MIGLIORAMENTO GENETICO DELLE PIANTE

FASI DEL MIGLIORAMENTO GENETICO

1) Introduzione di specie coltivabili in una regione

2) Selezione all'interno delle varietà locali -> omogeneità genetica (svantaggiosa sul lungo termine, es. suscettibilità a malattie)

3) Incroci intra- ed interspecifici per creare variabilità e selezionare nuovi genotipi

Fino al XVII-XVIII secolo, l'uomo ha semplicemente selezionato genotipi migliori basandosi sul fenotipo, sfruttando la variabilità genetica esistente

In seguito, ha iniziato a combinare in modo controllato tale variabilità per ottenere piante con specifici tratti migliorati: incroci intra- ed interspecifici


D. Joſeph Gottlieb Kölreuters

Vorläufige Nachricht

von einigen

das Geſchlecht der Pflanzen

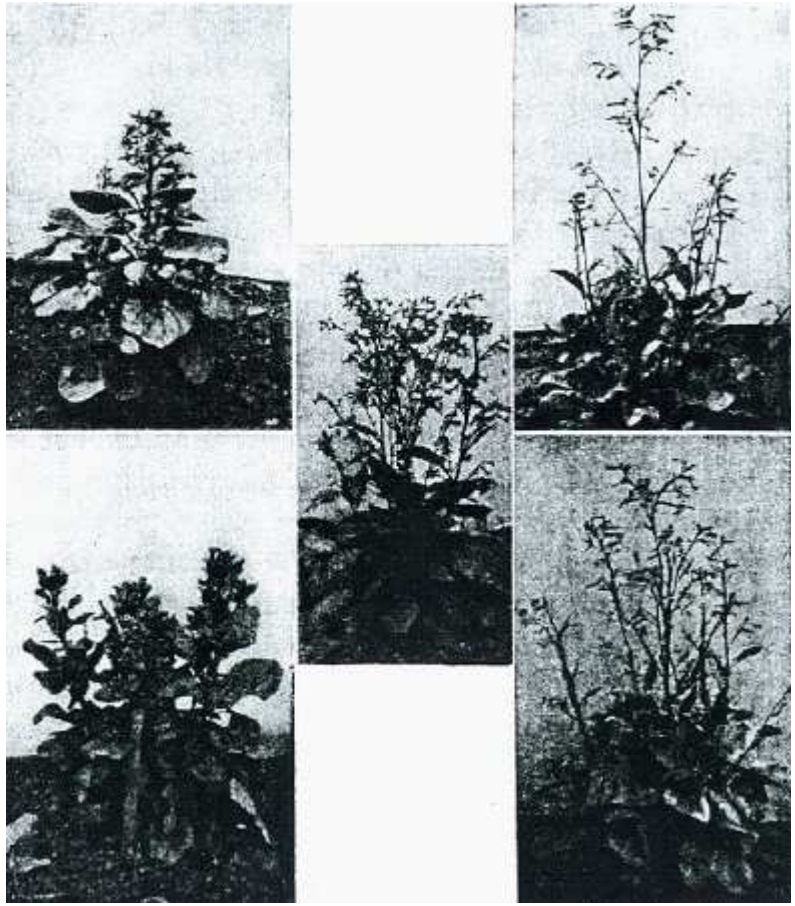
betreffenden Verſuchen

und Beobachtungen.

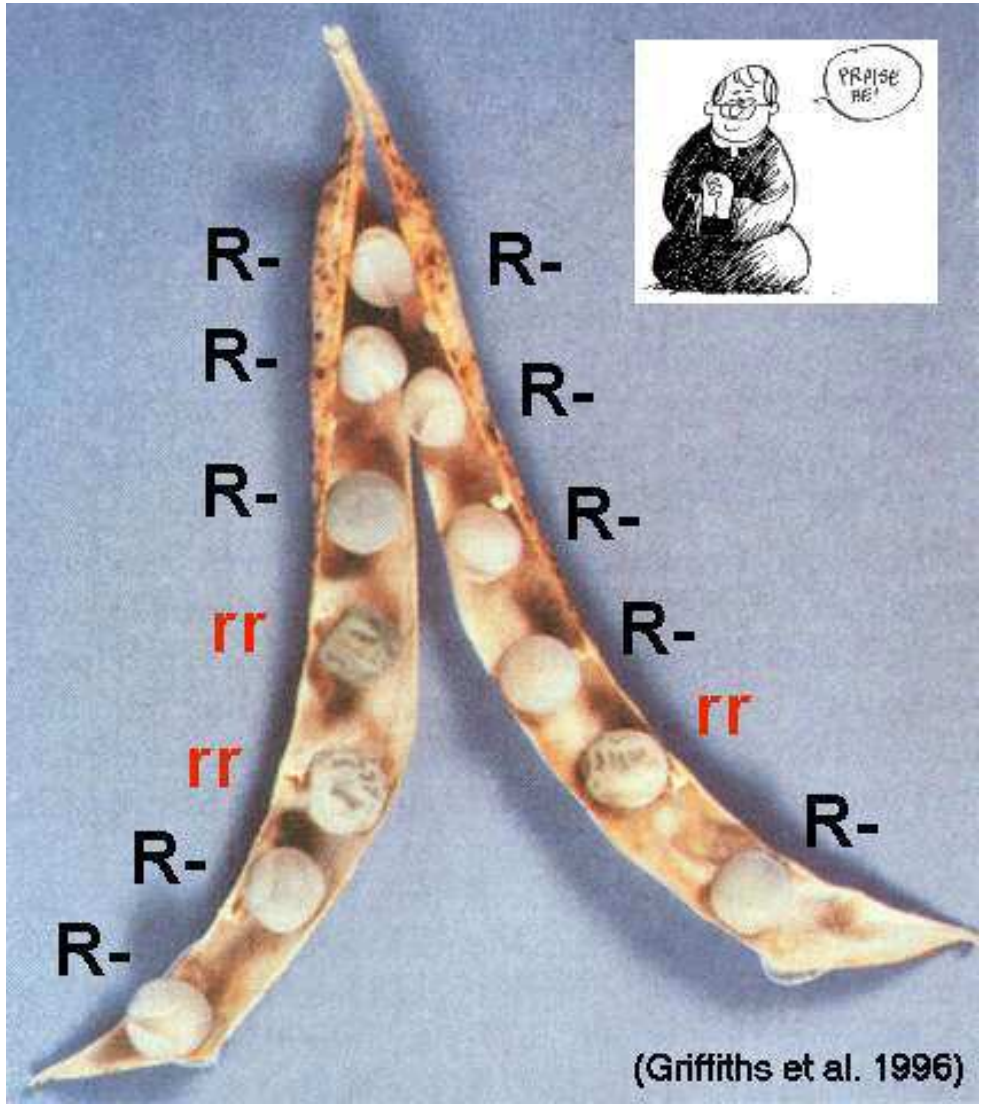
Leipzig,

in der Gleditſchiſchen Handlung.

1761.



Mendel: leggi dell'ereditabilità



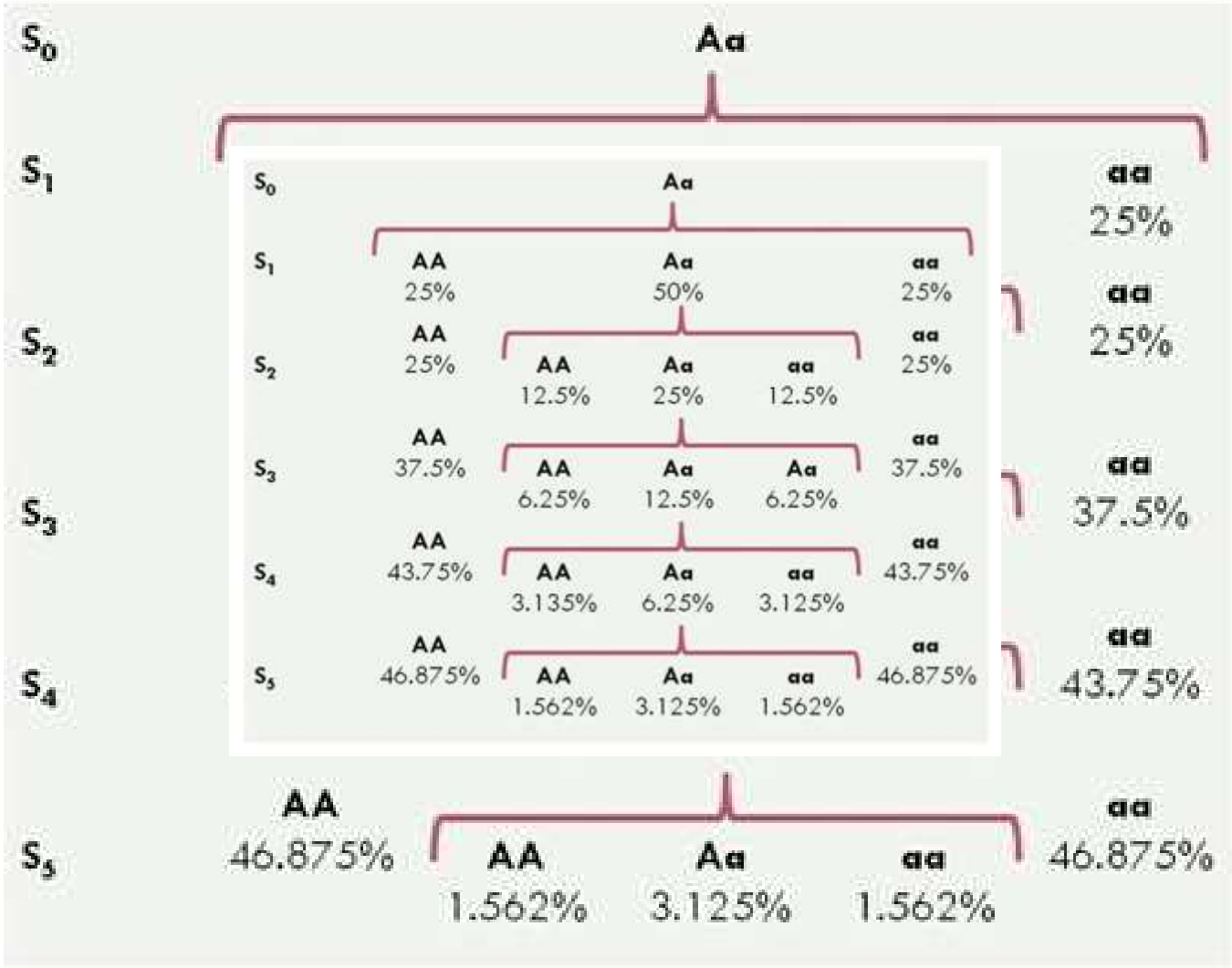
METODI D'INCROCIO

Dipende dal metodo d'impollinazione e riproduzione della specie:

1) **AUTOGAMIA**: auto-impollinazione -> alto grado di **omozigosità**

- Necessario demasculare i fiori ed impollinare a mano per effettuare incroci artificiali

Aumento dell'omozigosità in popolazioni autogame



METODI D'INCROCIO

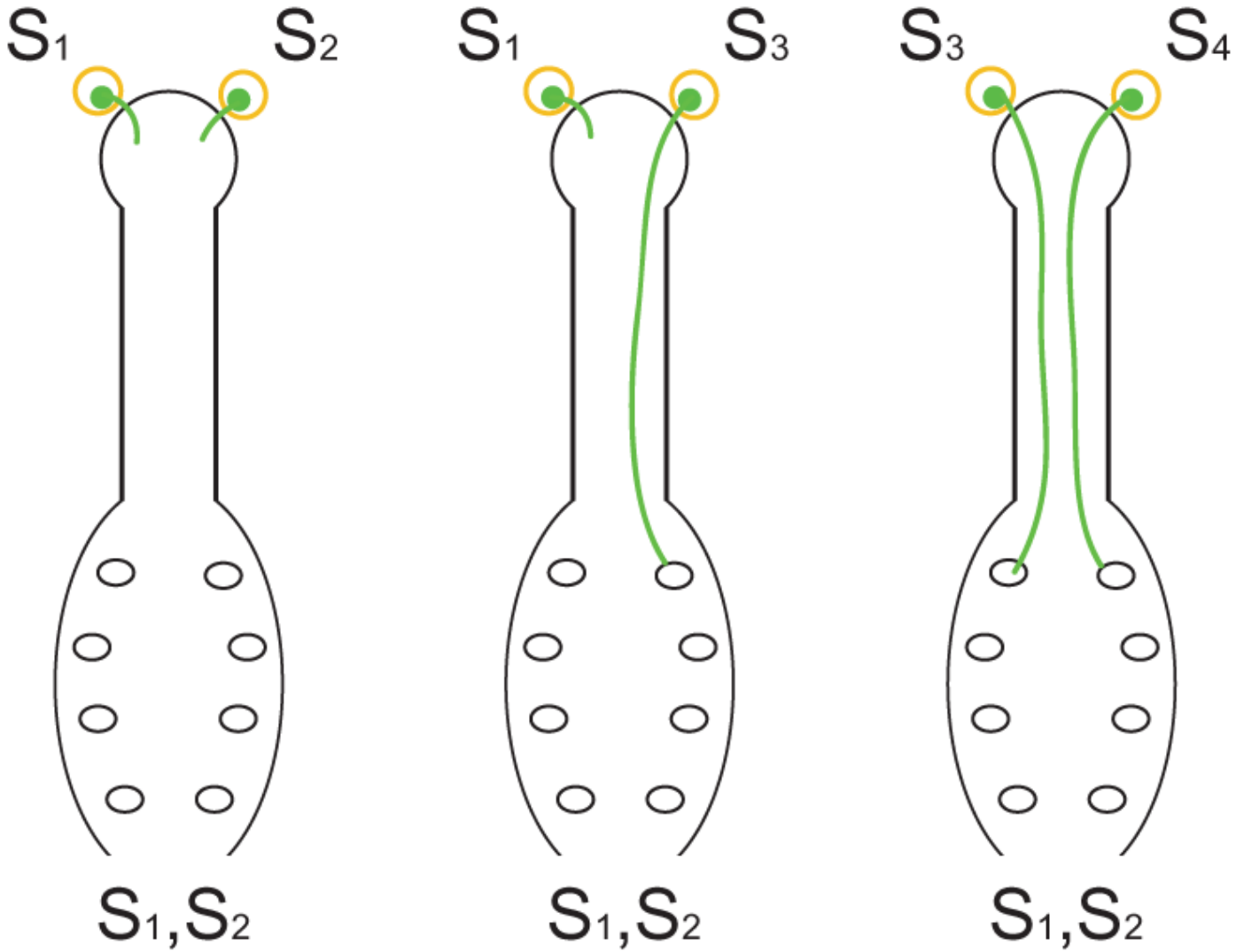
Dipende dal metodo d'impollinazione e riproduzione della specie:

1) **ALLOGAMIA**: impollinazione incrociata -> alto grado di **eterozigosità**

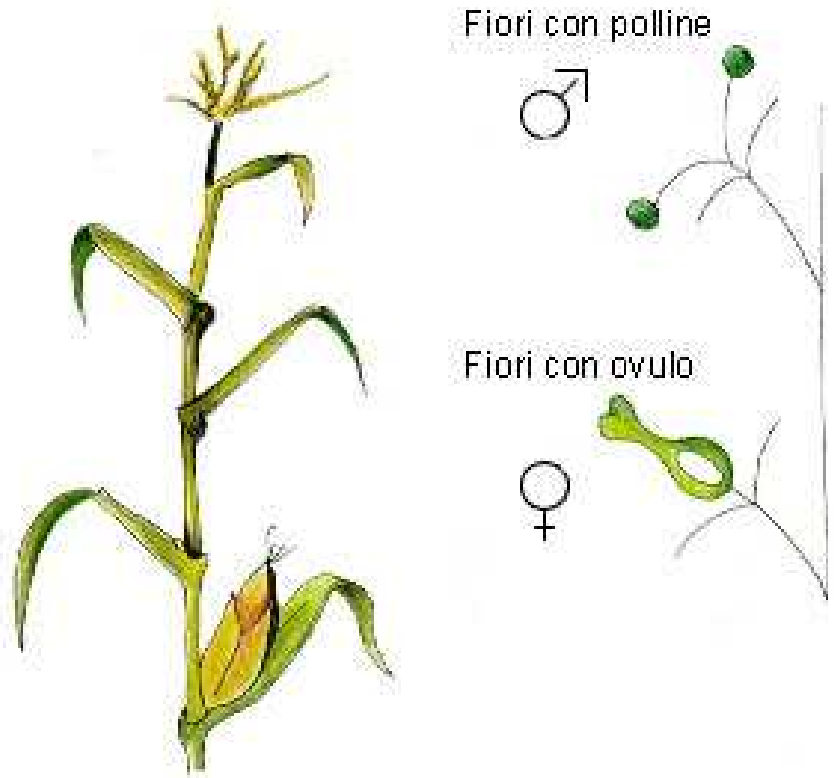
- Necessario prevenire fecondazione con polline non desiderato per effettuare incroci artificiali

- Varietà vendute come popolazioni non omogenee di piante eterozigoti

Autoincompatibilità genetica



Separazione fisica fiori maschili e femminili sulla stessa pianta (es. mais)



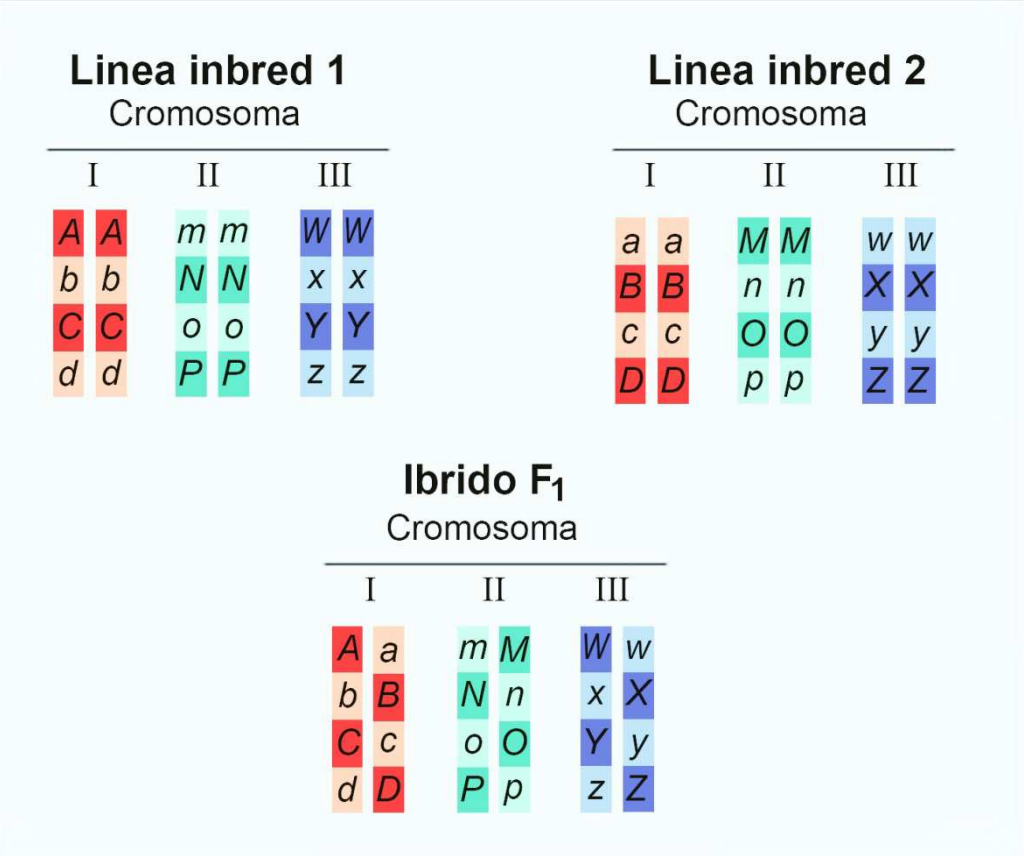
Specie bisessuale con fiore diclino

Separazione fisica fiori maschili e femminili sul piante diverse (es. kiwi)

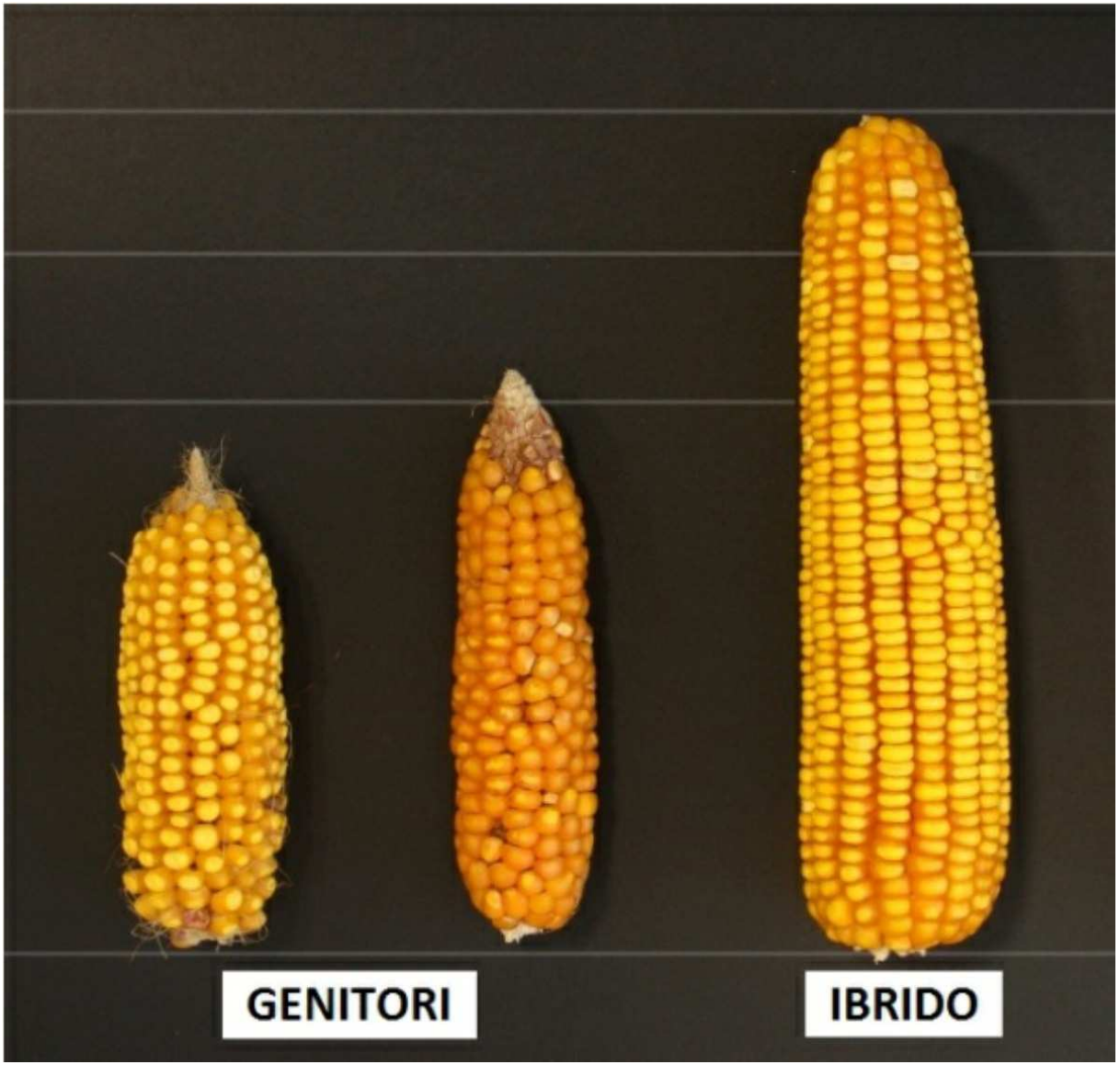
PRODUZIONE DI IBRIDI F1

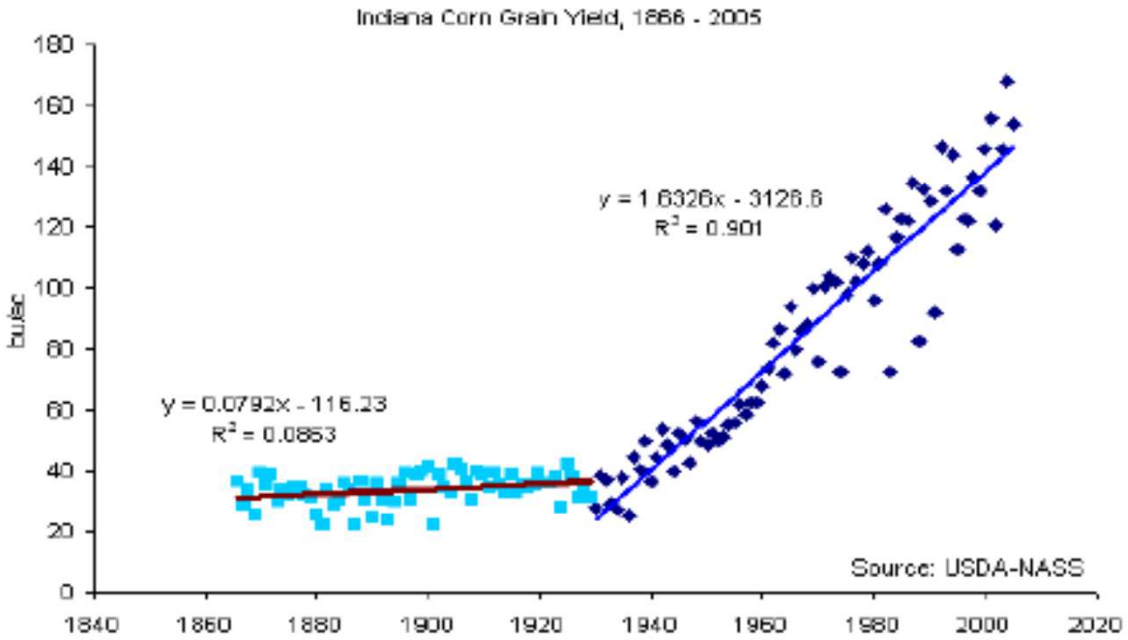
Possono essere ottenuti sia da piante autogame che allogame

1) Costituzione di linee INBRED (pure) per autoimpollinazione
2) Impollinazione incrociata tra linee inbred selezionate per ottenere ibrido F1



ETEROSI: gli ibridi tra due linee pure sono più vigorosi, resistenti e produttivi delle linee da cui derivano

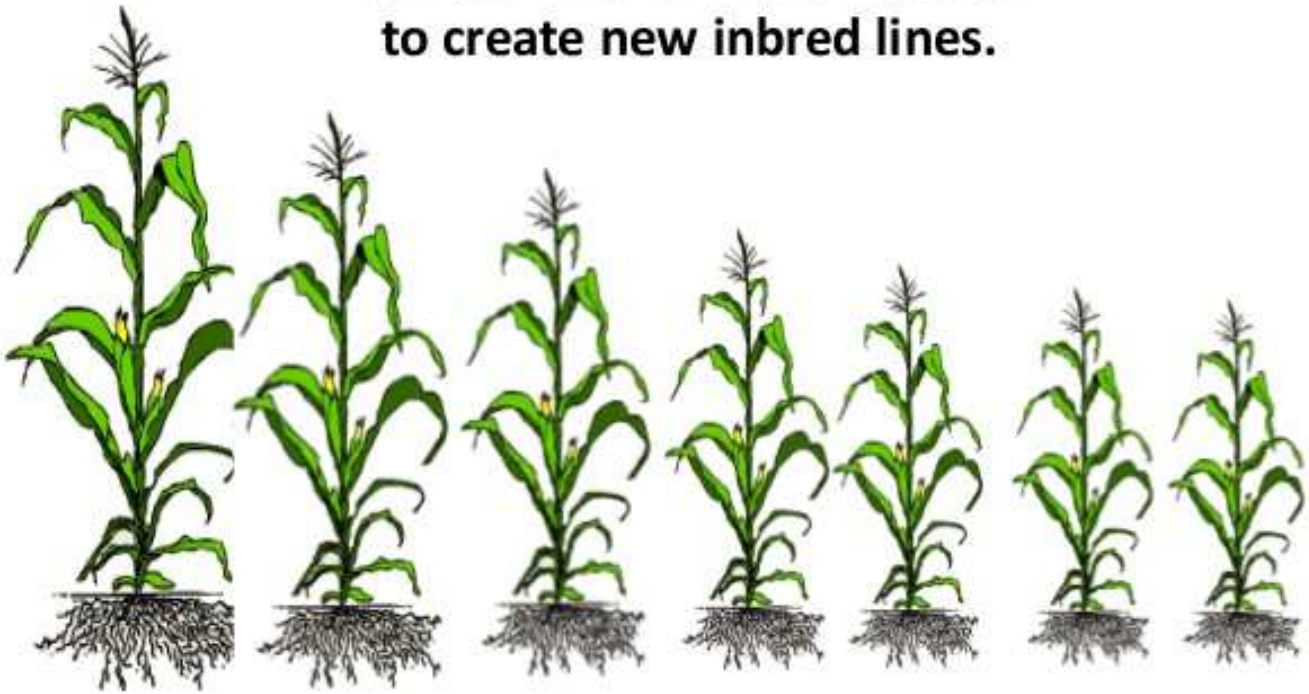




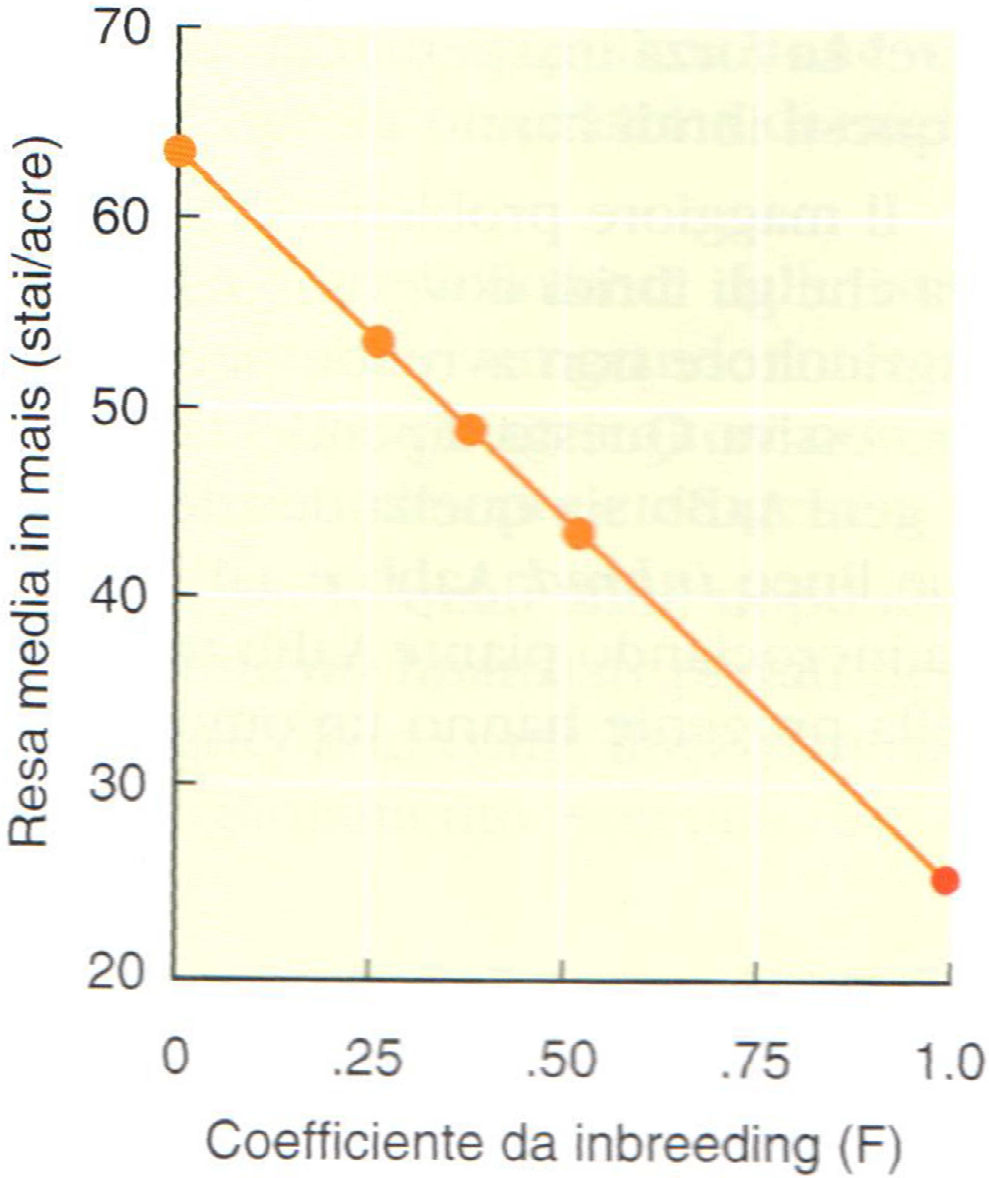
Adoption of hybrid genetics has tripled US corn yield since 1940

Depressione da inbreeding

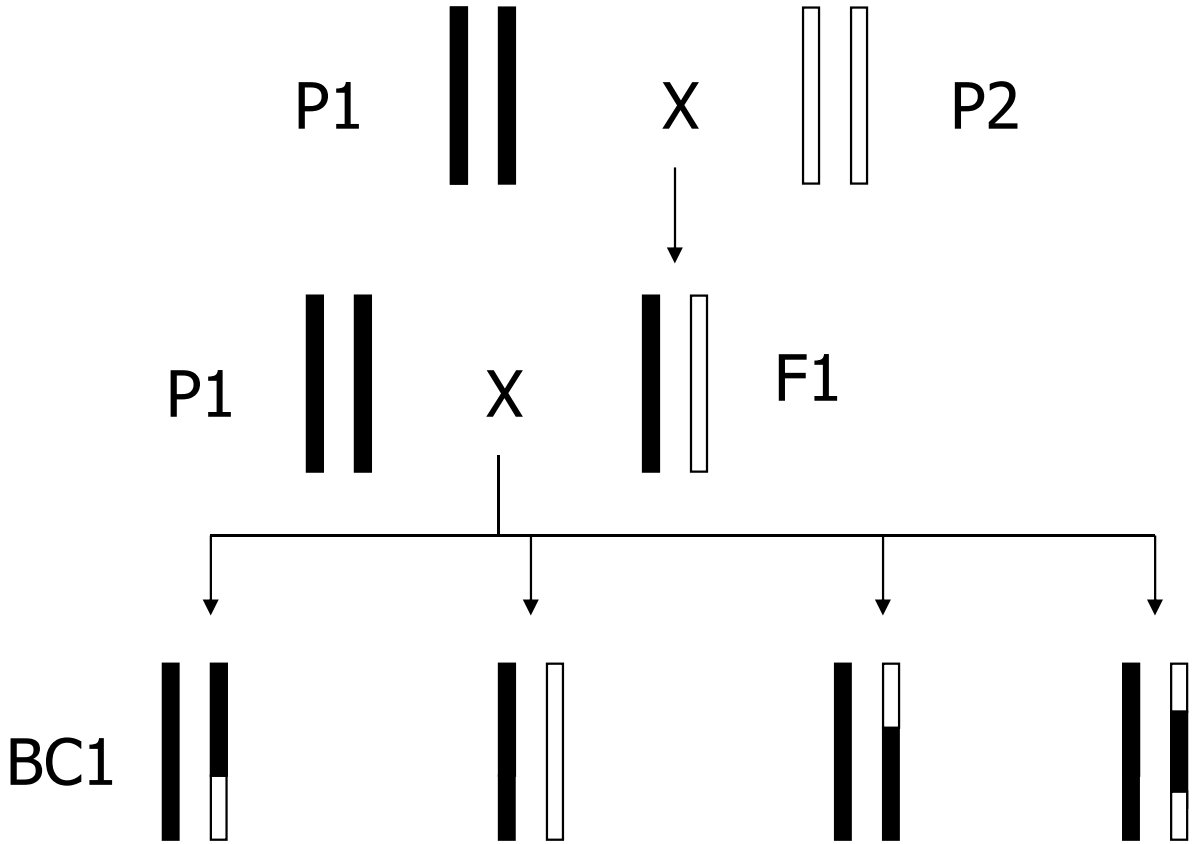
Historically 7+ generations of self-pollination were needed to create new inbred lines.



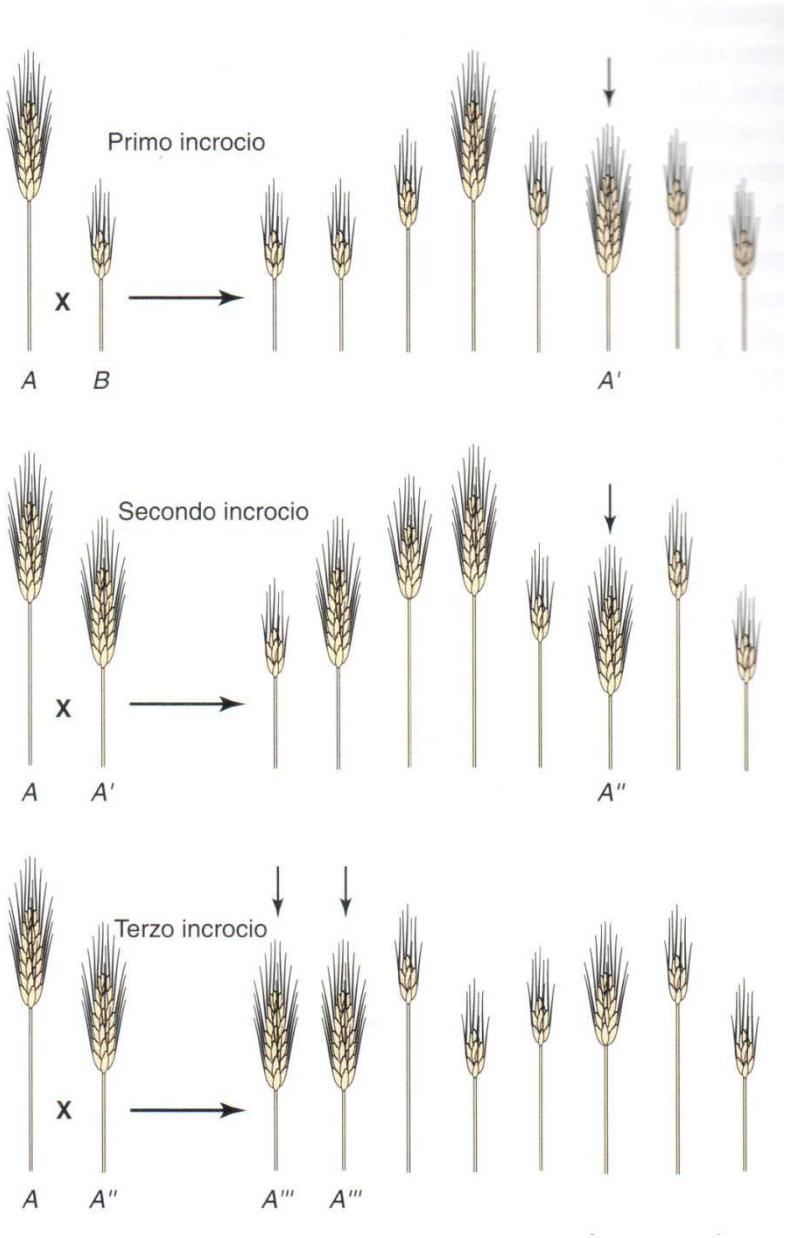
Inbreeding depression


70
60
50
40
30
20
Resa media in mais (stai/acre)
0
.25
.50
.75
1.0
Coefficiente da inbreeding (F)

Backcross



INTRODUZIONE DI SINGOLI CARATTERI TRAMITE BACKCROSSING



I vantaggi raggiunti dai selezionatori

Contadino romano: 1000 kg frumento per ettaro

In Italia 1920: 1000 kg frumento per ettaro

Adesso: 4000 kg frumento per ettaro

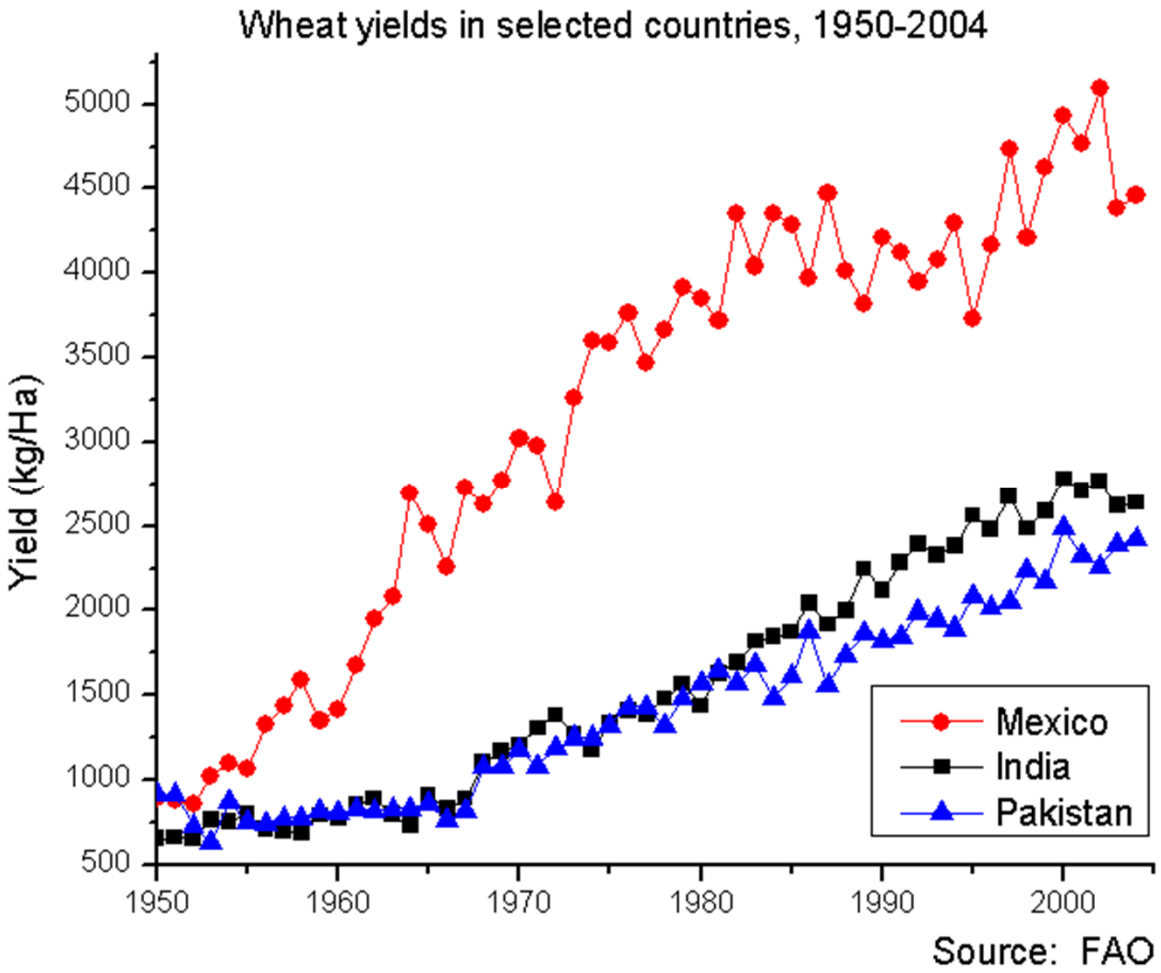
Pratiche agricole più efficiente e selezioni

RIVOLUZIONE VERDE

La Rivoluzione verde

“l’uomo che disinnescò la bomba dell’aumento della popolazione umana”

Norman Borlaug
breeding wheat for Mexico
Nobel Peace Prize 1970



La rivoluzione verde

Agricoltura ad alta resa con elevato input di sostanze chimiche

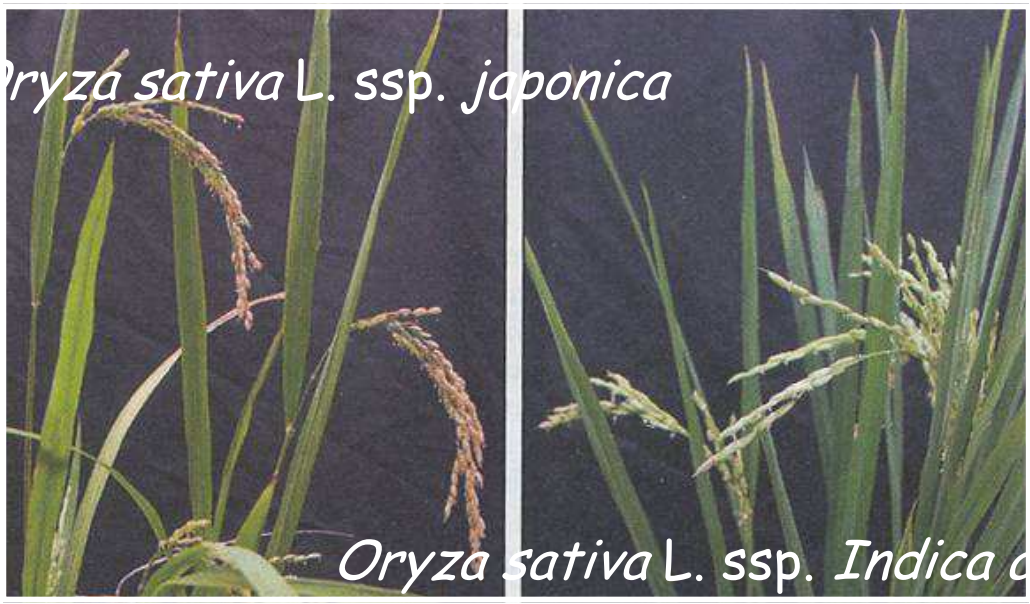
Alta resa: migliore assorbimento dei nutrienti, maggior biomassa

Rapida maturazione: nel riso dalla semina alla raccolta in 125 giorni contro i 210 previsti (in Asia due cicli)

Habitus di crescita: semi-nano (90 cm contro i 120 del frumento)

Giappone, Filippine, USA

Riso: varietà Japonica e Indica



La rivoluzione verde si basa sulle tecniche del miglioramento genetico

Alta resa

Rapida maturazione

Habitus di crescita semi-nano

Resistenza alle malattie

Adattabilità alle condizioni locali

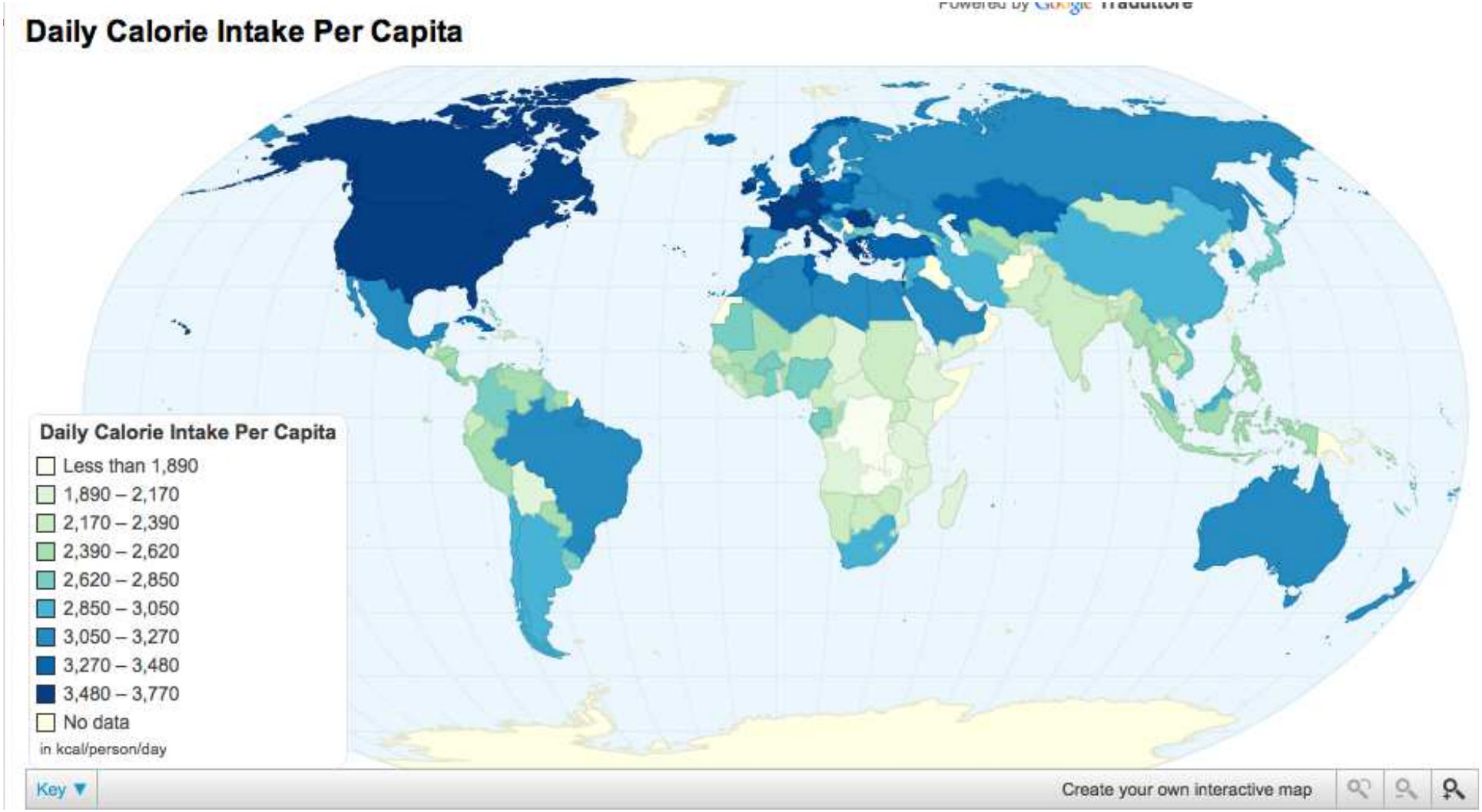
Applicazione di fertilizzanti inorganici

erbicidi e fitofarmaci

Tecnologia di irrigazione, macchine….

Le conseguenze….

Quante calorie al giorno?



http://chartsbin.com/view/1150

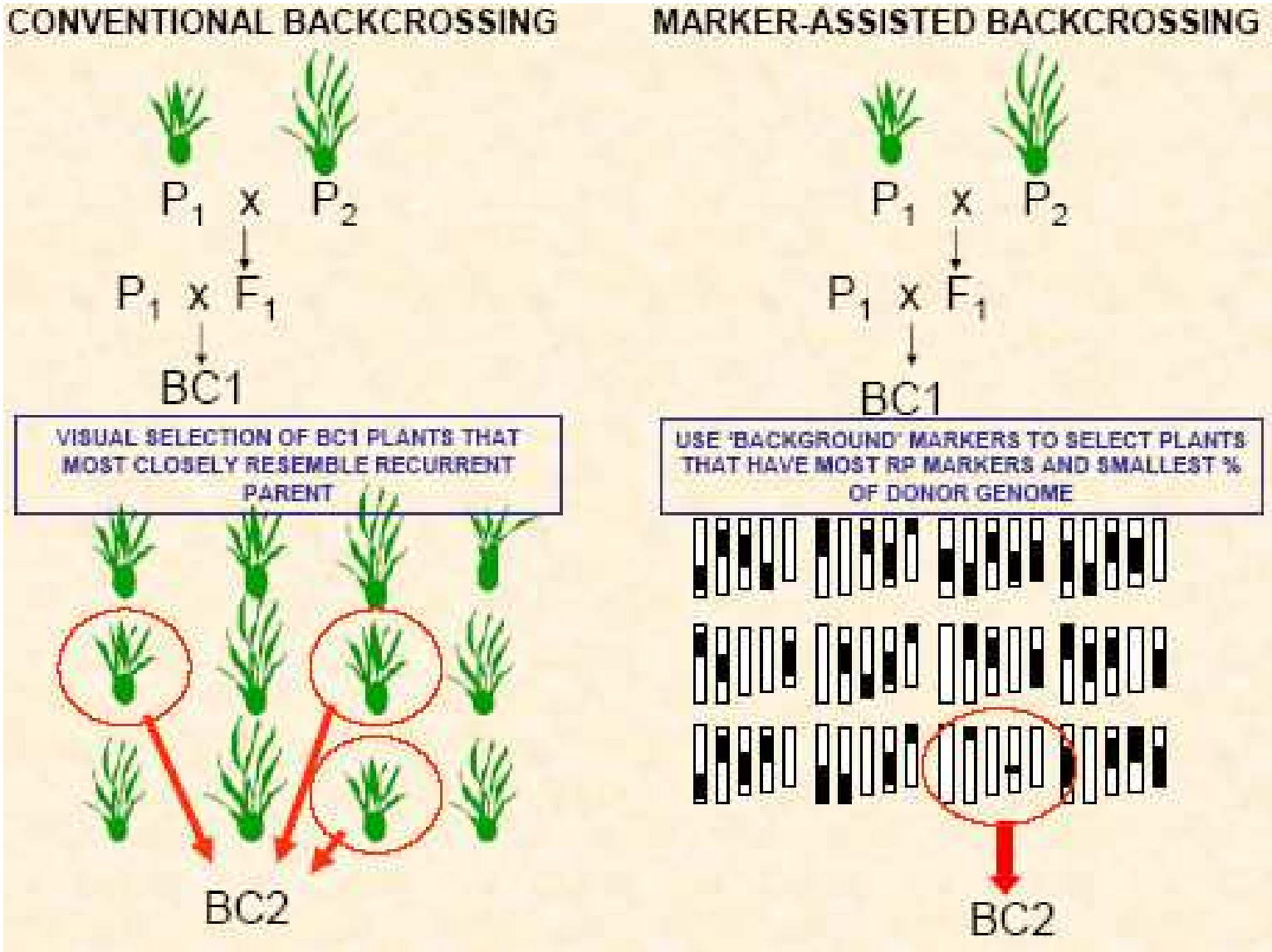
VARIABILITA’ INDOTTA PER MUTAGENESI

MARKER ASSISTED SELECTION (MAS)

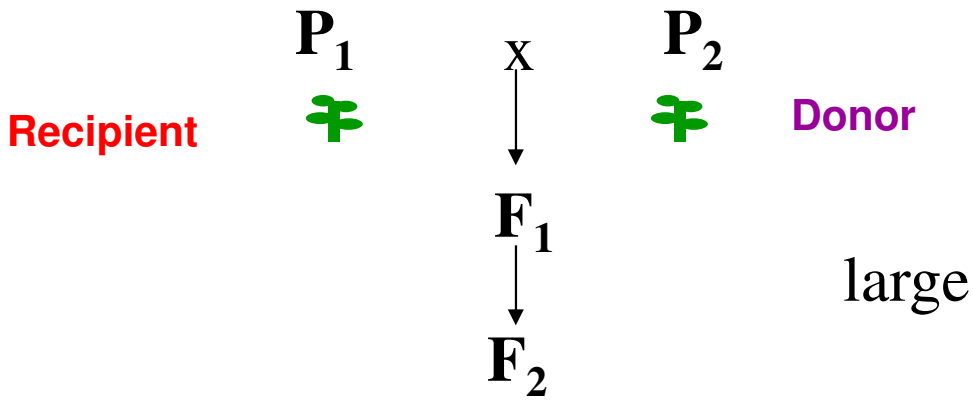
Definition:

Marker assisted selection (MAS) refers to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening

Assumption: DNA markers can reliably predict phenotype


CONVENTIONAL BACKCROSSING
P1 x P2
P1 x F1
BC1
VISUAL SELECTION OF BC1 PLANTS THAT MOST CLOSELY RESEMBLE RECURRENT PARENT
BC2
MARKER-ASSISTED BACKCROSSING
P1 x P2
P1 x F1
BC1
USE 'BACKGROUND' MARKERS TO SELECT PLANTS THAT HAVE MOST RP MARKERS AND SMALLEST % OF DONOR GENOME
BC2

CONVENTIONAL PLANT BREEDING



large populations consisting of thousands of plants

PHENOTYPIC SELECTION

Salinity screening in phytotron

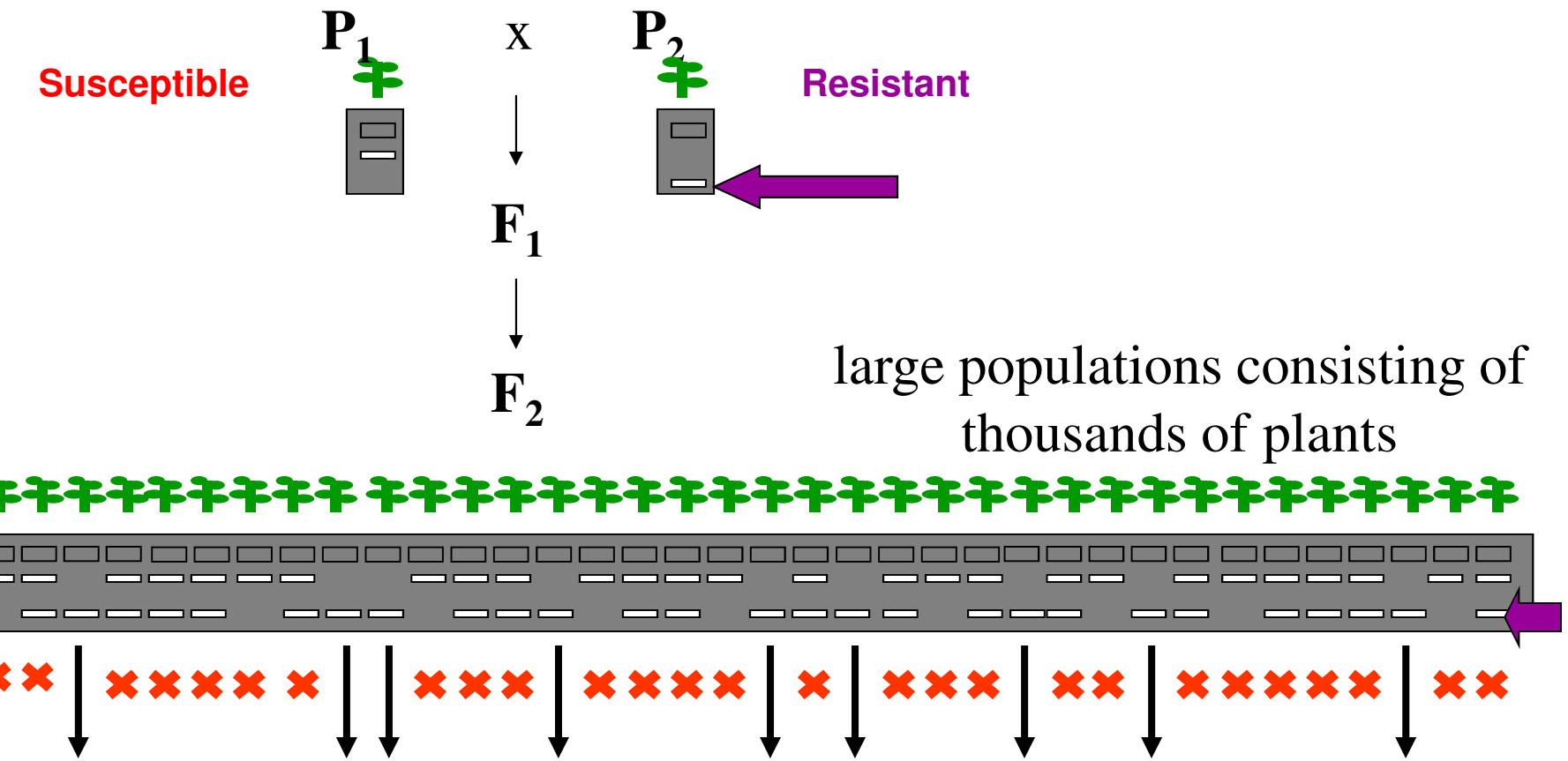
Bacterial blight screening

Phosphorus deficiency plot

Glasshouse trials

Field trials

MARKER-ASSISTED BREEDING



MARKER-ASSISTED SELECTION (MAS)

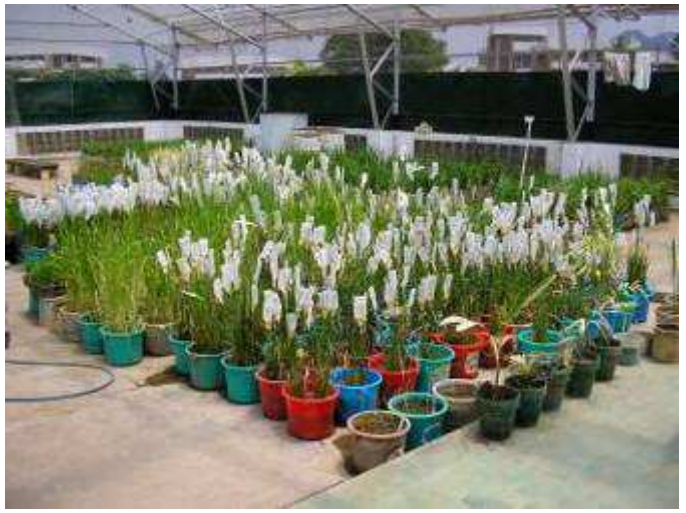
Method whereby phenotypic selection is based on DNA markers

Advantages of MAS

- **Simpler method compared to phenotypic screening**
  - Especially for traits with laborious screening
  - May save time and resources
- **Selection at seedling stage**
  - Important for traits such as grain quality
  - Can select before transplanting
- **Increased reliability**
  - No environmental effects
  - Can discriminate between homozygotes and heterozygotes and select single plants

Potential benefits from MAS

- more accurate and efficient selection of specific genotypes
  - May lead to accelerated variety development
- more efficient use of resources
  - Especially field trials



Crossing house

Backcross nursery

Overview of 'marker genotyping'

(1) LEAF TISSUE SAMPLING

↓

(2) DNA EXTRACTION

↓

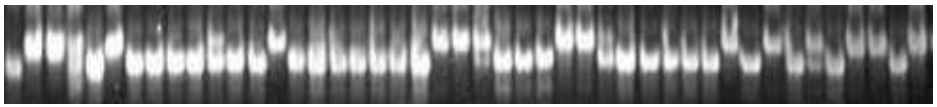
(3) PCR

↓

(4) GEL ELECTROPHORESIS

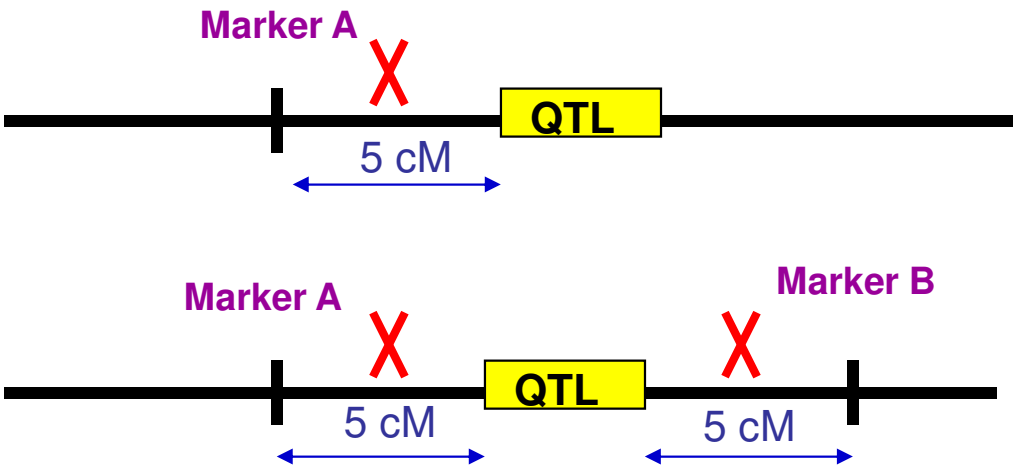
↓

(5) MARKER ANALYSIS



Markers must be tightly-linked to target loci!

- Ideally markers should be <5 cM from a gene or QTL



RELIABILITY FOR SELECTION

Using marker A only:

$1 - r_A$ = ~**95%**

Using markers A and B:

$1 - 2\, r_A r_B$ = ~**99.5%**

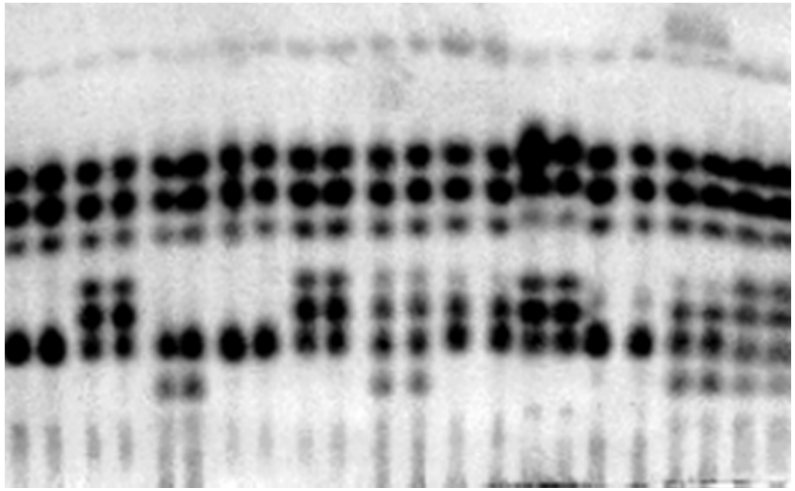
- Using a pair of flanking markers can greatly improve reliability but increases time and cost

Markers

- What makes a good marker:
  - co-dominant (so homozygotes and heterozygotes can be distinguished)
  - many alleles at each locus (so most individuals will be heterozygous and different from each other)
  - many loci well distributed throughout the genome
  - easy to detect, especially with automated machinery
- No system is perfect

MARCATORI

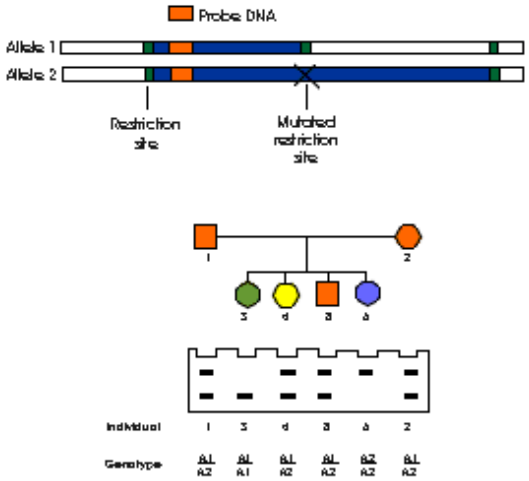
- Isozimi: enzimi con differente mobilità elettroforetica (spesso alleli diversi dello stesso gene)
- Di solito co-dominanti, dimeri possono confondere
- Spesso poco polimorfici
- Ogni enzima richiede condizioni specifiche

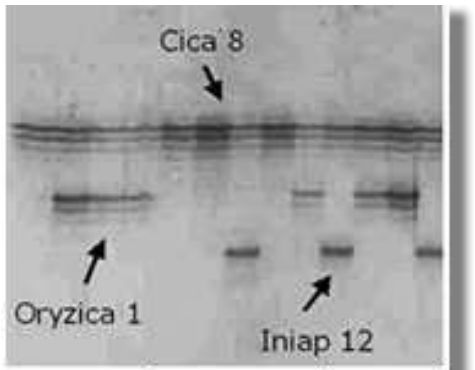


MARCATORI MOLECOLARI

- Restriction Fragment length Polymorphisms (RFLPs). The original DNA-based marker system. These markers are (usually) single nucleotide polymorphisms which create or destroy a restriction site. Thus, they have only 2 alleles per locus. The original detection technique, Southern blots, were expensive, time-consuming and finicky (and radioactive too).

- Microsatellites (SSRs). Lots of loci well scattered throughout the genome. Most loci have multiple alleles that are easily distinguishable. Detection is PCR-based, and there is some problem with DNA polymerase stuttering in PCR (which is also how new alleles are generated). The main problem is the need for gel electrophoresis to detect the alleles.

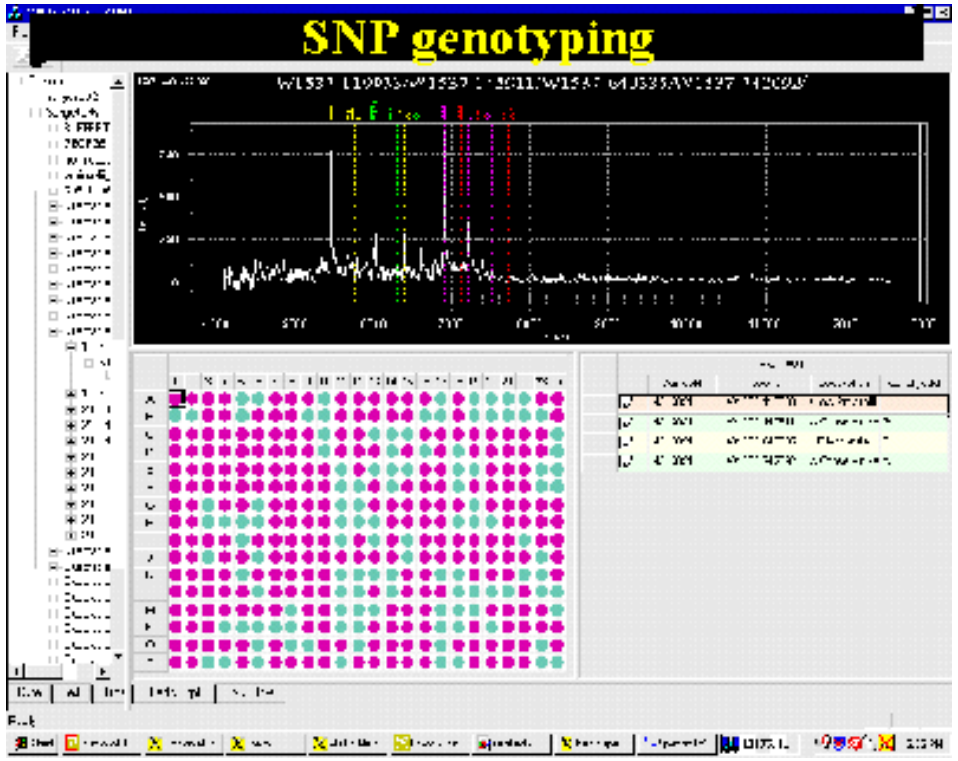




Crop specific microsatellite used to trace genetic introgression

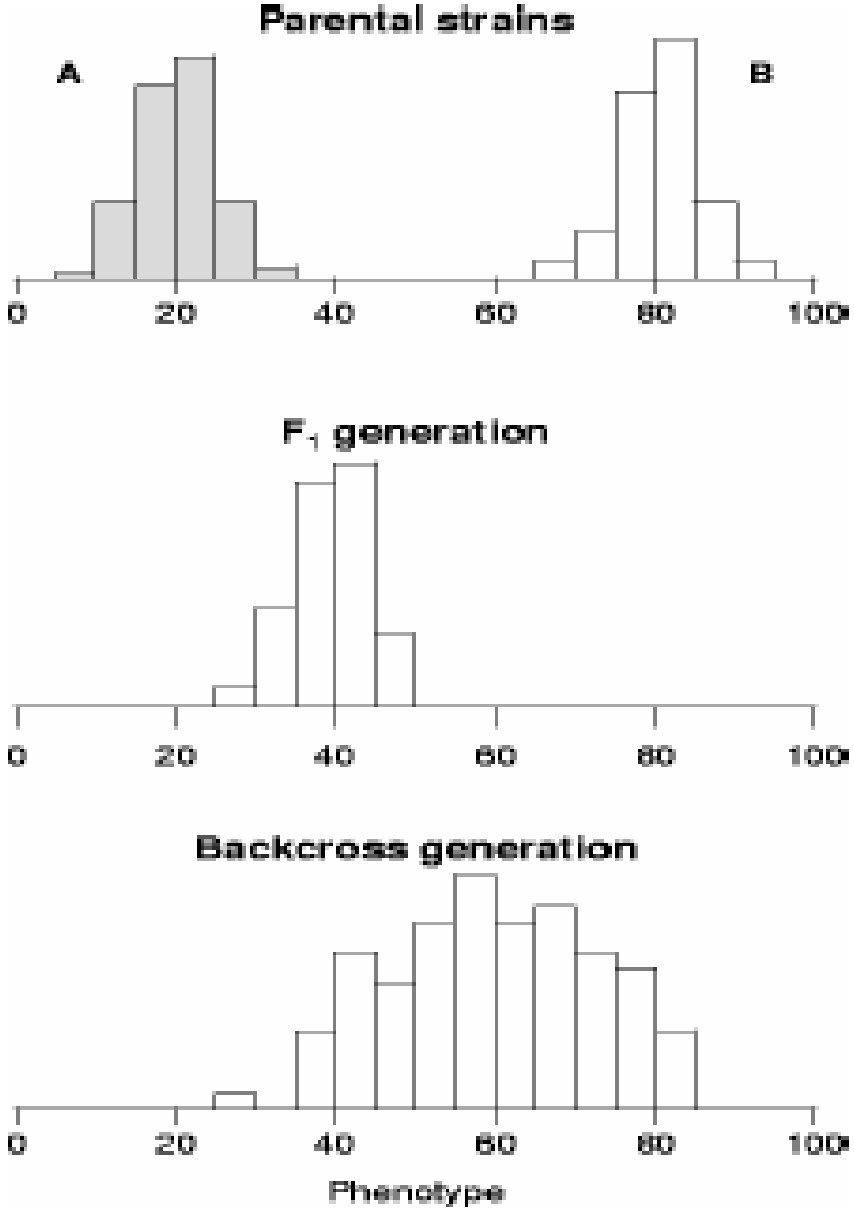
Single Nucleotide Polymorphisms

- Single Nucleotide Polymorphisms (SNPs). The current method of choice. Each locus has a maximum of 4 alleles (with 2 being the usual case). But, there are very large numbers of SNP loci, often several per gene even within exons. And, detection can be done with assays that don't require electrophoresis and so are very fast and easy to automate.
  - At present there are approximately 12 million human SNPs recorded in the NCBI database.



Quantitative trait loci (QTLs)

- QTLs determine the genetic component of variation in quantitative traits.
- Quantitative traits are usually encoded by many genes (polygenes).

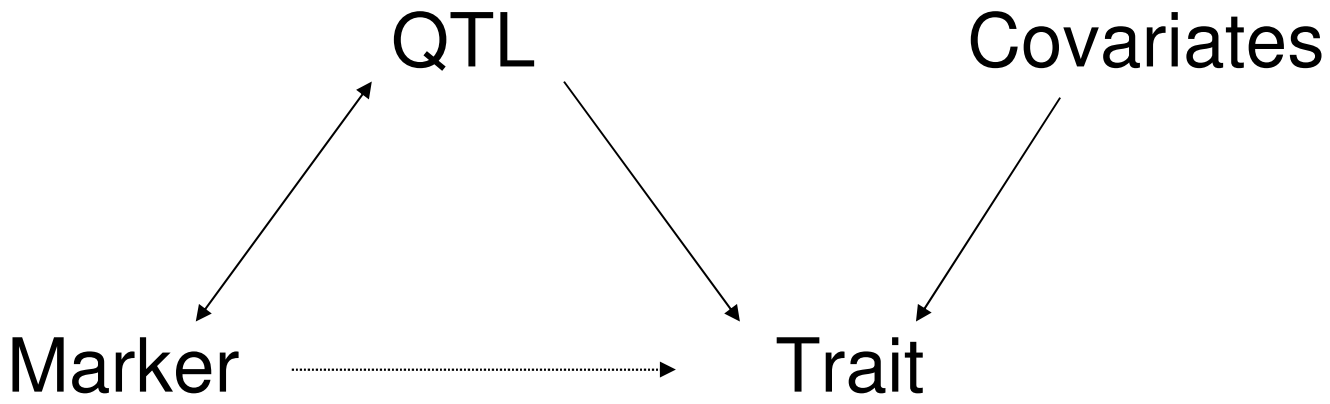

Parental strains
A
B
0
20
40
60
80
100
F1 generation
0
20
40
60
80
100
Backcross generation
0
20
40
60
80
100
Phenotype

Goals of QTL analysis

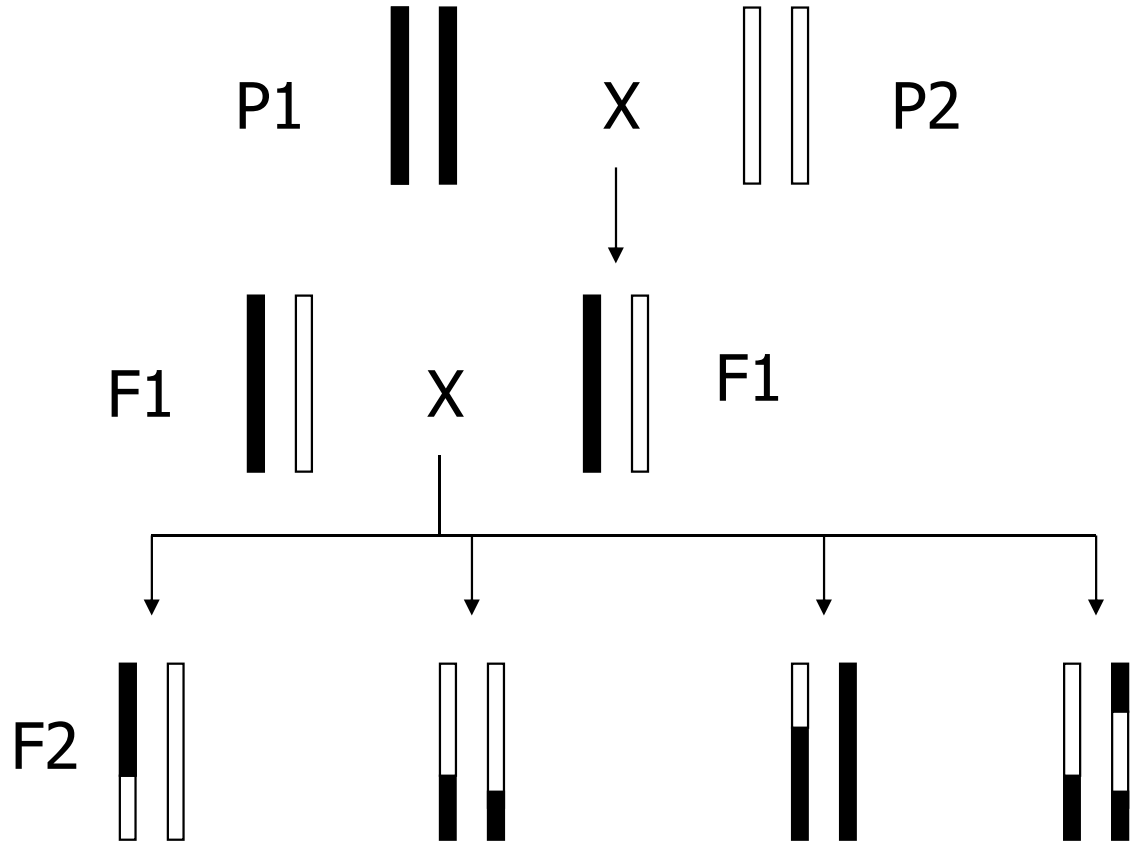
- Detect genetic effects
- QTL mapping: inference of the QTL location on chromosome

QTL mapping in experimental crosses

Experimental crossing creates associations between genetic marker loci and traits to allow localization of QTL.



Intercross



Data structure for a backcross experiment

- Phenotypes:

  y_i = quantitative measurement of trait

- Genotypes:

  x_{ij} = 0/1 coded for AA/AB at marker j

- Covariates:

  $\mathbf{Z}_i$ = environmental factors, demographics, etc.

where i = 1, …, n; j = 1, …, M.

Model and Assumptions

- No interference in recombination process
- Independence
- Normality

$$y_i|X \sim N(\mu_X, \sigma_X^2)$$

- Homoscedasticity

$$\sigma_X^2 = \sigma^2$$

LOD SCORE

- In genetica, acronimo di l(ogarithm) of od(ds) score («punteggio del logaritmo della probabilità»), parametro usato per calcolare l'associazione (linkage) e determinare la probabilità di ricombinazione tra diversi marcatori genici.

LOD SCORE

- Test statistico spesso usato per l'analisi del linkage in popolazioni animali, umane e vegetali.
- Compara le probabilità di riscontrare i valori osservati se i due loci sono effettivamente sullo stesso cromosoma rispetto alla probabilità di osservare gli stessi valori esclusivamente per caso.
- Valori positivi di LOD implicano la presenza di linkage, laddove valori negativi indicano che il linkage è meno probabile.

LOD SCORE

Una descrizione sommaria del suo funzionamento è:

- Costruzione di un albero genealogico;
- Stimare la frequenza di ricombinazione;
- Calcolare il LOD score per ognuna delle stime;
- La stima con il LOD score massimo sarà considerata la migliore.

$$LOD = Z = \log_{10} \frac{\text{probabilità di nascita con un certo valore di linkage}}{\text{probabilità di nascita con linkage assente}} = \log_{10} \frac{(1-\theta)^{NR} \times \theta^{R}}{0.5^{(NR+R)}}$$

NR = numero di prole non-ricombinante,
R = numero di prole ricombinante.

0.5 al denominatore -> ogni allele completamente unlinked (e.g. alleli su cromosomi distinti) ha il 50% di possibilità di ricombinare
Teta = frazione ricombinante, ed è uguale a R / (NR + R)

LOD score > 3.0 è considerato prova di linkage (probabilità 1000:1 che il linkage osservato non avvenga per caso).
LOD score < -2.0 è considerato sufficiente per escludere la probabilità di linkage.

LOD Score Mapping

- The lod score method is an example of a maximum likelihood procedure.
- The point of the maximum likelihood procedure is to estimate the value of a parameter that can't be directly observed, in this case the recombination fraction.
- The likelihood (probability) of an observed set of data (the phenotypes seen in a family, in this case) is calculated as a function of that parameter.
- The parameter value that gives the maximum likelihood is taken as the best estimate of the parameter.

LOD curve

- Likelihood profile
- A clear peak is taken as the QTL
- 1.5-LOD support interval

Breeders' QTL mapping 'checklist'

- LOD & R^2 values will give us a good initial idea but probably more important factors include:

1. *What is the population size used for QTL mapping?*
2. *How reliable is the phenotypic data?*
   - *Heritability estimates will be useful*
   - *Level of replication*
3. *Any confirmation of QTL results?*
4. *Have effects of genetic background been tested?*
5. *Are markers polymorphic in breeders' material?*
6. *How useful are the markers for predicting phenotype? Has this been evaluated?*

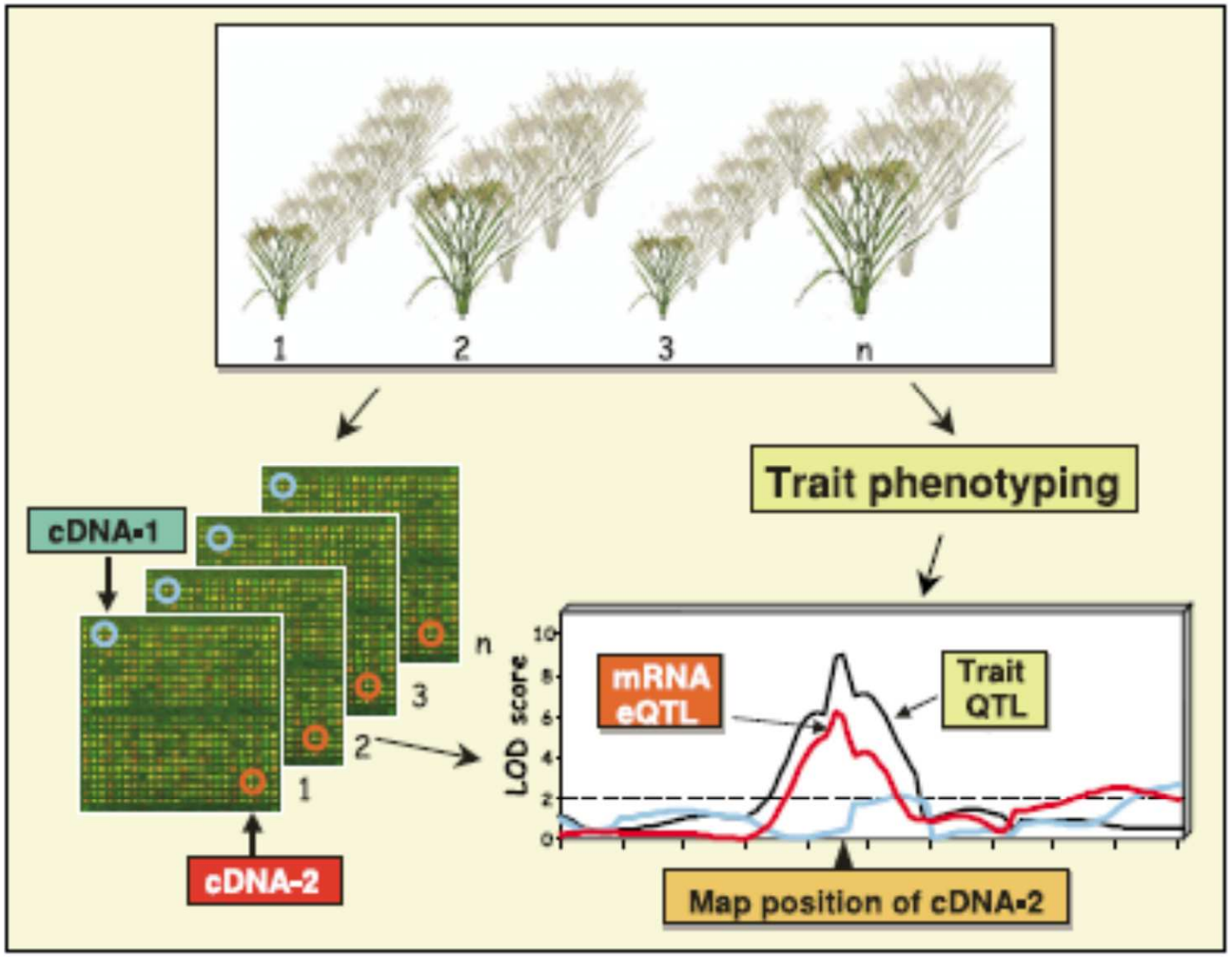


Figure 5. Expression profiling of a mapping population at the mRNA level via microarray analysis to identify expression QTLs (eQTLs) for specific cDNAs. Correspondence between an eQTL peak for a specific cDNA (e.g. cDNA-2) and a QTL peak for a trait causally linked to the function of the protein encoded by the cDNA provides circumstantial evidence supporting the role of the cDNA as a candidate gene for the target trait.

Current status of molecular breeding

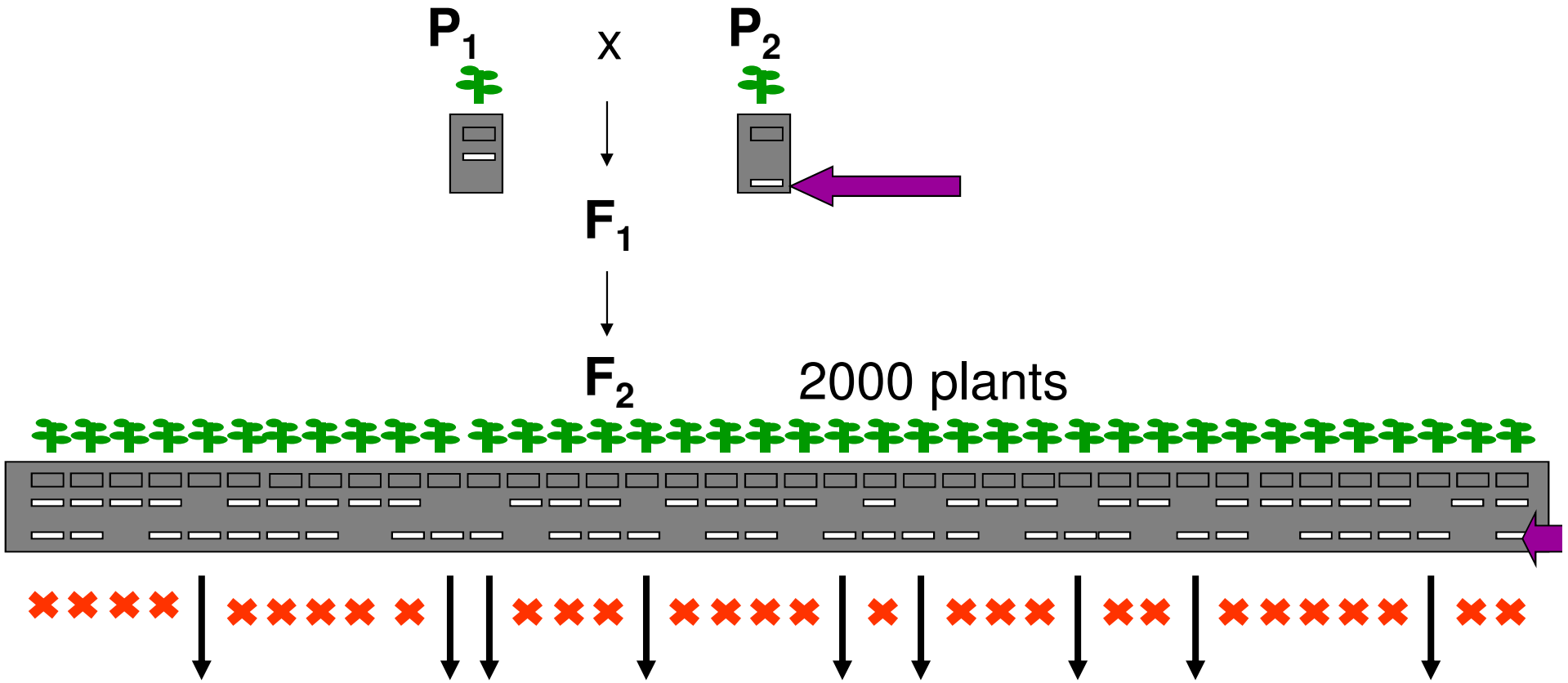
- A literature review indicates thousands of QTL mapping studies but not many *actual* reports of the application of MAS in breeding

- *Why is this the case?*

Some possible reasons to explain the low impact of MAS in crop improvement

- Resources (equipment) not available
- Markers may *not* be cost-effective
- Accuracy of QTL mapping studies
- QTL effects may depend on genetic background or be influenced by environmental conditions
- Lack of marker polymorphism in breeding material
- Poor integration of molecular genetics and conventional breeding

Cost of MAS in context: Example 1: Early generation MAS



USD **$640** to screen 2000 plants with a single marker for one population

Reliability of QTL mapping is critical to the success of MAS

- Reliable phenotypic data critical!
  - Multiple replications and environments
- Confirmation of QTL results in independent populations
- “Marker validation” must be performed
  - Testing reliability for markers to predict phenotype
  - Testing level of polymorphism of markers
- Effects of genetic background need to be determined

ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina*

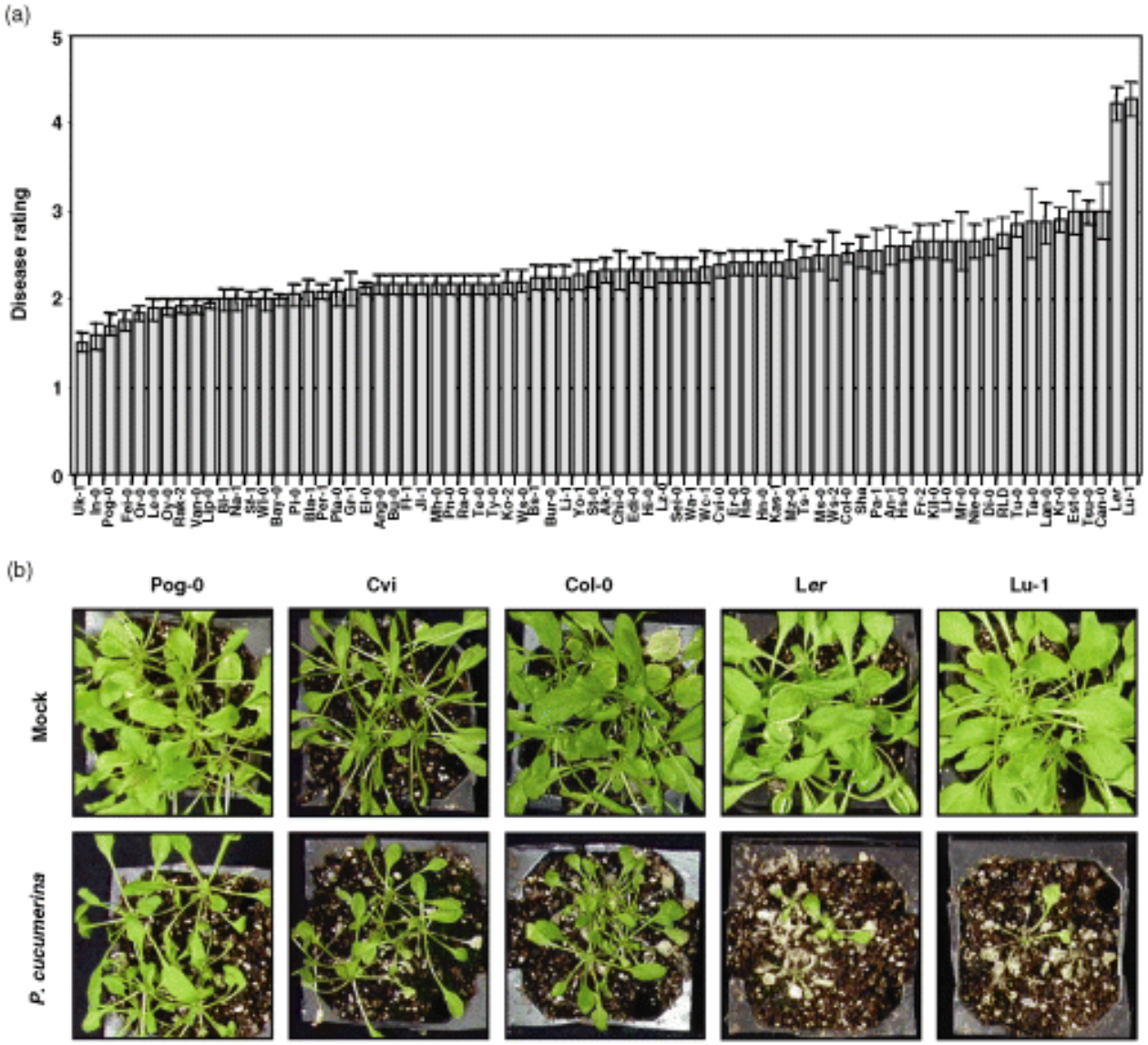
Francisco Llorente1, Carlos Alonso-Blanco2, Clara Sánchez-Rodriguez1, Lucía Jorda1 andAntonio Molina1,*

The Plant Journal
Volume 43, Issue 2, pages 165–180, July 2005

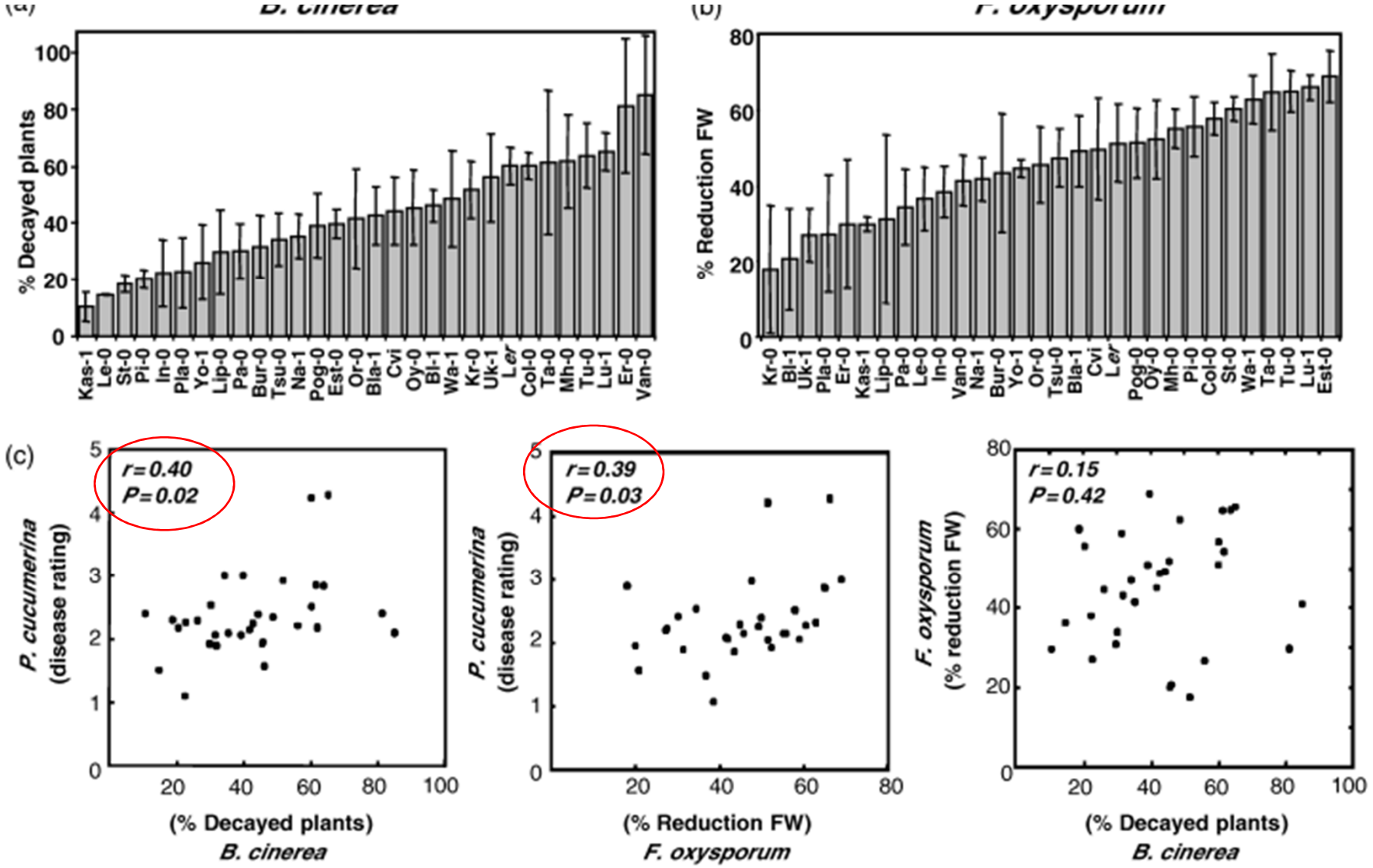
Plectosphaerella cucumerina

Fungo ascomicete necrotrofico che causa marciumi di frutti, foglie e del colletto in molte specie ortive

Diversa suscettibilità a *Plectosphaerella cucumerina* in accessioni di Arabidopsis



Risposta differenziale di accessioni di Arabidopsis ad altri funghi necrotrofi (*Botrytis cinerea* e *Fusarium oxysporum*)



QTL likelihood maps for *Plectosphaerella cucumerina* resistance in a L*er*/Cvi RIL population

- Full quantitative trait locus analysis in a RIL population derived from the cross between the **moderately susceptible Cvi** and the **highly susceptible Ler** (Alonso-Blanco et al., 1998).

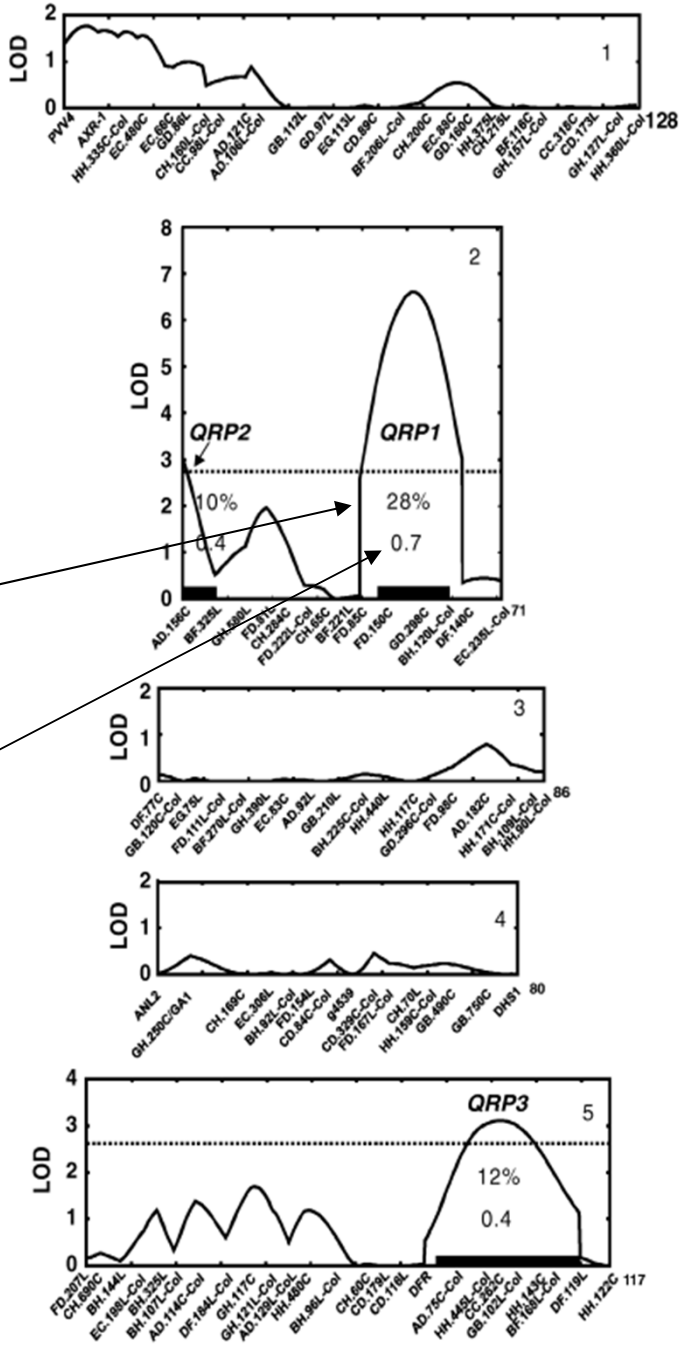
- Plants from **72 RILs Ler/Cvi**, as well as the parental accessions Ler and Cvi, were inoculated with a spore suspension of the fungus and their mean DRs were estimated.

Tre QTL responsabili per circa 50% della variabilità fenotipica

Soglia per rilevare i QTL =2.6 LOD

% della varianza fenotipica totale

Effetto additivo di ogni QTL (in Disease Rating): i valori positivi indicano che i genotipi La-er mostrano aumentata suscettibilità della popolazione Cvi



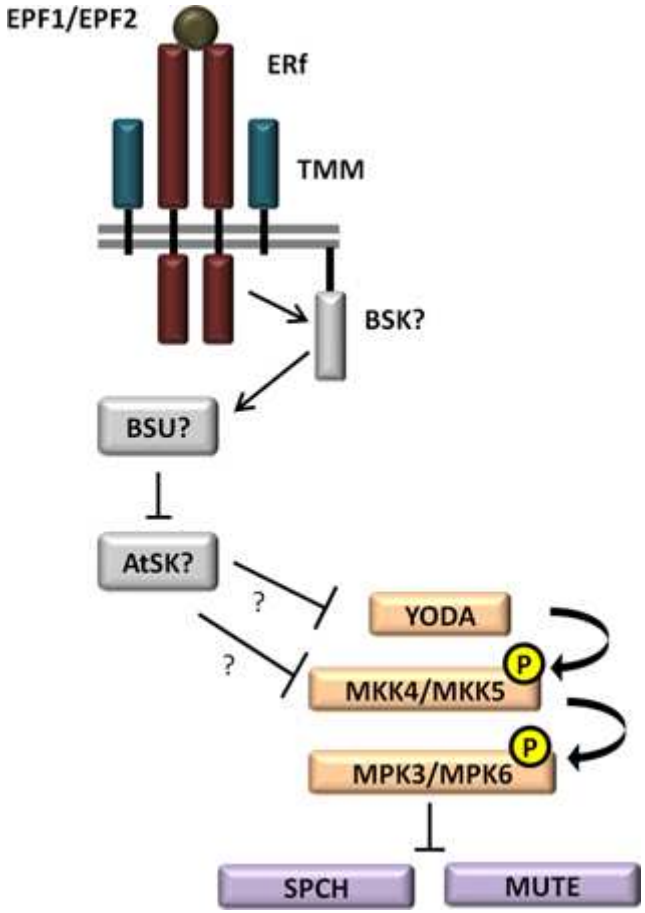
QRP1 (sul chr 2, vicino al gene *ERECTA*, o *ER*) è il locus con l'effetto più forte

Ler porta una mutazione loss-of-function (allele *er-1*) nel gene *ER* (Torii et al., 1996).

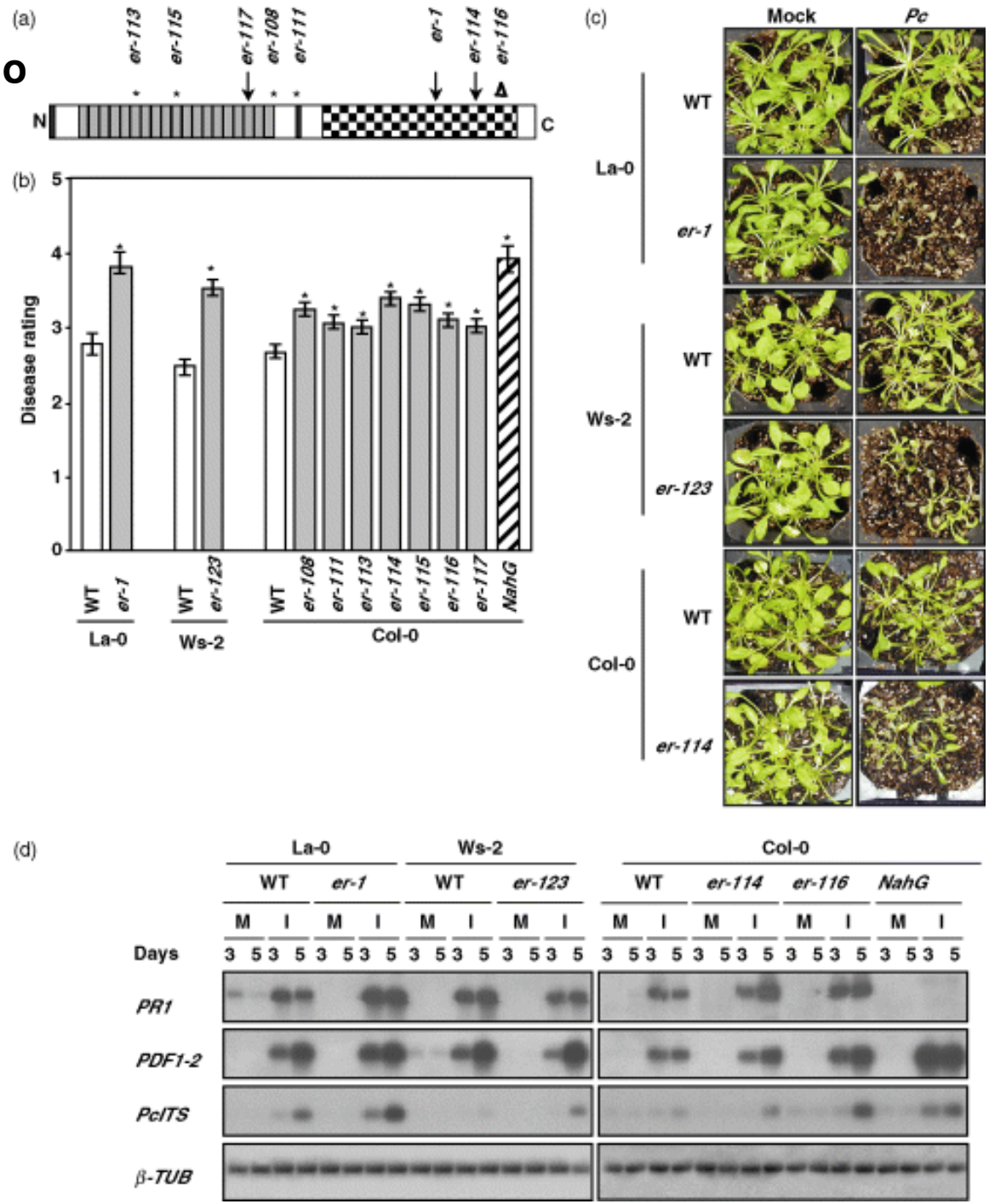
Stesso QTL osservato anche in una popolazione RIL Ler/Col

-> IPOTESI: il gene ERECTA è responsabile per il QTL QRP1

ERECTA: receptor-like kinase coinvolta in numerose funzioni



Differenti alleli loss-of-function per *ERECTA* rendono le piante più suscettibili al fungo



Tre mutanti nel pathway di trasduzione a valle di ERECTA sono più suscettibili al fungo