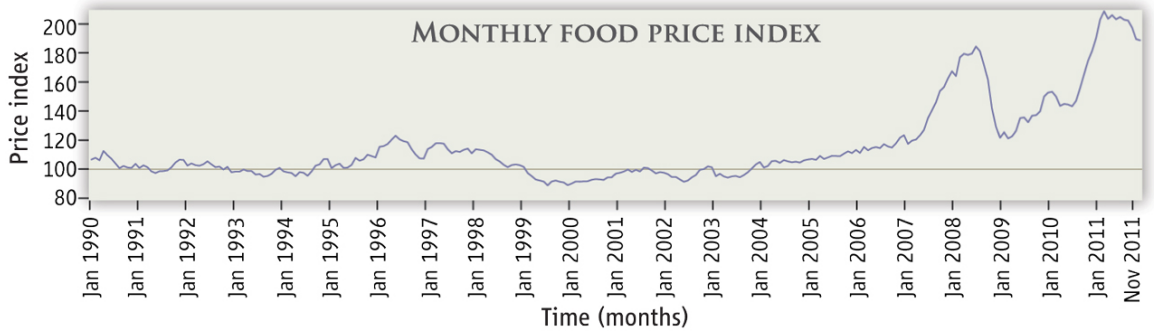
Projected population increase under different assumptions of reproduction and mortality



Food
Τρόφιμα

Current Food Production vs. Future Food Production In Relation to Growing World Populations Over Time

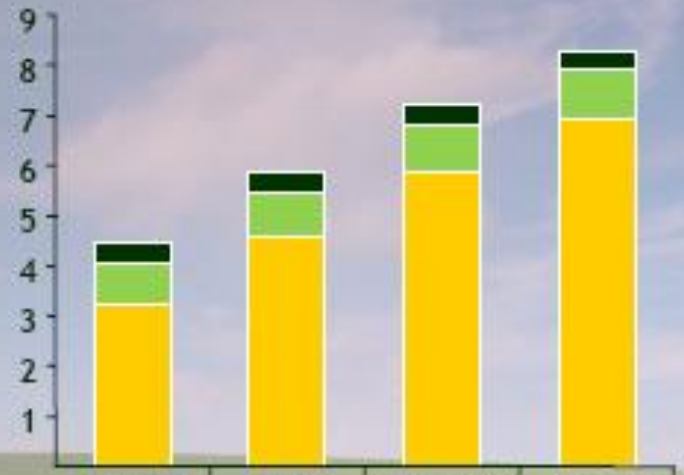




ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

In the next 50 years we have to produce more food than ever before in the history of humankind

GROWING WORLD POPULATION (B)



RISING CEREAL DEMAND (MMT)

TRANSITION NATIONS DEVELOPED NATIONS DEVELOPING NATIONS

- World population continues to increase
- Per capita food consumption continues to rise
- Consumers continue to demand improved taste, convenience, and nutrition

Source: FAO, WHO

Agricultural **F** production

Food
Τρόφιμα

Feed
Τροφές

Fiber
Ίνες

Flowers
Άνθη

Farmaceuticals and Parfums
Φάρμακα και Αρώματα

Fuels
Καύσιμα

Projections

- We need to make as much progress in production efficiency in the next 30 years as we have made in the previous 12,000
- We need to double food production by 2050

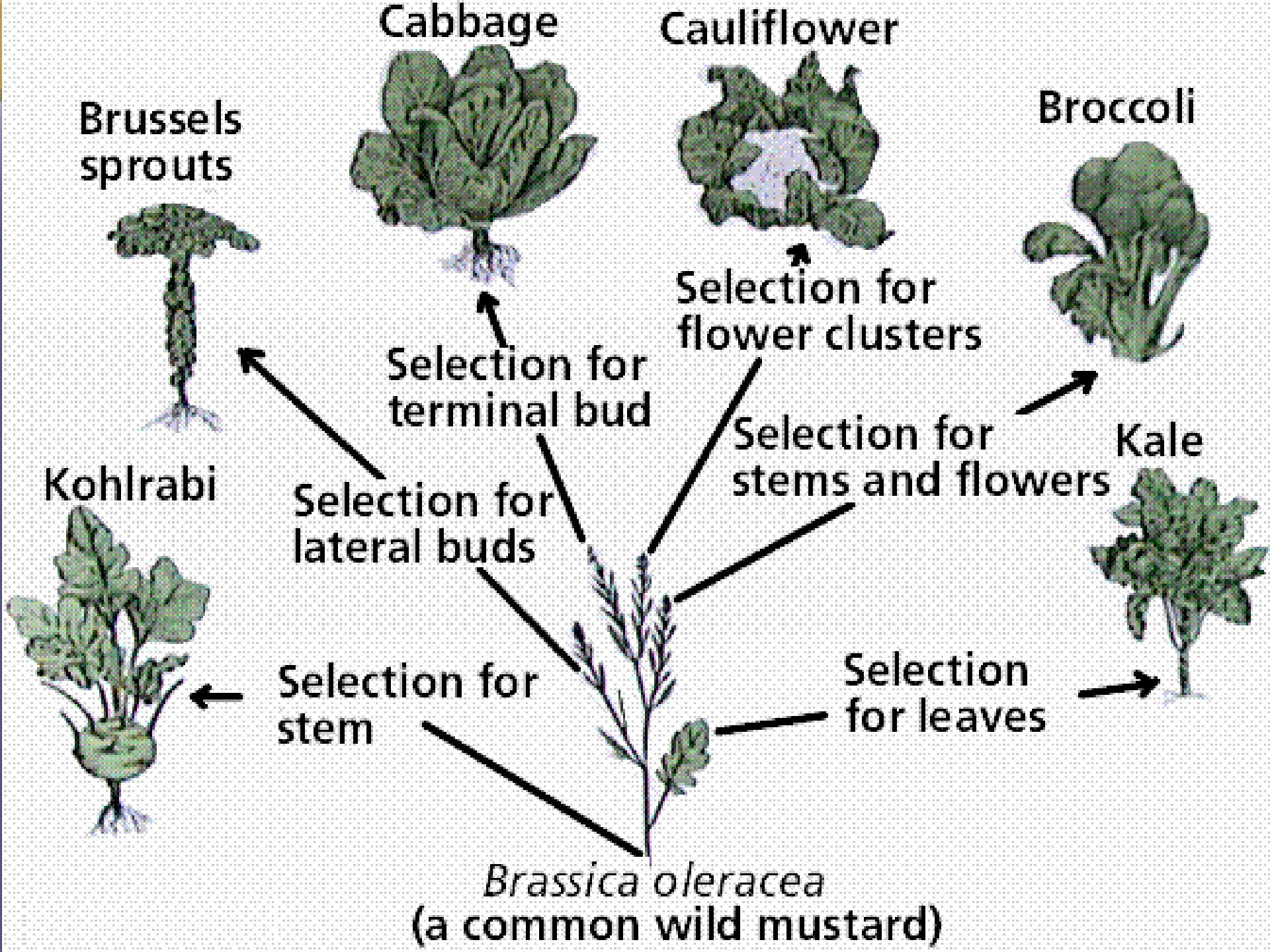
The success of plant breeding

- Increases in yield are derived both from improved varieties and from improved management.
- In vegetable crops, research suggests about a 50-50 split between genetic gain and gain attributed to management.
- Genetic gain in grain yield of 75 Kg ha^{-1} yr^{-1} for corn can be attributed to breeding.
  - 1 ton/acre increase in yield every 30 years.
  - Maize yields have increased 60% to 120% in the U.S. since 1940 (Cooper et al., 2004. Genomics, Genetics, and Plant Breeding: A Private Sector Perspective. Crop Science. 44:1907-1913).
- "Green revolution" varieties have increased yields 2 to 3 fold in many "developing" nations (Knight, 2003).

Green revolution

"Short" wheat disease resistant ('50s)

Norman Borlaug


Cabbage
Cauliflower
Brussels sprouts
Broccoli
Selection for flower clusters
Selection for terminal bud
Selection for stems and flowers
Kale
Kohlrabi
Selection for lateral buds
Selection for stem
Selection for leaves
Brassica oleracea
(a common wild mustard)

Breeding success story

teosinde

Breeding →

Καλαμπόκι

Breeding success story

wheat

Aegilops tauschii

Einkorn
AA

Emmer
AABB

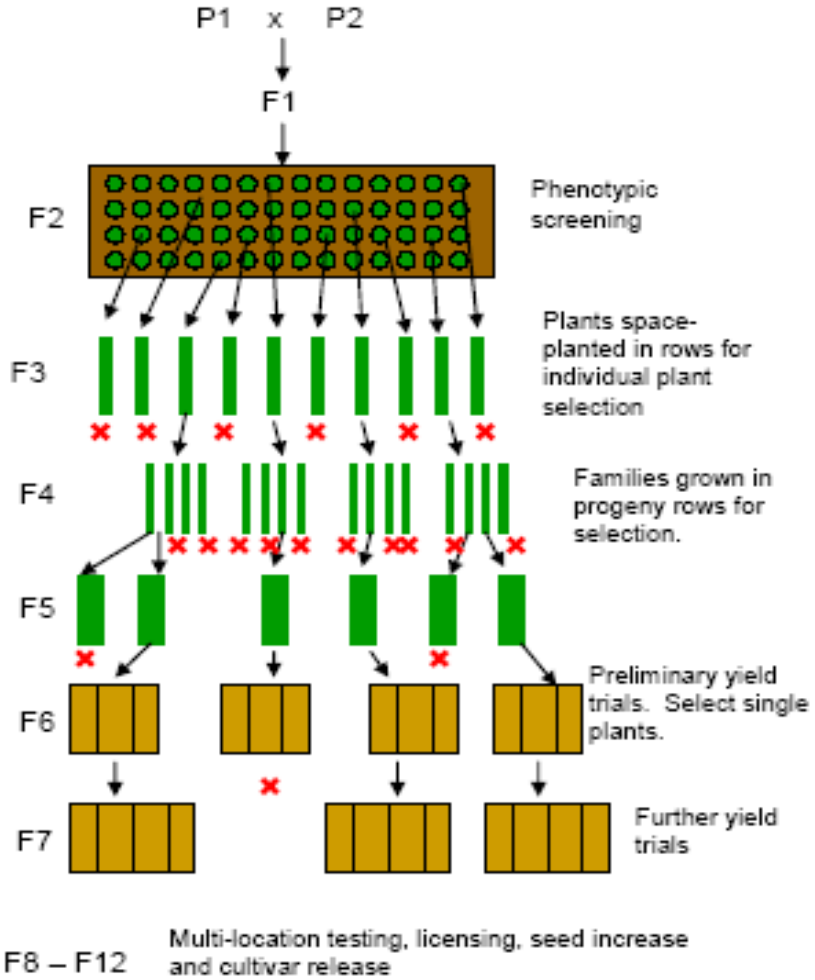
spelt
AABBDD

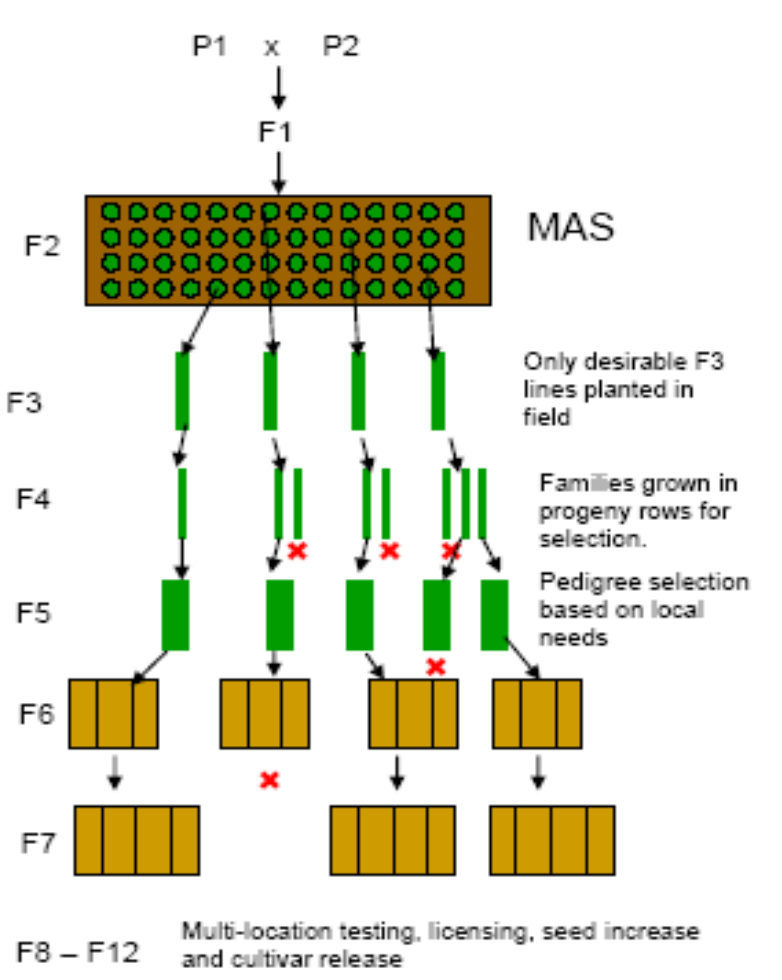
Triticum aestivum
AABBDD

Is conventional breeding obsolete?

- Easy traits to manipulate via GMO techniques are single gene – these are also easily manipulated using conventional breeding.
- Conventional breeding can manipulate multiple traits simultaneously
- Conventional breeding can manipulate genetically complex "quantitative traits"
    - Traits that are influenced by the environment
    - Traits that are conditioned by multiple genes
- Selection on phenotype is a powerful approach to bring about directed changes. (Robust but can be slow; requires that genetic variation exist for the trait of interest)
- Complex genotype x environment systems that agriculture operates under means that "methodology" of evaluation will always be important.

Early generation marker assisted selection





Early generation selection scheme (proposed by Ribaut & Betran (1999). Note that many lines can be discarded in an early generation which permits the evaluation of fewer lines in later generations.

Molecular Breeding

What is a microsatelite?

- **Repeats with a motive (1-6 repeats)**

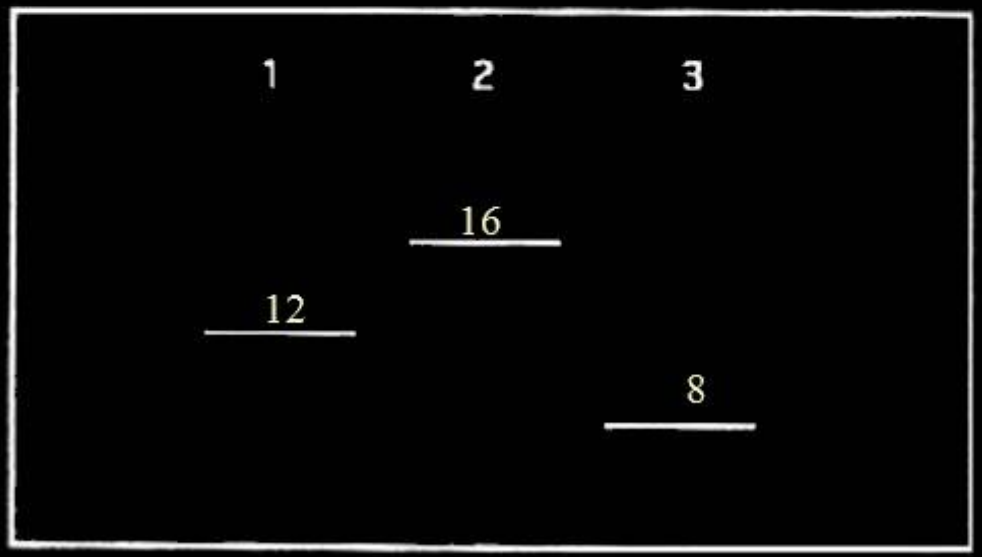
Dinucleotide	(CT) 6	- CTCTCTCTCTCT
Trinucleotide	(CTG) 4	- CTGCTGCTGCTG
Tetranucleotide	(ACTC) 4	- ACTCACTCACTCACTC

- **Classes**

Perfect repeat when repeat tract pure for one motif	CTCTCTCTCTCT
Compound SSR when repeat tract pure for two motifs	CTCTCTCACACA
Imperfect SSR if single base substitution	CTCTCTACTCTCT
Region of cryptic simplicity if complex but repetitive structure	GTGTCACAGAGT

SSRs

Ενίσχυση μέσω τεχνικής της Αλυσιδωτής Αντίδρασης της Πολυμεράσης (PCR)



Πηκτή ανάλυσης

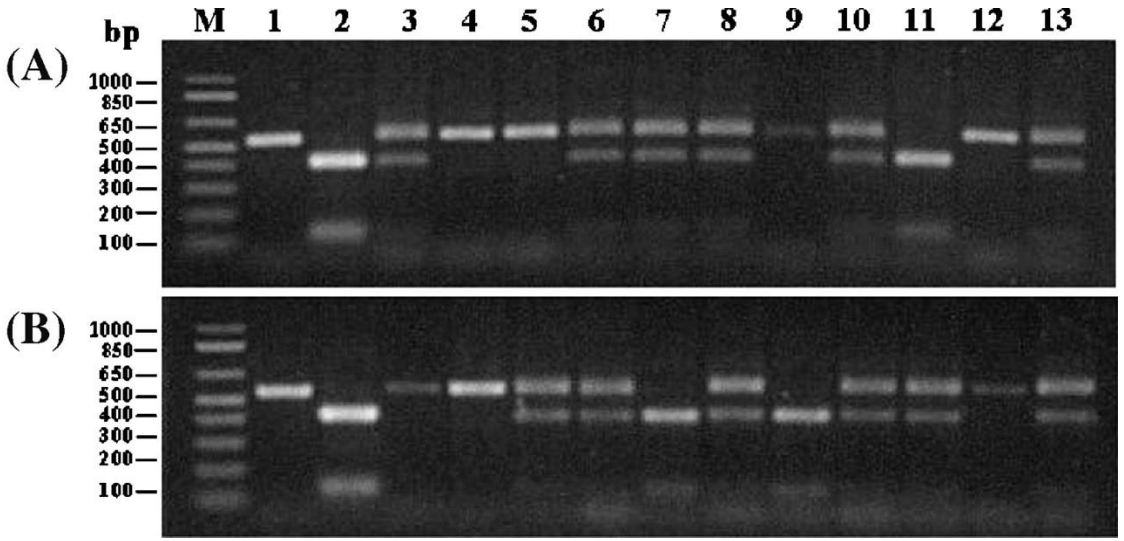
Metaphor Αγαρόζη

Πηκτή Πολυακρυλαμίδης

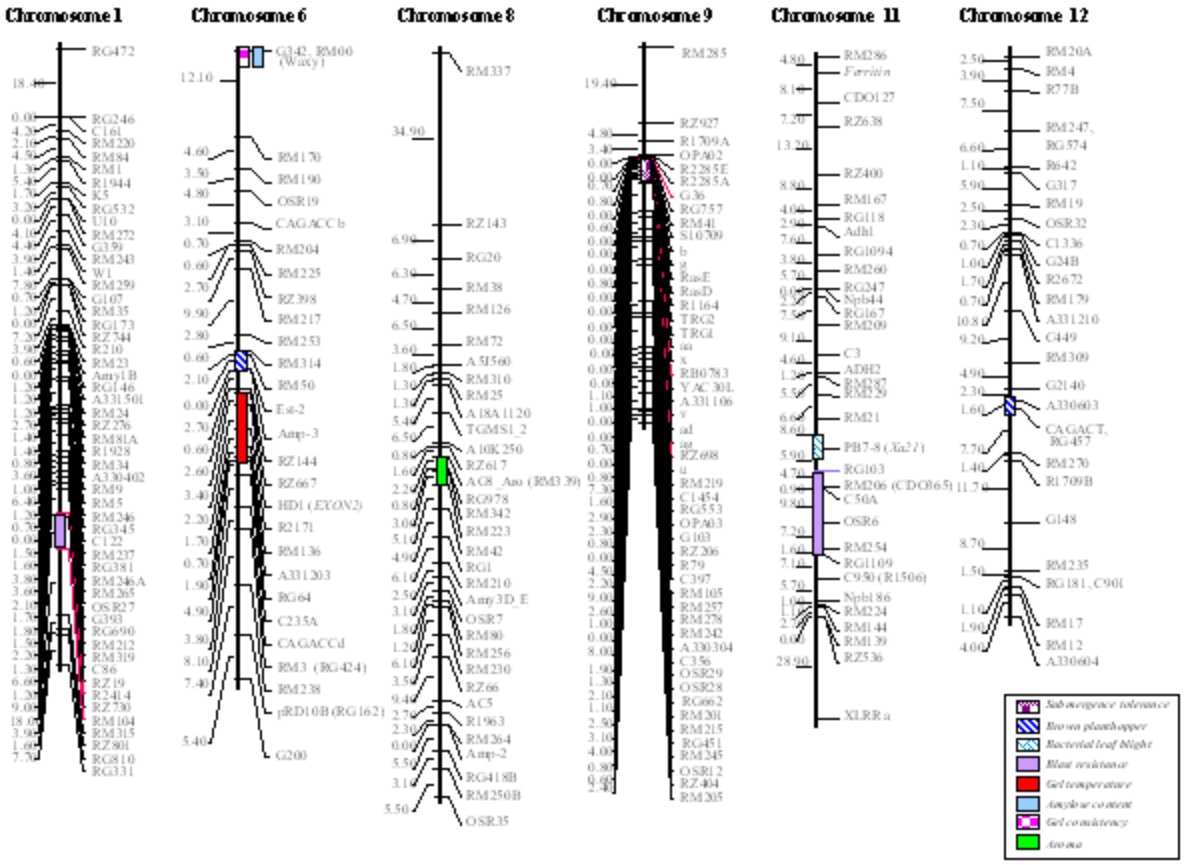
Ανάλυση με πρόγραμμα (software) → Εύρεση αριθμού και μεγέθους διαφορετικών Αλληλομόρφων

Molecular breeding

Marker assisted selection

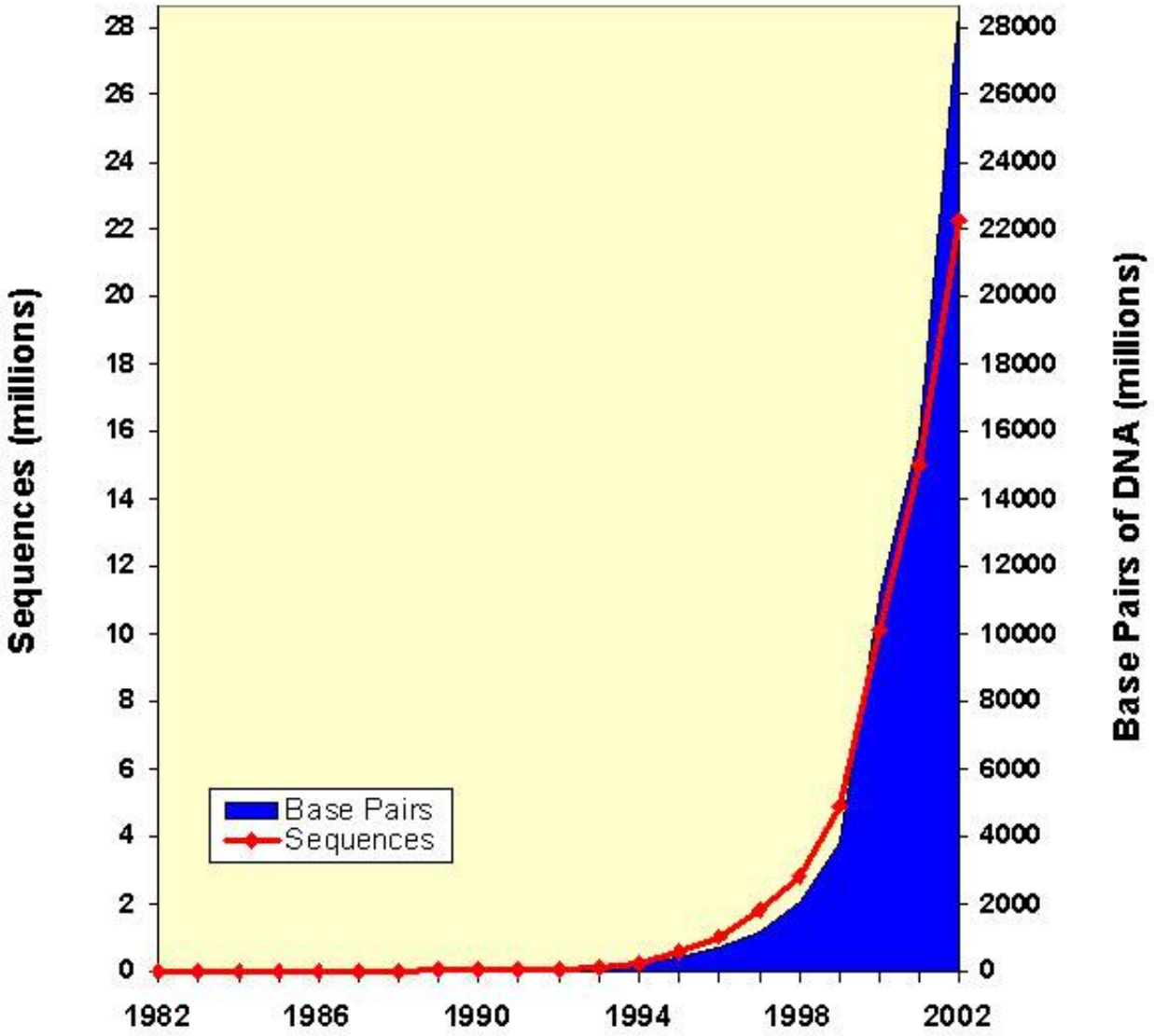


Molecular Breeding

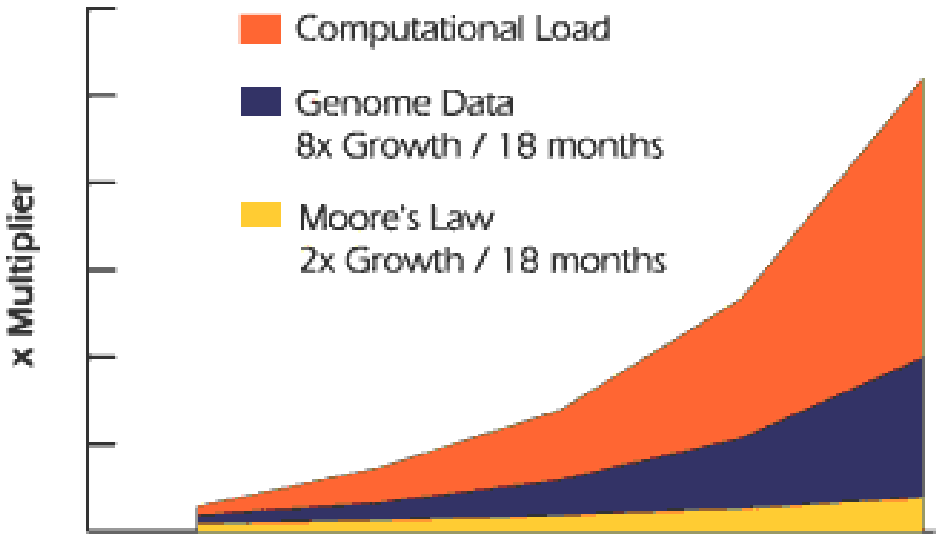


Using genomic maps

Growth of GenBank

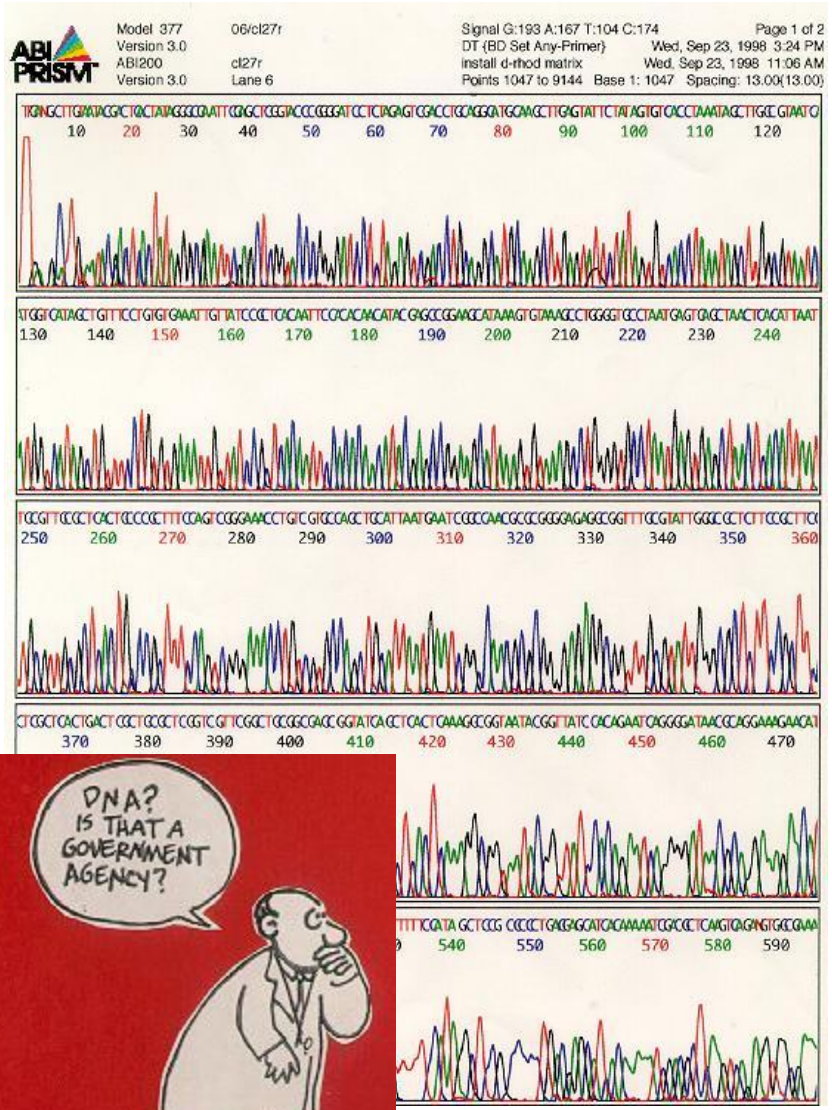


The challenge of scale - computational

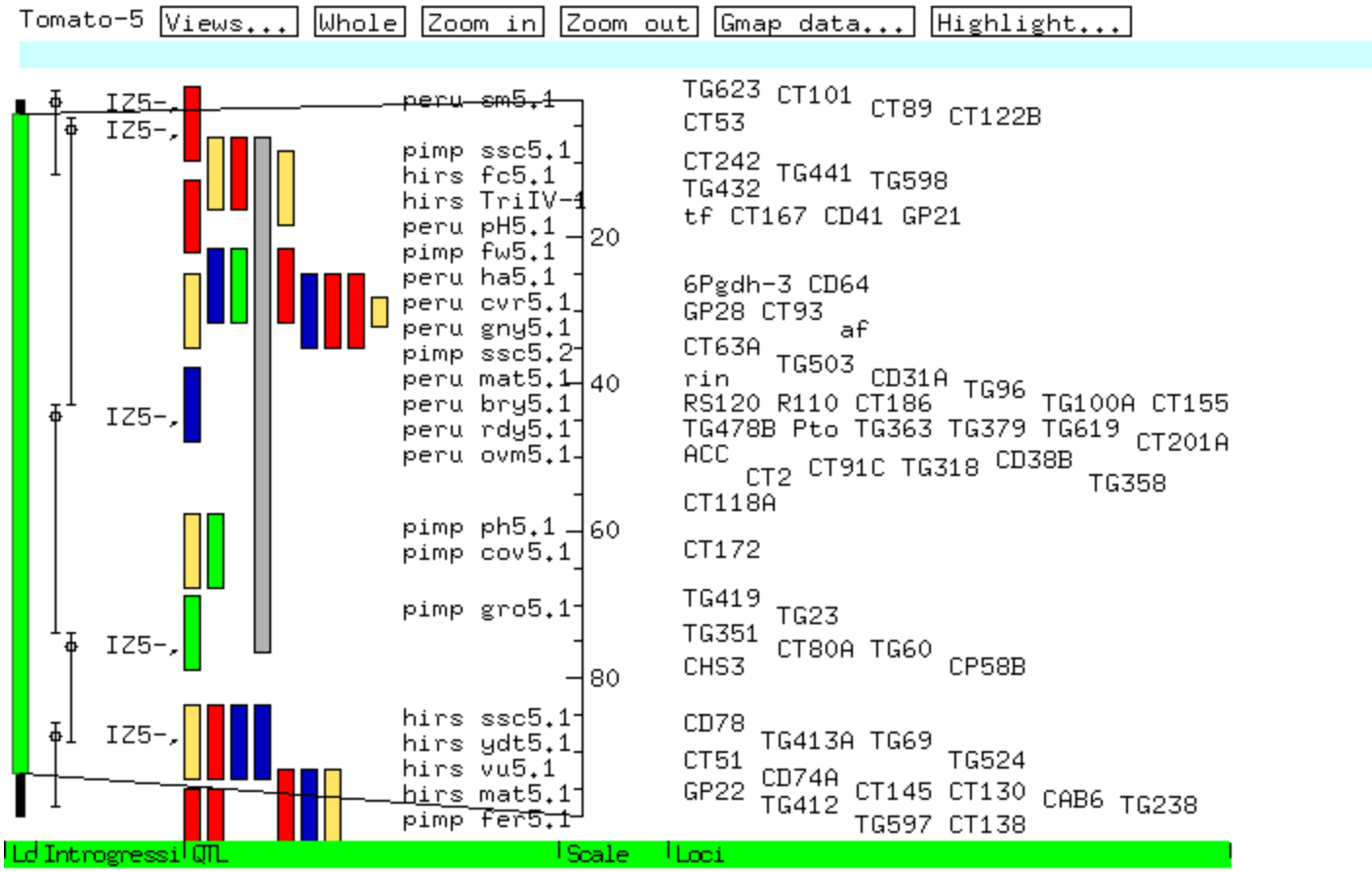


- High-throughput
- Pipelines
- Standardization
  - Controlled vocabulary
- Validation

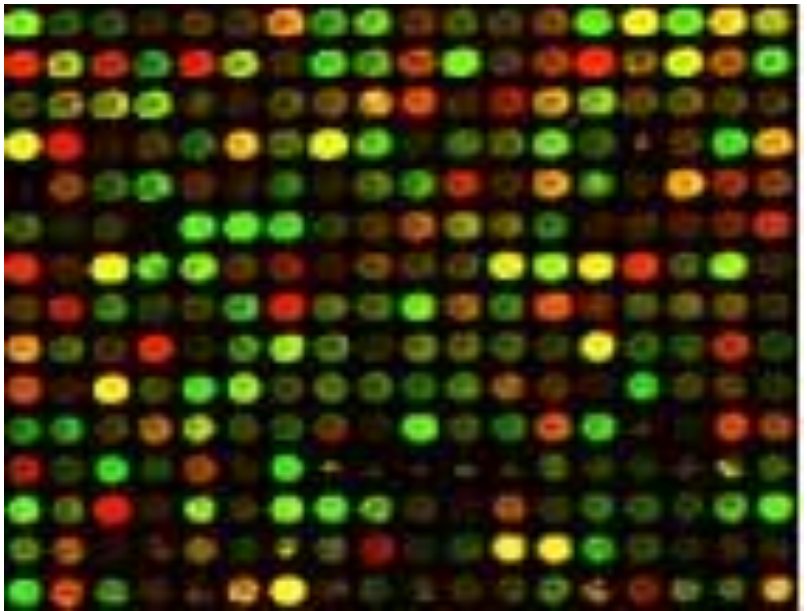
How can we use new information ?



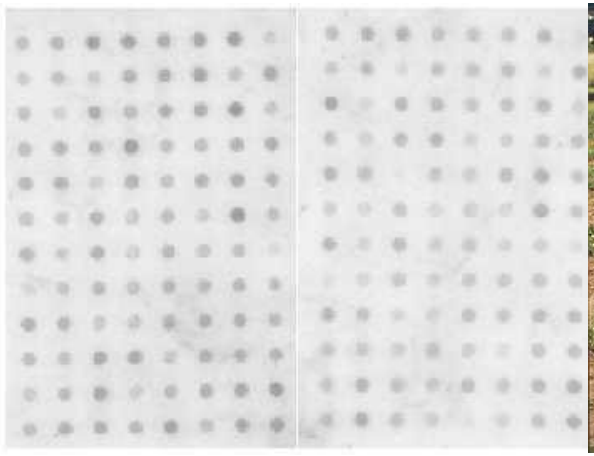
The results of “Structural Genomics” gives us many new tools to improve crops through “map-based” breeding



There is a broader context to the methodology e.g., quantitative methods used in plant breeding are currently being applied to the analysis of “DNA chip” experiments



- “When considering the handling of undesirable variation it is inevitable that the discussion will centre on agricultural field trials, since modern experimental technique was initiated and has reached its greatest elaboration in this realm”
- K. Mather, The Control of Error in Statistical Analysis in Biology, University Paperbacks, Methuen & Co., LTD., London.



Tobacco plant breeding

Combination

conventional

Marker assisted selection

Study genetic diversity → Parent selection

Parent selection

Crosses → Assessment of parents and progeny

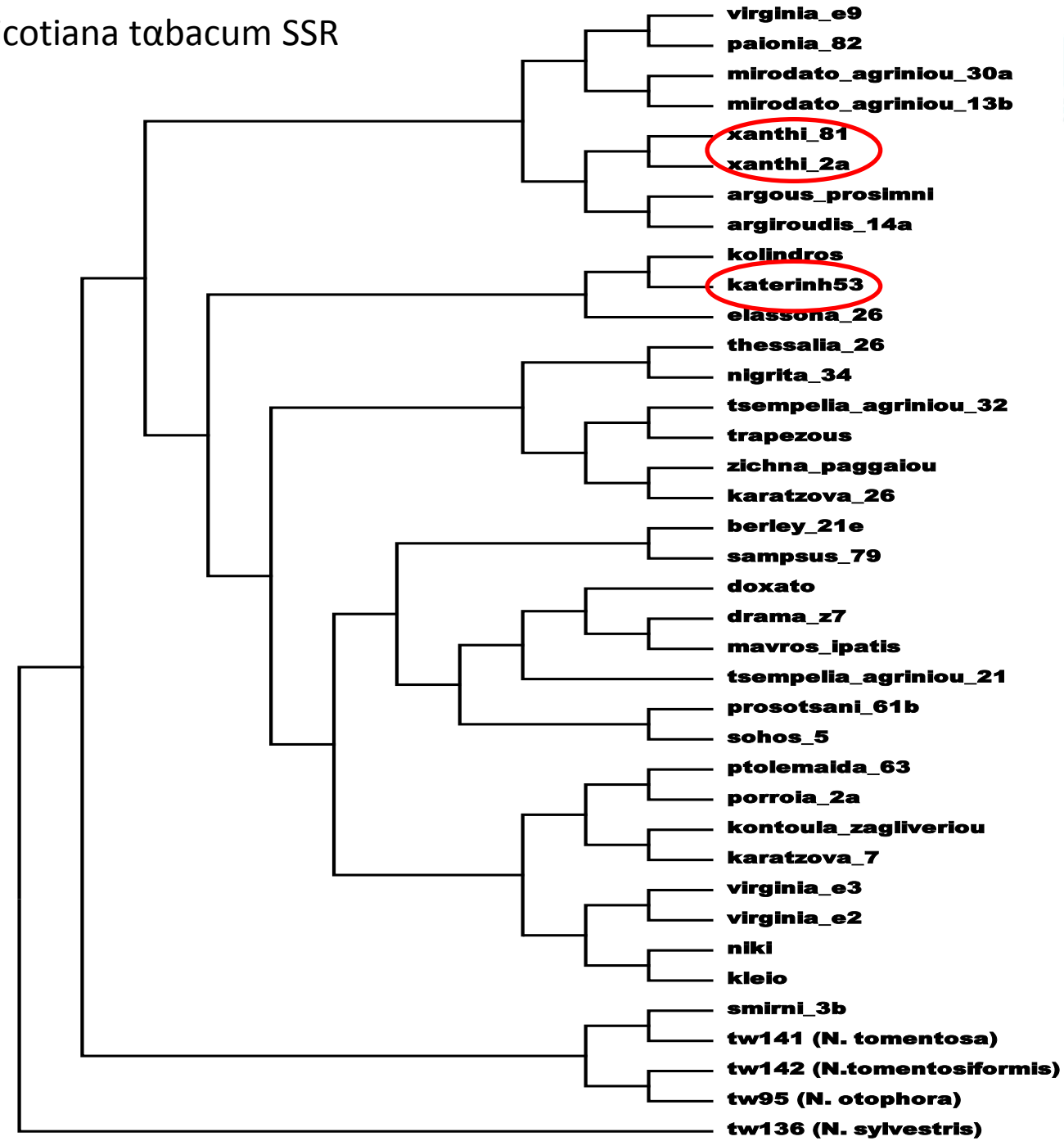
Selection

back crosses

selection → Assessment of parents and progeny

Pure lines → Assessment of progeny

Nicotiana tαbacum SSR



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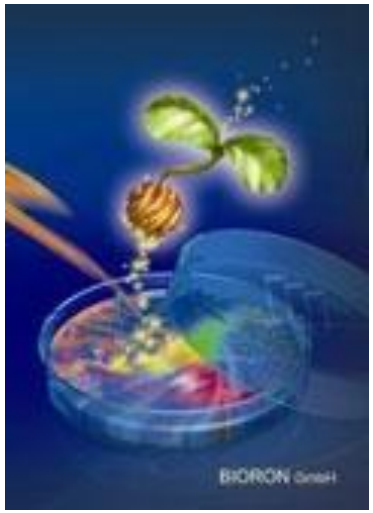
Genetic Engineering



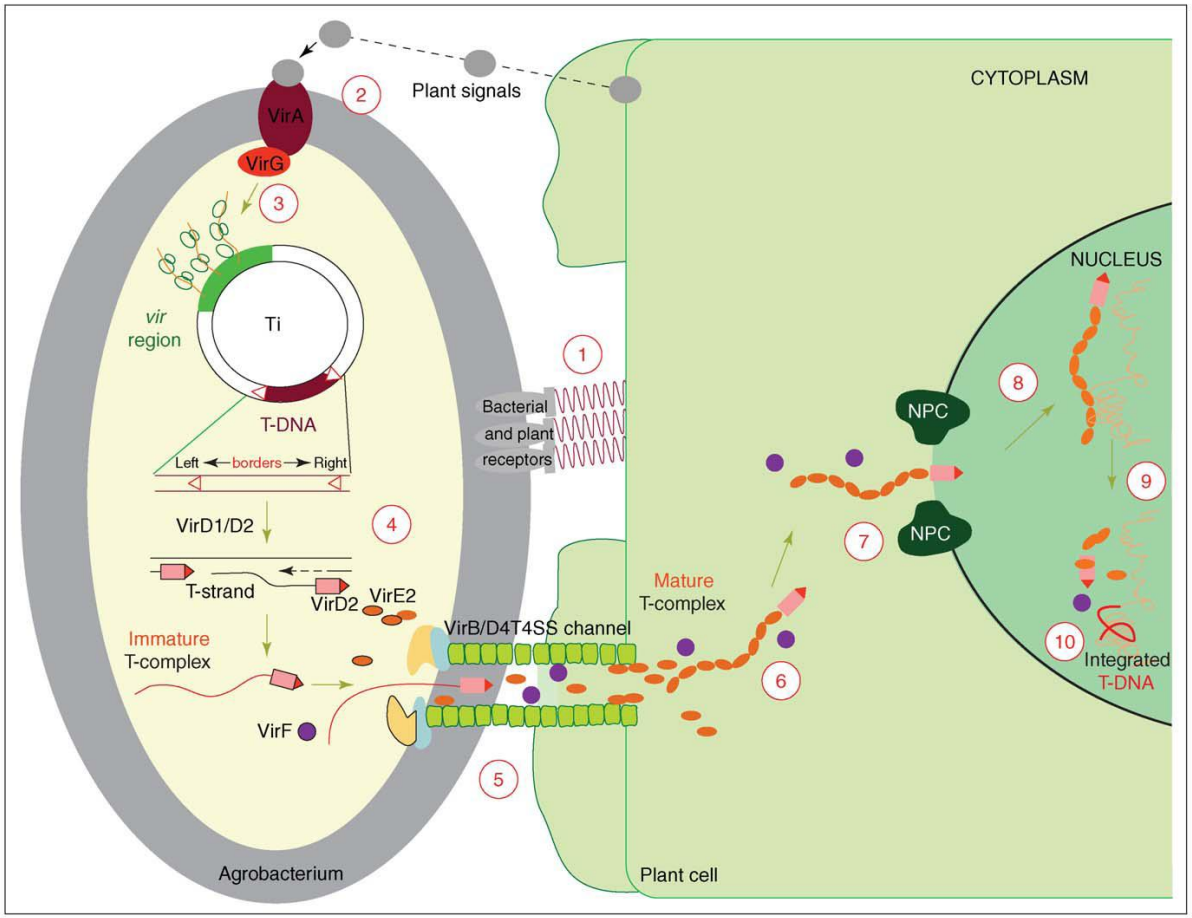
Developing disease resistant plants

- Over expression of cpd1 as a means for cercospora resistance

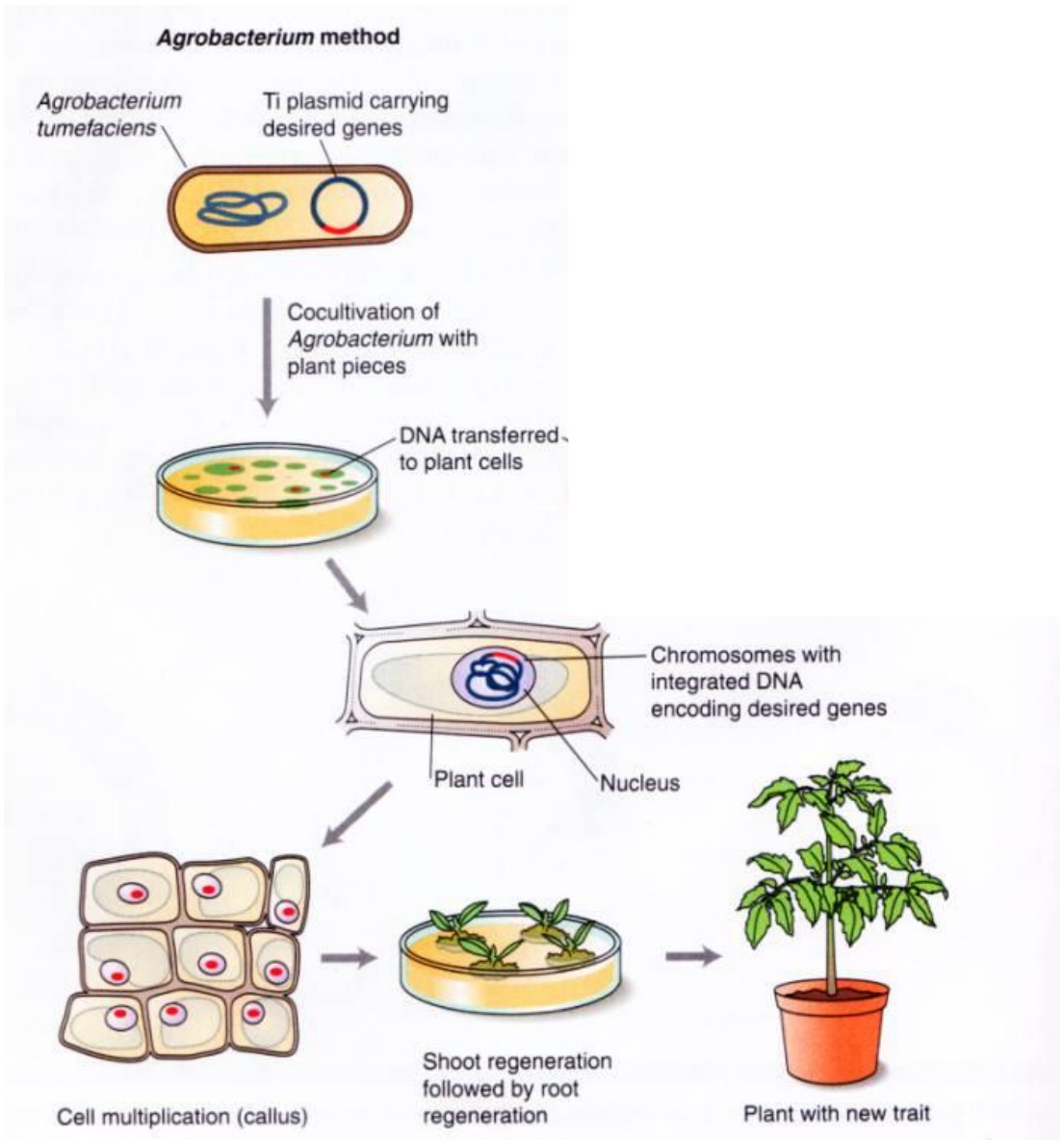
Cercospora infection

Cercopsora on beta vulgarisleaves

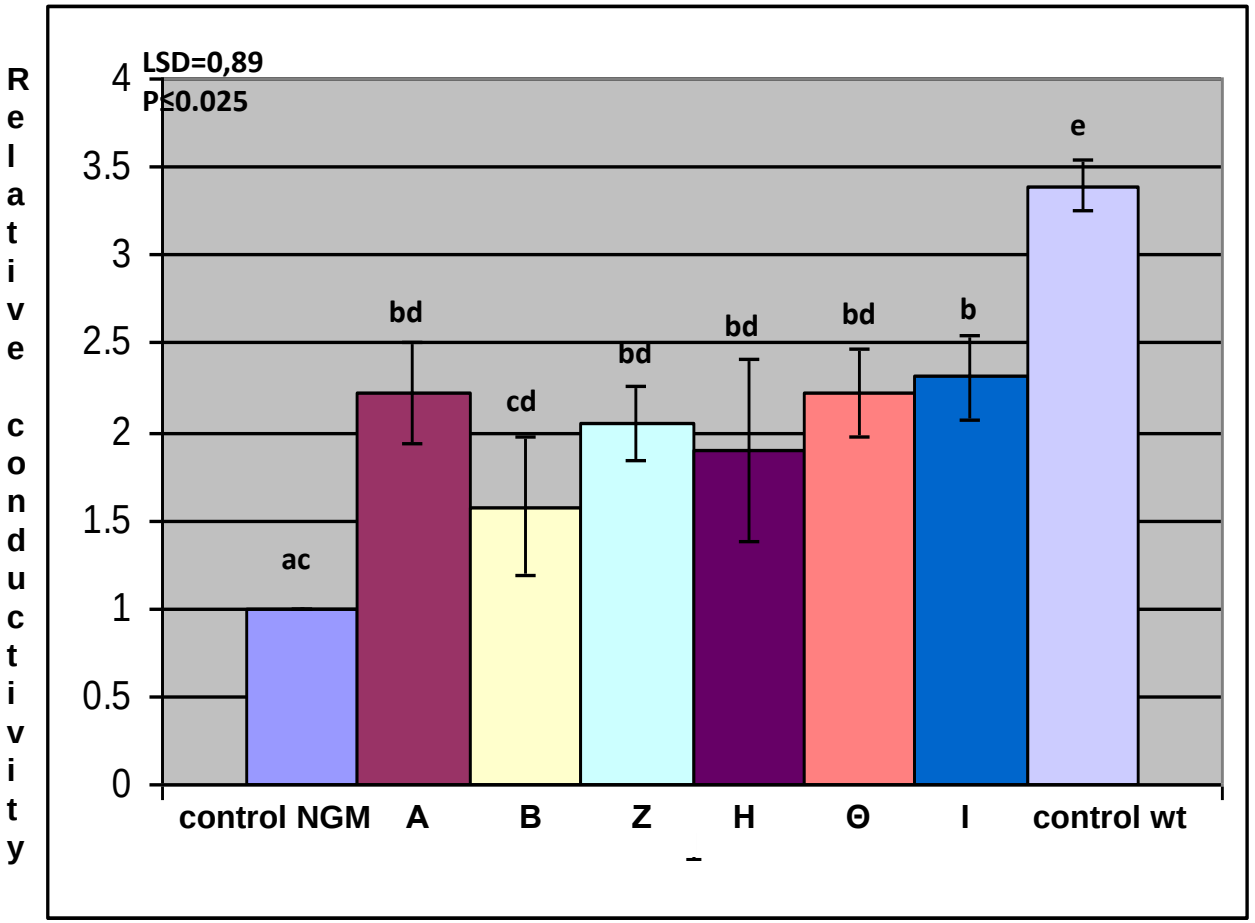
Genetic engineering through agrobacterium tumefaciens



Γενετική τροποποίηση φυτών



Relative resistance of transgenic plants



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Available online at www.sciencedirect.com
ScienceDirect
Biomolecular Engineering 24 (2007) 245–251
Biomolecular Engineering
www.elsevier.com/locate/geneanabioeng

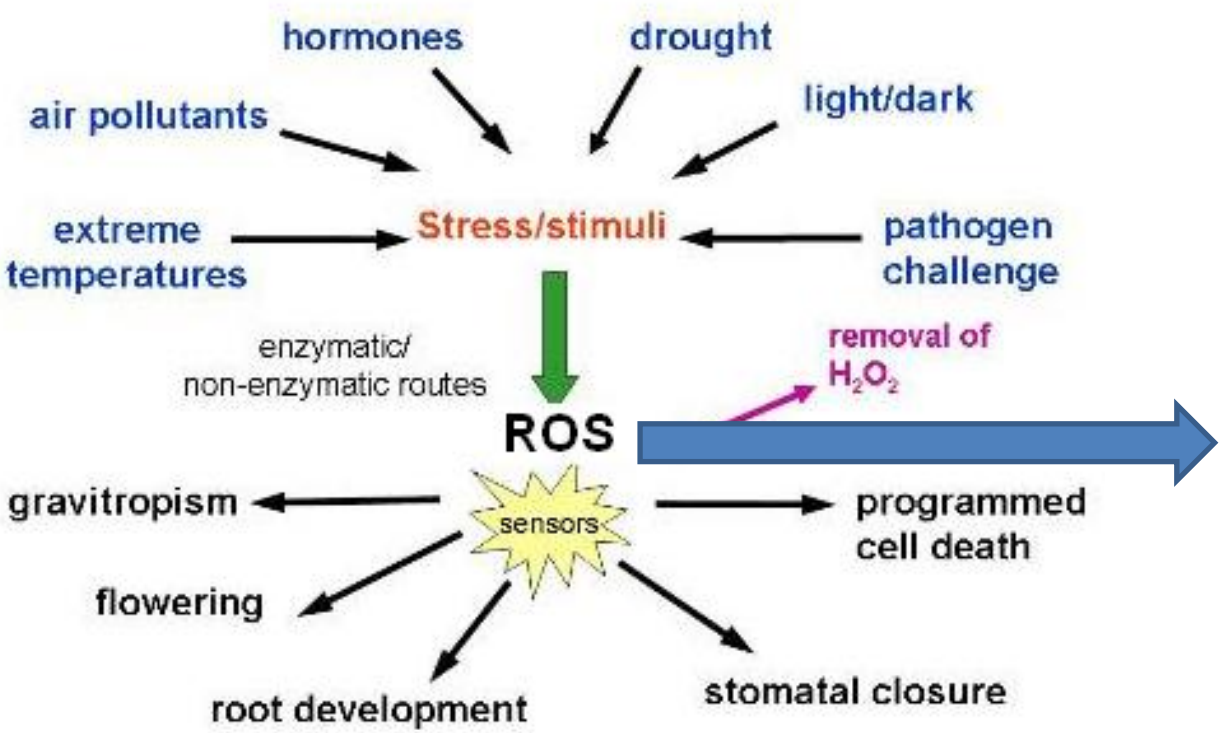
Expression of the yeast *cpd1* gene in tobacco confers resistance to the fungal toxin cercosporin

Madesis Panagiotis[a], Kalantidis Kritonas[a], Nianiou Obeidat Irini[a], Chatzidimitriou Kiriaki[a], Panopoulos Nicolaos[b], Tsaftaris Athanasios[a,c,*]

Developing stress tolerant plants

Oxidative stress

ROS: (O_2^{*-} ,H_2O_2, OH^{*-} ,$^1O_2$)



ROS damage proteins, nucleic acids, lipids etc

Oxidative stress

Addressing ROS

Natural Defense :

- Avoid
- Plant Morphology

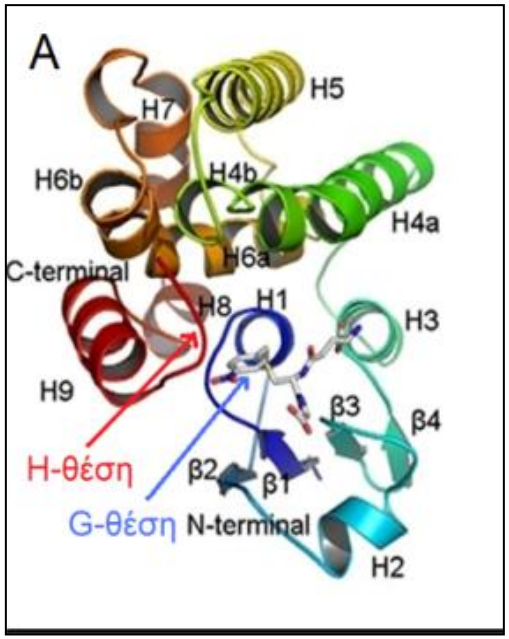
Chenical Defense:

- Vitamins
- Flavonoids
- carotenoids,
- **GSH**

***Enzyme Defense*:**

- Superoxide dismoutase SOD,
- Catalase CAT,
- ascorbate peroxidase APX
- glutathione reductase, GR
- dehydroascorbate reductase, DHAR
- υπεροξειδάσες της γλουταθειόνης (GPX)
- **glutathione transferase, GST**

Structure of GST

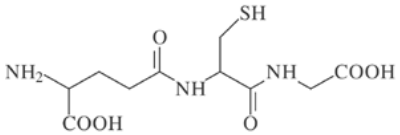


Current Chemical Biology, 2011, *5*, 000-000

Structure and Antioxidant Catalytic Function of Plant Glutathione Transferases

Evangelia Chronopoulou[1], Irene Axarli[1], Irini Nianiou-Obeidat[2], Panagiotis Madesis[3], Athanasios Tsaftaris[2,3] and Nikolaos E. Labrou[1]*

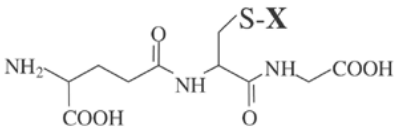
Function of GST



Glutathione

+ Xenobiotic (X)

GST



Glutathione-S-Conjugate

Current Chemical Biology, 2011, *5*, 000-000

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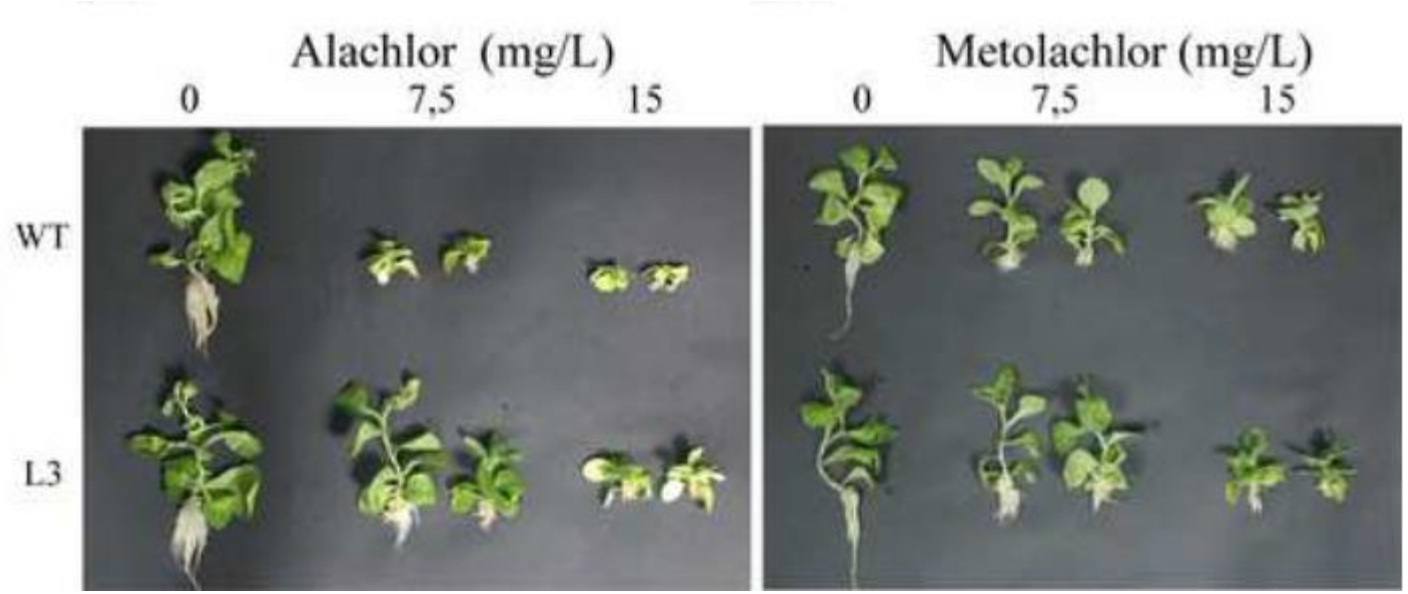
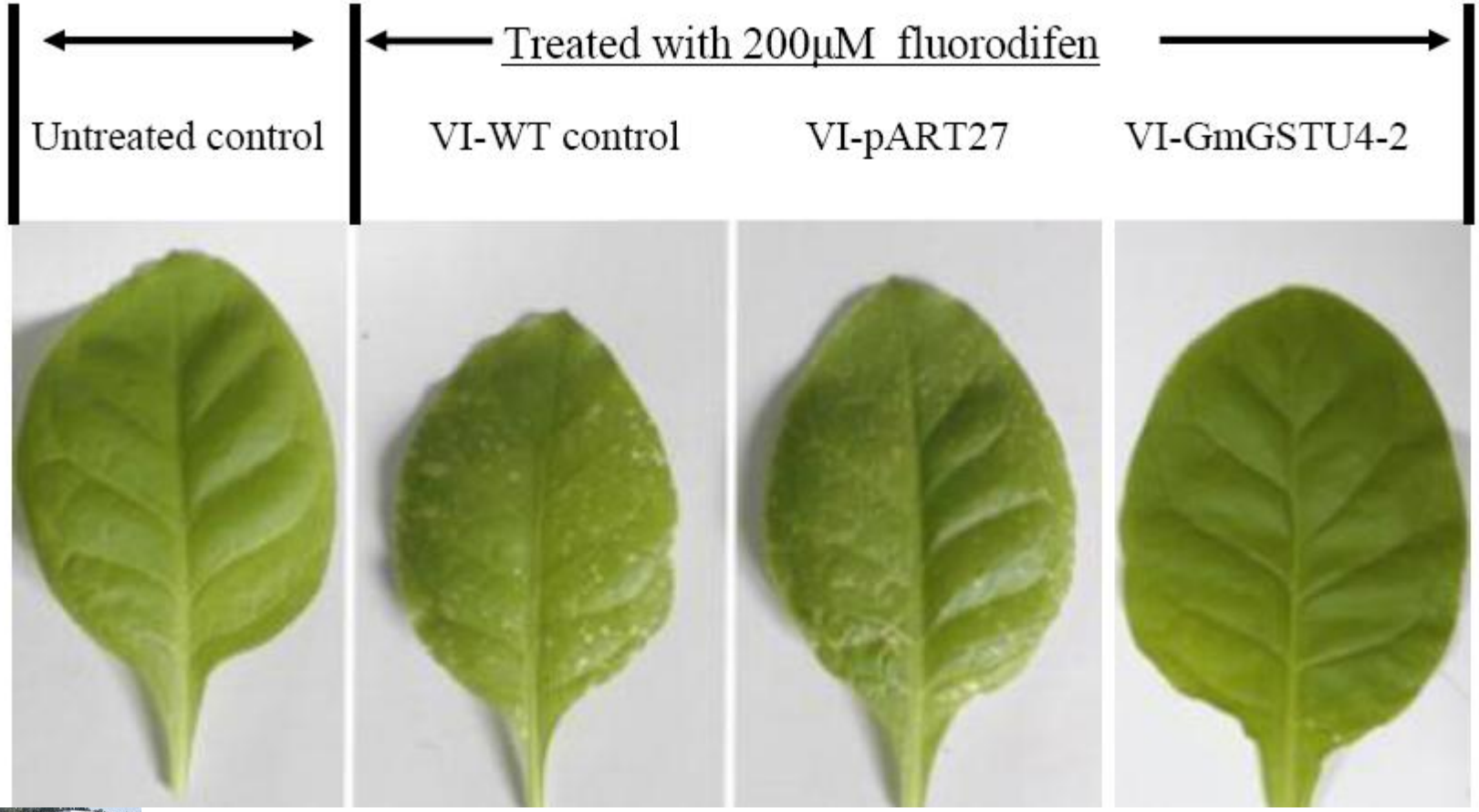
Alachlor resistance through *gst4* over-expression in tobacco



Fluorodifen resistance through *gst4* over-expression in tobacco

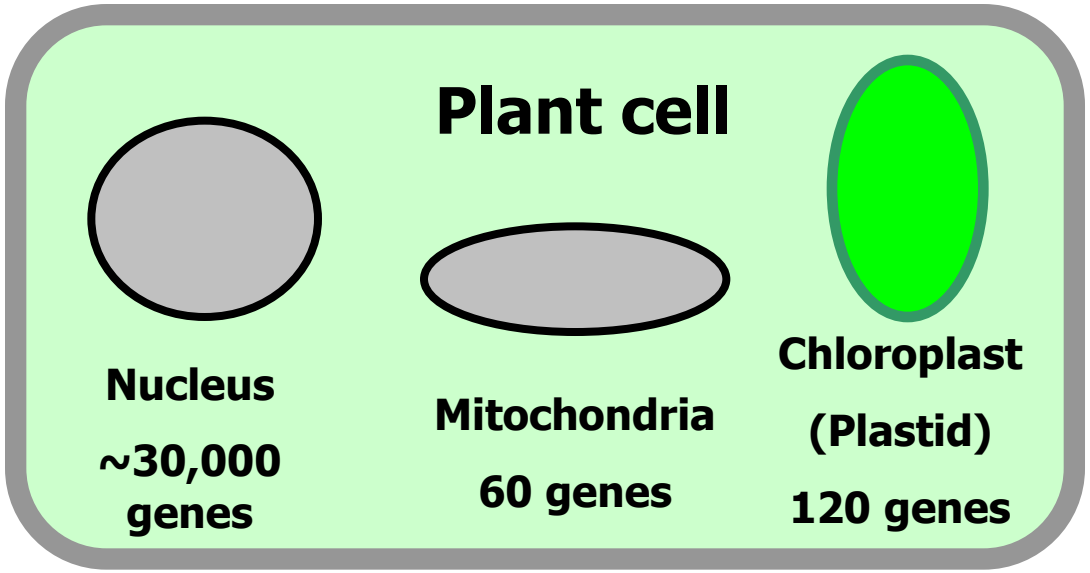


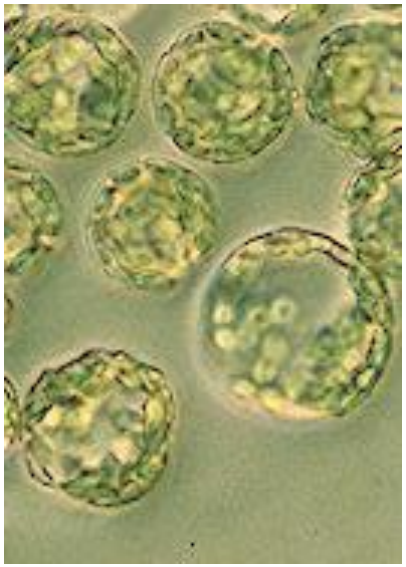
Salt resistance

NaCl

Chloroplast genetic engineering

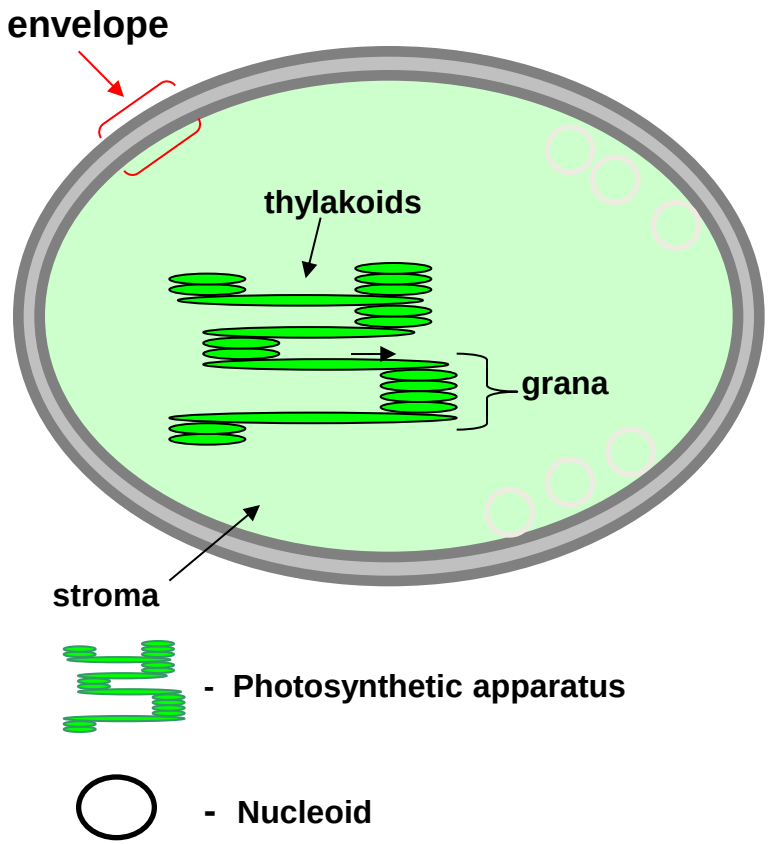
Plants contain multiple genomes

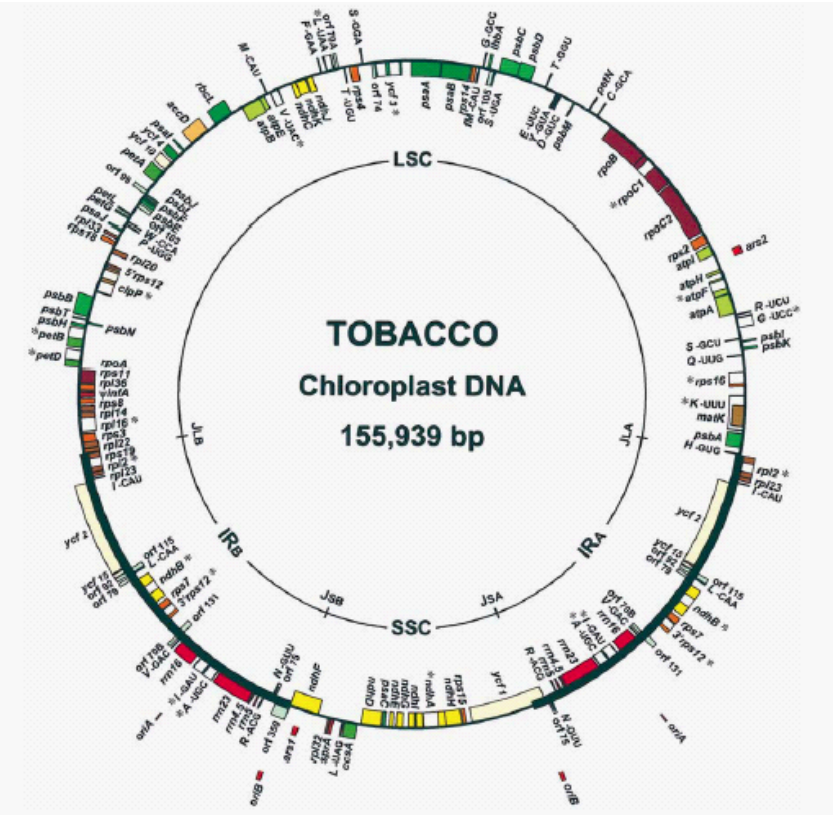




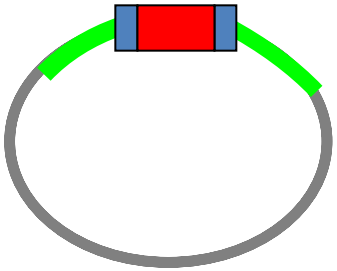
- Three genomes in all plant cells
- 100 chloroplasts per cell
- 100 genomes per chloroplast (10,000 per cell)

Chloroplast structure

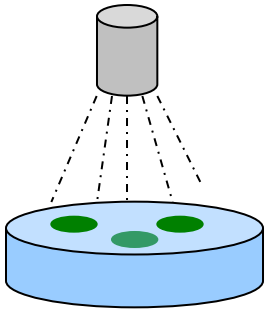




Tobacco chloroplast transformation



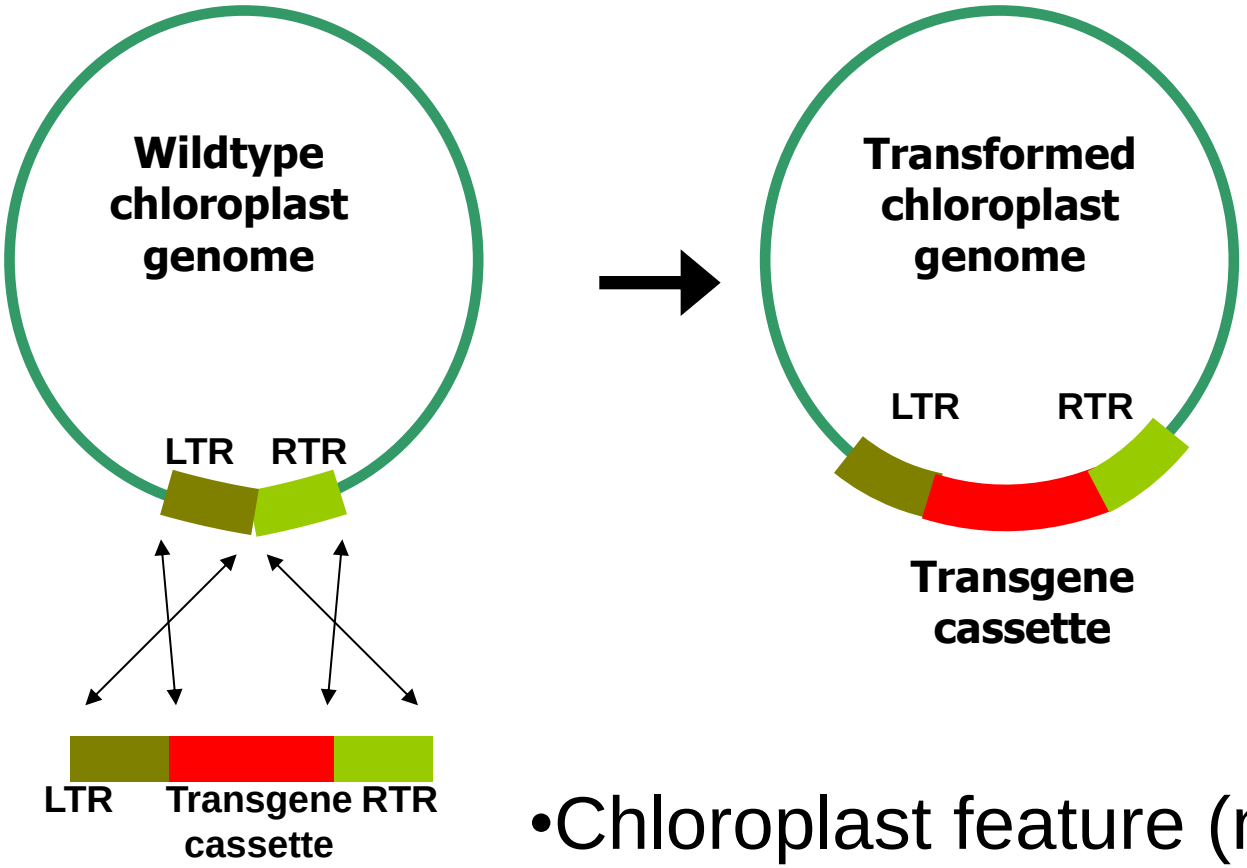
Plasmid stock purified and used for particle bombardment of leaf tissue



Three cycles of regeneration from leaf tissue produces homoplasmic plants

Plants regenerated from leaf tissue under antibiotic selection

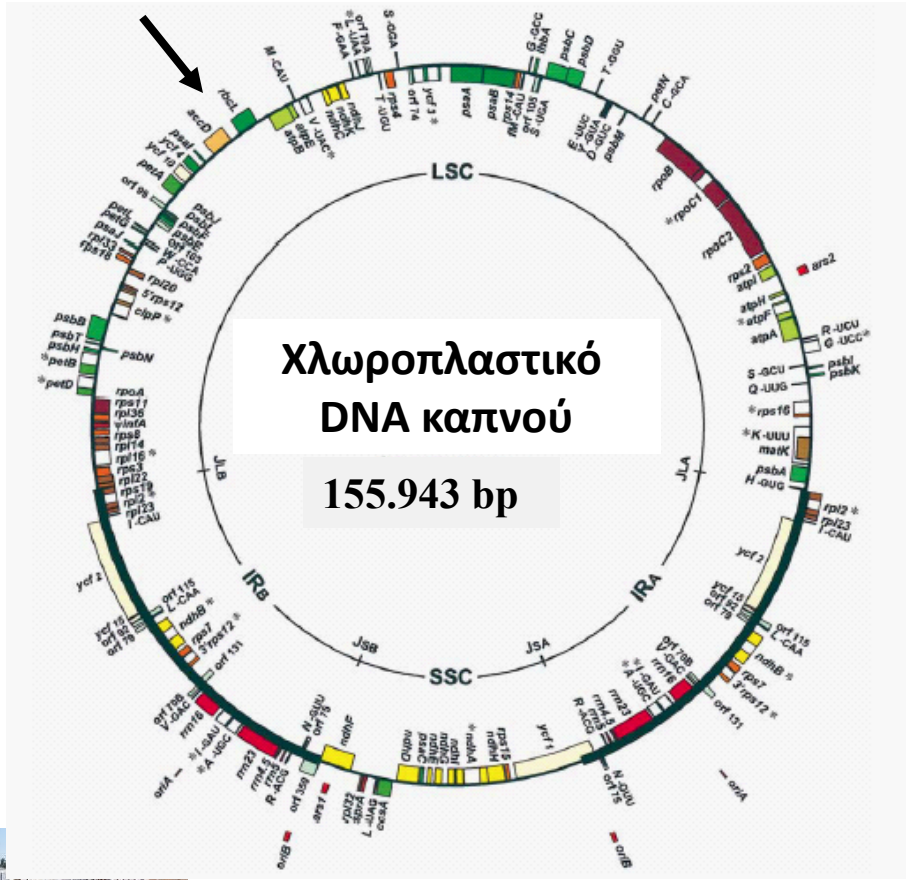
Transgene insertion proceeds by homologous recombination

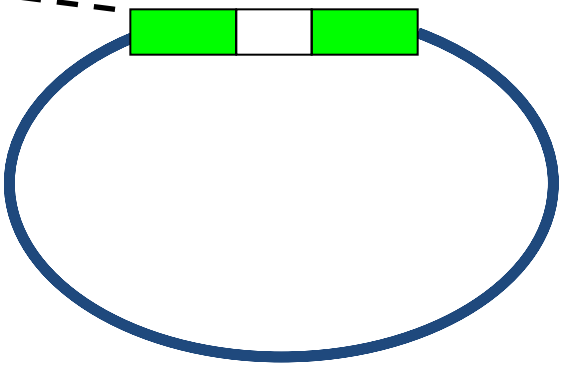


- Chloroplast feature (not nuclear)
- Targeted and stable integration

Vector structure

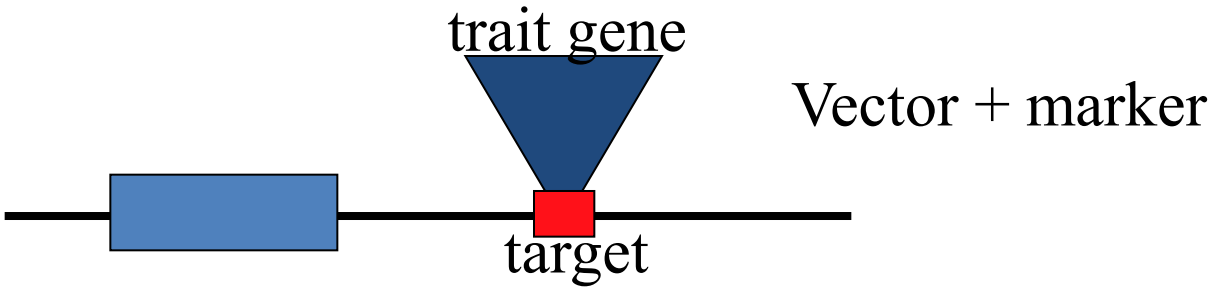
atpB rbcL(LTR)	*PROM*	*aadA*	*TERM*	*PROM*	*Target cDNA*	*TERM*	*accD* (RTR)





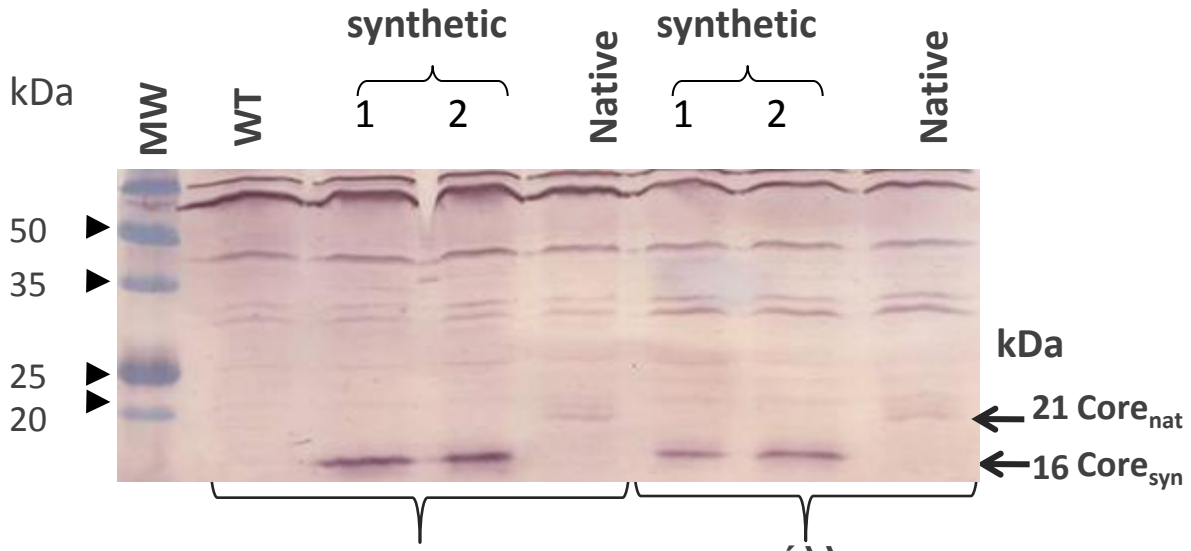
Clean gene transformation

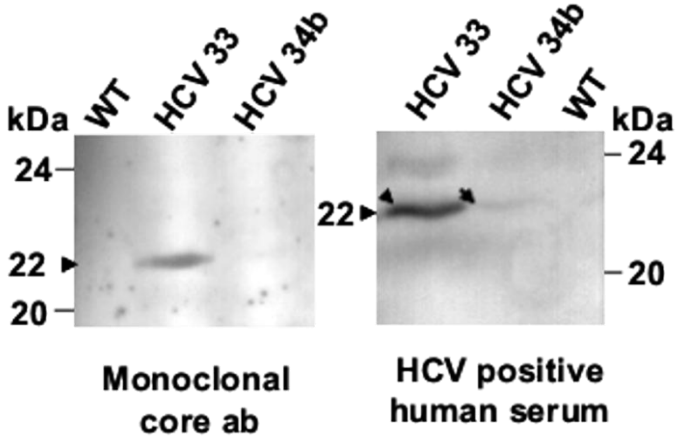
- Precise integration without vector sequences or plant marker genes

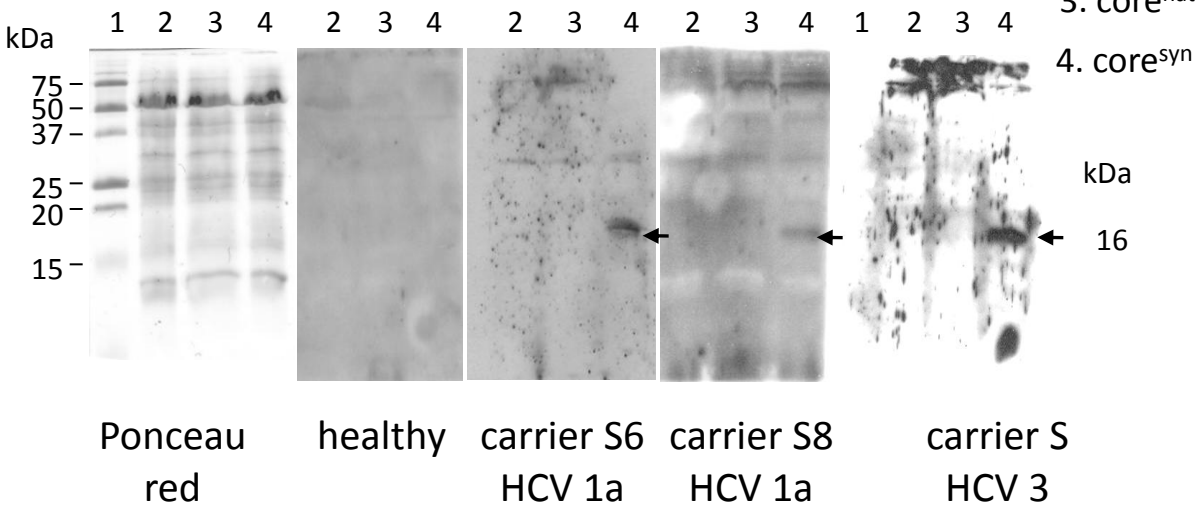


- Success of transgenic plants
  - Not only trait genes
  - improved transformation technologies

HCV core protein over-expression







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Thank you
for your attention