

# **Genetics of Host-Pathogen Interactions**

## Outline

1. History of resistance gene discovery
2. Gene-for-gene hypothesis
3. Mapping and cloning of resistance (R) genes
4. Mapping and cloning of avirulence (Avr) genes
5. Forward genetics
6. Reverse genetics

# History of Genetic Studies

## Early genetic studies

Observation of resistance and its genetic basis

pre-1900

**1905 Biffen:** Wheat resistance to rust is a single recessive character, First scientific investigation of genetic basis of resistance

**1911 Barrus:** First to demonstrate variability in a pathogen species to different cultivars

- series of **cultivars** showed differential responses to different **isolates** of the pathogen
- essentially among the first to look at a matrix of host and pathogen types

**1922 Stakman and Levine**

- Established concepts of pathogen "**physiological races**" and host "**differentials**"
- "physiological races" were morphologically indistinguishable, but clearly differed in their reactions to a series of "tester" lines known as "differentials"
  - since the basis of variability in the pathogen was not shown to be genetic, therefore termed "physiological"

**1931 Craigie:** Concept of "physiological race" extended by studies that showed that these "races" were genetically determined (not solely physiological) and that new races could develop

## Harold Flor's Experiments (1946, 1947)

Unique contribution is that he studied inheritance of resistance and susceptibility in the host and inheritance of virulence and avirulence in the pathogen

Host: flax (*linum usitatissimum*)

Pathogen: *Melampsora lini*

Cultivar Bison is "**universal susceptible**", a critically important new concept

Bison x Series of resistant lines ==> F1

F1s selfed, looked at segregation in F2s

Saw resistance segregates differently to different races

Moreover, a cross could yield progeny resistant to two pathogens, but in such cases, for example, a resistant:susceptible ratio of 3:1 ratio might be seen for one and 15:1 for the second

Therefore, the host genes that distinguished reaction to a pathogen depended on the **isolate**

Flor **also** crossed the fungal races:

1) Host line #1 : one gene for resistance

Crosses of pathogen ==> 1 gene segregated for virulence or avirulence.

2) Host line #2 : two genes for resistance

Crosses of pathogen ==> 1 or 2 genes segregating for virulence or avirulence

# "GENE FOR GENE" HYPOTHESIS

The Pattern of Interactions with Two Genes in the Host and Two Genes in the Pathogen. (Note P = Avirulence Gene)

Pathogen	Host			
	A	B	C	D
	R1R1R2R2	R1R1r2r2	r1r1R2R2	r1r1r2r2
1. P1P1P2P2	R	R	R	S
2. P1P1p2p2	R	R	S	S
3. p1p1P2P2	R	S	R	S
4. p1p1p2p2	S	S	S	S

- IN THE F2 CROSS OF THE HOST:

USE OF PATHOGEN 1 ==> SEGREGATION OF R1 AND R2 BOTH SEEN

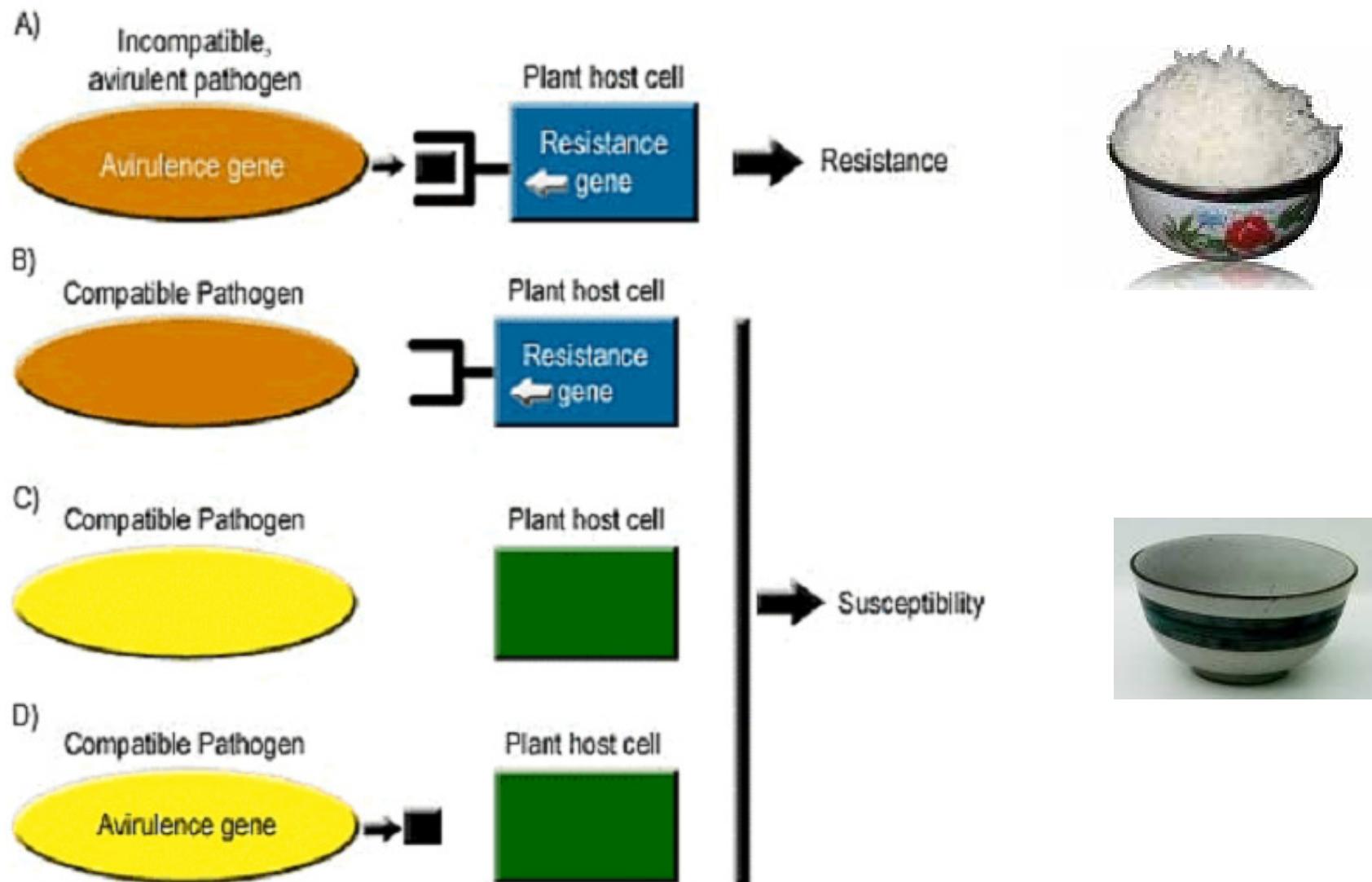
USE OF PATHOGEN 2 ==> SEGREGATION OF ONLY R1 OBSERVED

USE OF PATHOGEN 3 ==> SEGREGATION OF ONLY R2 OBSERVED

USE OF PATHOGEN 4 ==> NO RESISTANCE SEEN

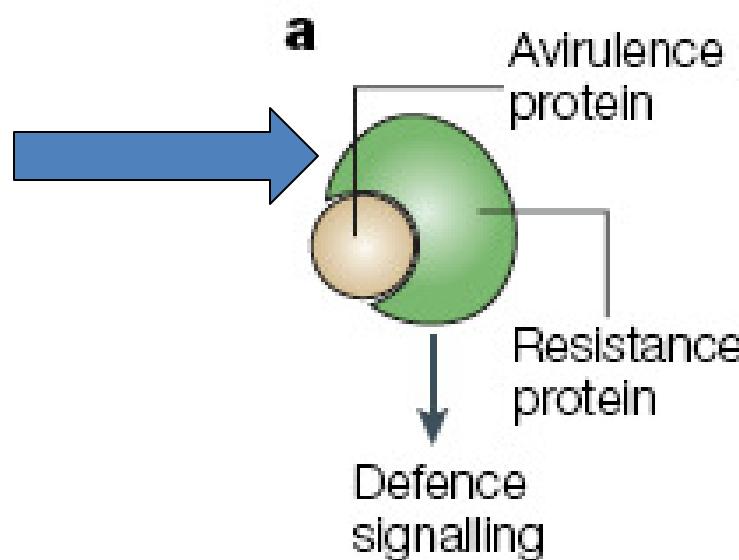
**==> "GENE FOR GENE" HYPOTHESIS - FOR EACH DOMINANT GENE FOR RESISTANCE IN THE HOST THERE IS A CORRESPONDING DOMINANT GENE FOR AVIRULENCE IN THE PATHOGEN**

# Gene-for-Gene Hypothesis (Flor, 1971)

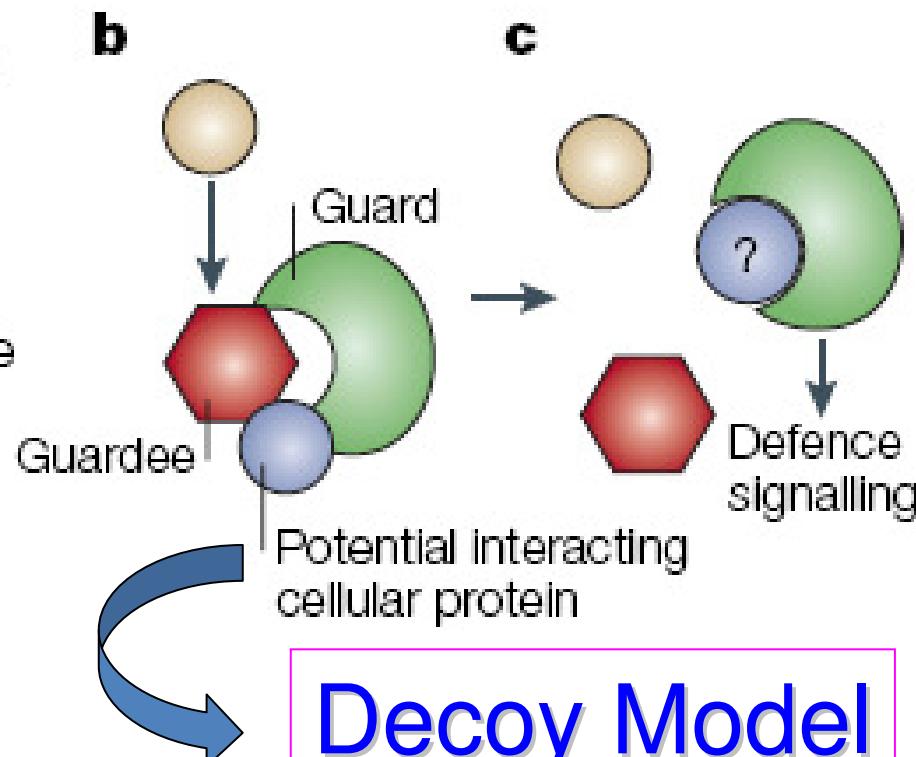


# Molecular Models of R and Avr Interactions

## Direct Interaction



## Indirect Interaction



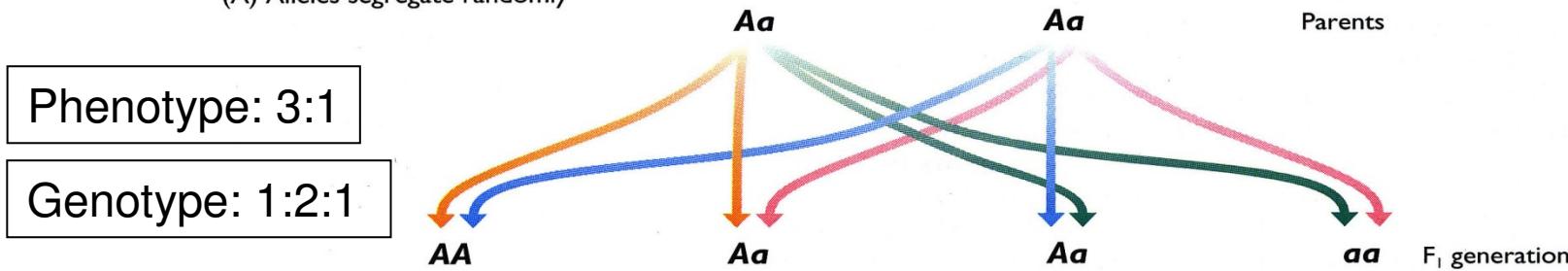
**Decoy Model**

Soosaar J. et al. 2005. Nature Review-Microbiology, 3:789

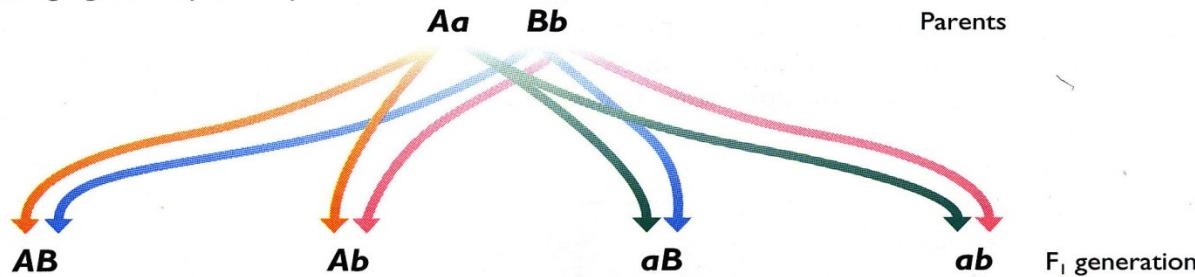
More discussions in the lecture on R protein signalling

# Review of Genetic Analysis

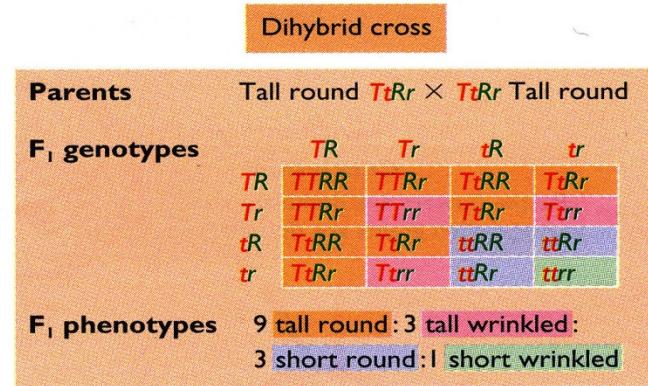
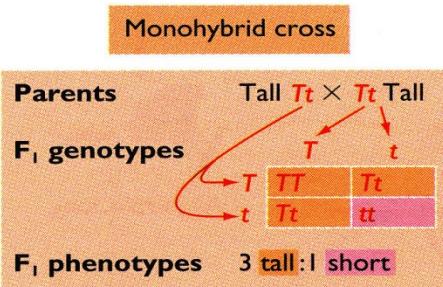
(A) Alleles segregate randomly



(B) Pairs of alleles segregate independently



(C) The outcomes of crosses are predictable



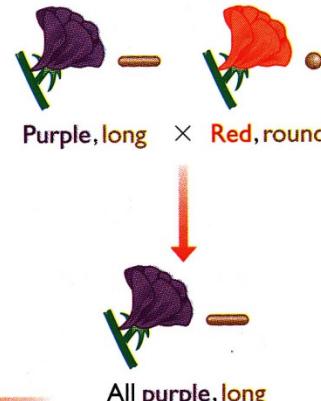
TA Brown, p 135

Mendel's Laws enable the outcome of genetic crosses to be predicted <sup>7</sup>

# Unlinked, linked and partial linked genes

Genes	Alleles
Flower color	Purple      Red
Pollen shape	Long      Round

## PARENTAL CROSS



## Conclusion

Purple flowers are dominant to red  
Long pollen grains are dominant to round

## If genes are unlinked

F<sub>1</sub> cross will give a ratio of  
9 purple, long : 3 purple, round:  
3 red, long : 1 red, round

## If genes are linked

F<sub>1</sub> cross will give a ratio of  
3 purple, long : 1 red, round

## Conclusion

Genes display partial linkage

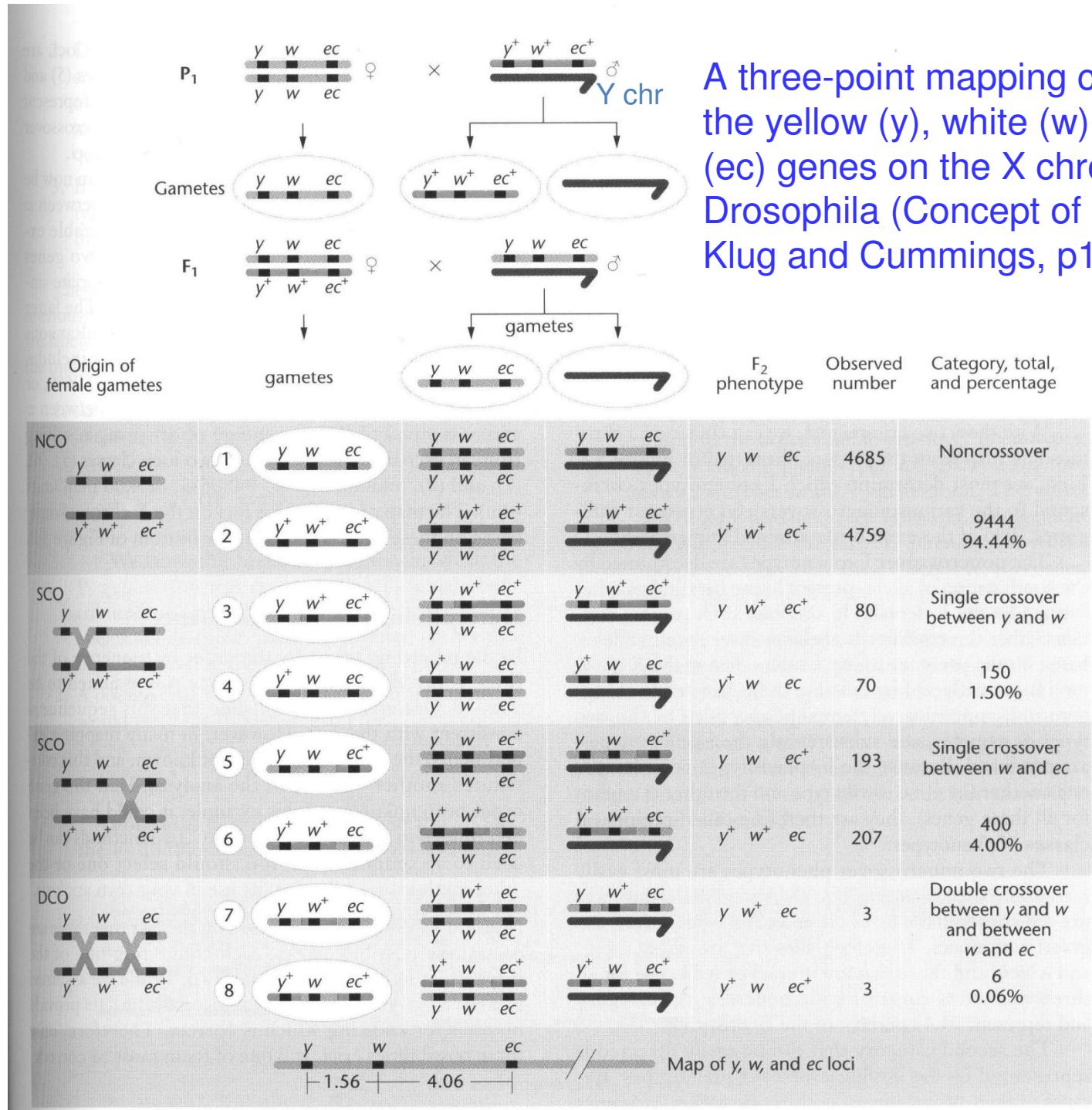
## F<sub>1</sub> CROSS



## Actual results

4831 purple, long  
390 purple, round  
391 red, long  
1338 red, round

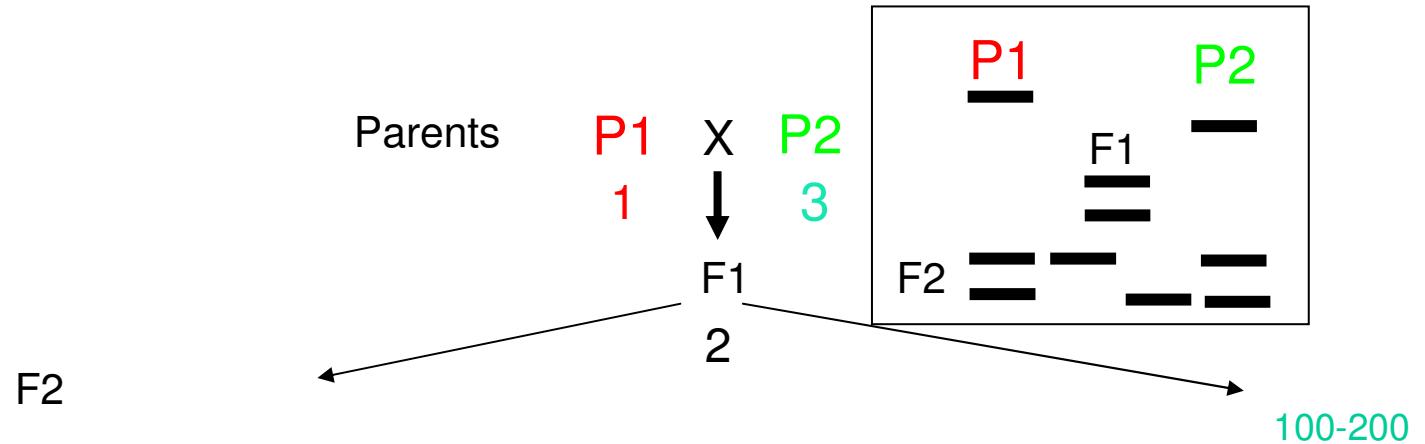
TA Brown, p 136



A three-point mapping cross involving the yellow (y), white (w) and echinus (ec) genes on the X chromosome of Drosophila (Concept of Genetics, Klug and Cummings, p123)

# How to make a linkage map for a self-pollinated plant

1. Making a cross



2. Marker genotype analysis

	Plt1	Plt2	Plt3	Plt4	Plt5	Plt6	Plt7	Plt8	Plt9	Plt10
Marker1	2	3	1	2	2	3	1	2	3	2
Marker2	2	3	1	2	2	1	1	2	3	2
Marker3	3	2	2	1	1	3	2	1	2	2
Marker4	3	2	2	3	1	3	2	1	2	2

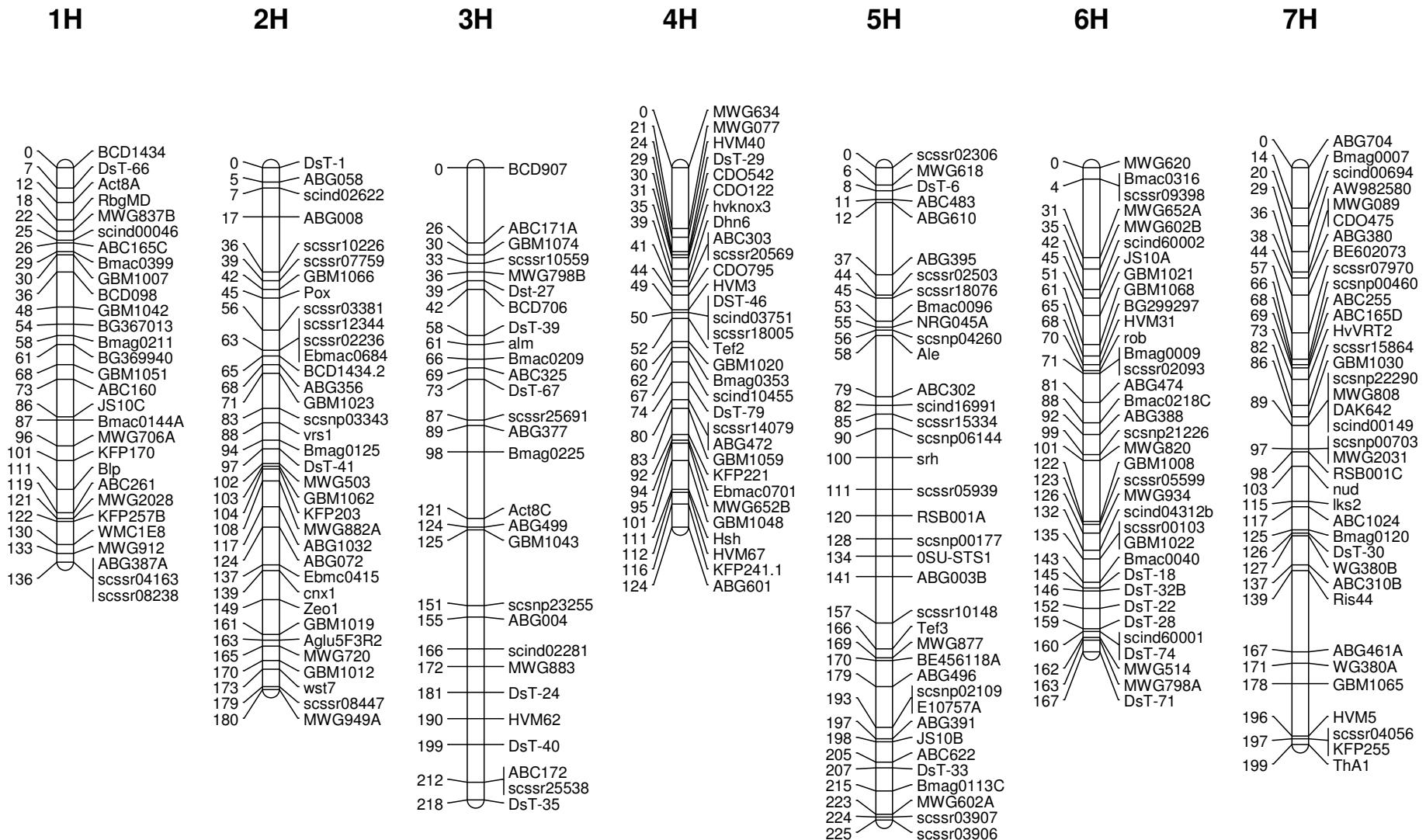
100-200

Marker 1 and 2, and marker 3 and 4 are linked

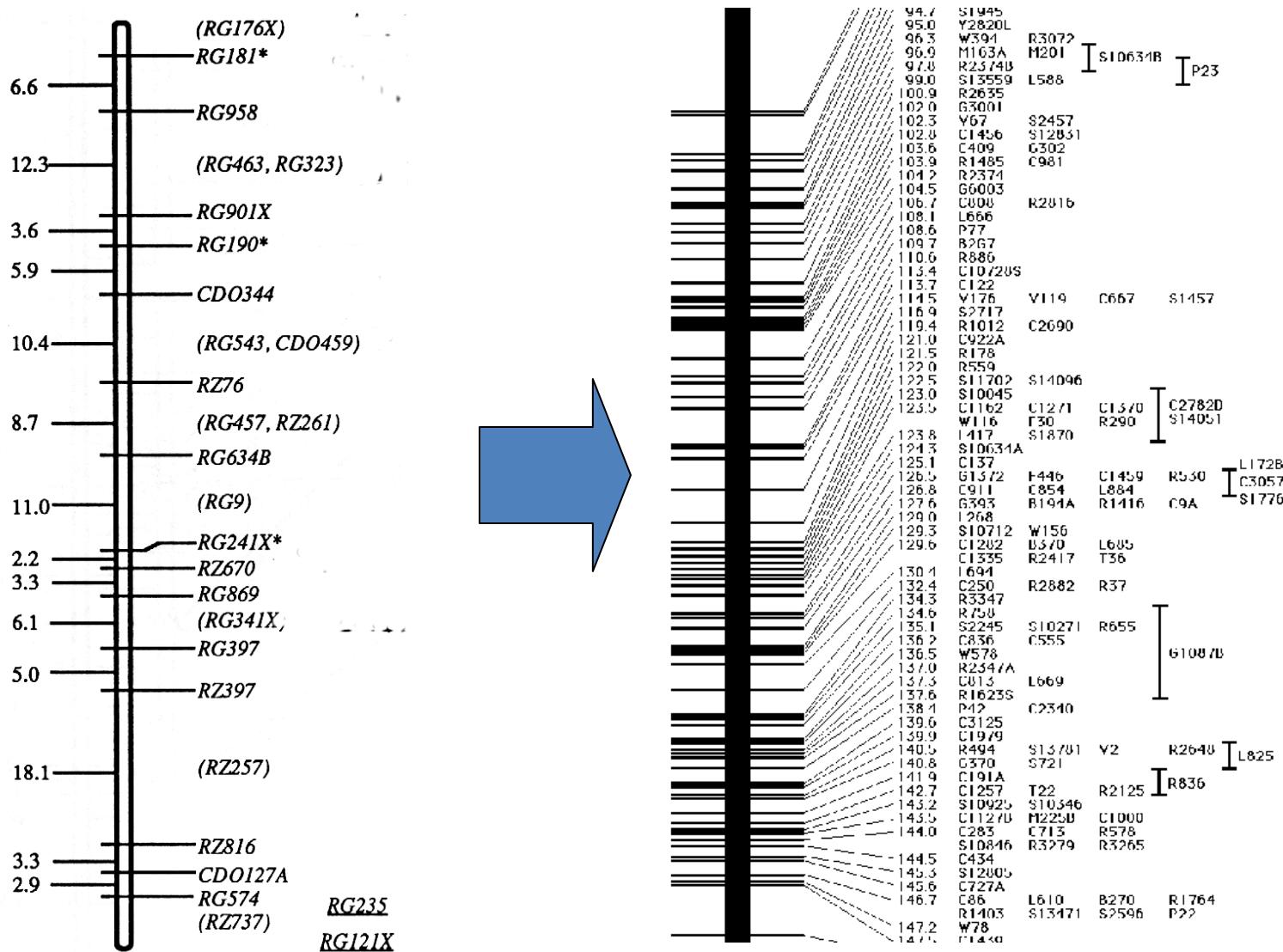
3. Linkage analysis

MAPMAKER: a linkage map construction software developed by Eric Lander. Website: <http://linkage.rockefeller.edu/soft/mapmaker/>

# Barley Linkage Map



# High-density molecular linkage map of rice chromosome 1



1990

2000

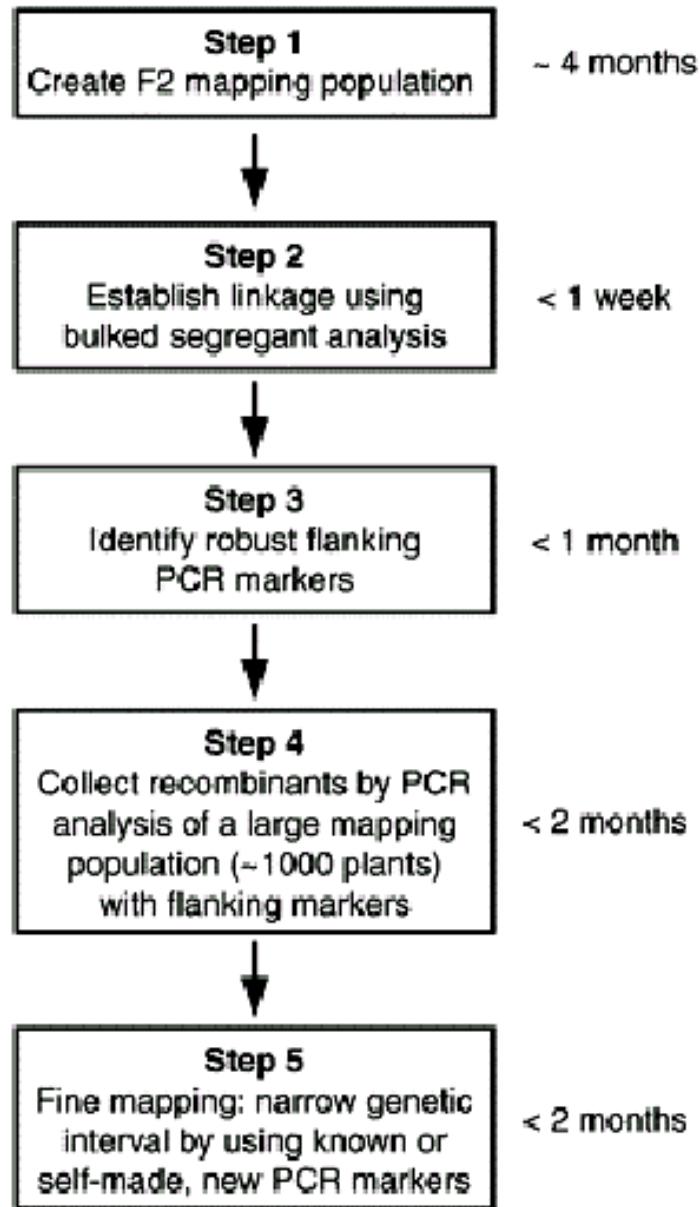
12

# **Map-based cloning strategies**

1. Tagging the gene with DNA markers
2. High resolution mapping
3. Physical mapping and chromosome walking
4. Sequencing of selected regions and candidate gene identification
5. Complementation: Phenotype and molecular analysis of transgenic plants

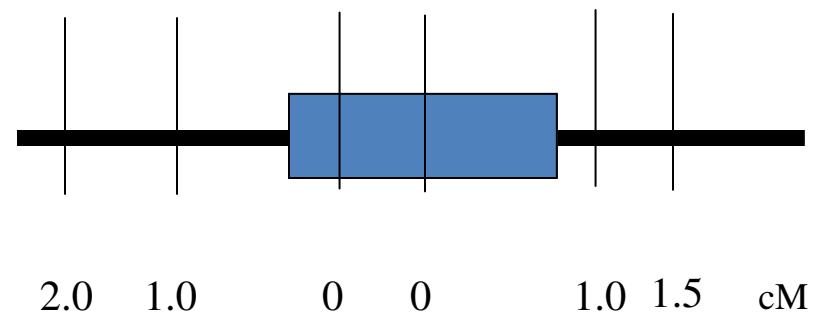
# DNA Markers for Gene Mapping and Cloning

1. **Restriction fragment length polymorphisms (RFLP)**
2. **RAPD-Random amplified polymorphic DNA (RAPD)**
3. **Microsatellite, simple sequence repeat (SSR) markers or short tandem repeat (STR)**
4. **Single nucleotide polymorphisms (SNPs)**
5. **Amplified fragment length polymorphism (AFLP)**

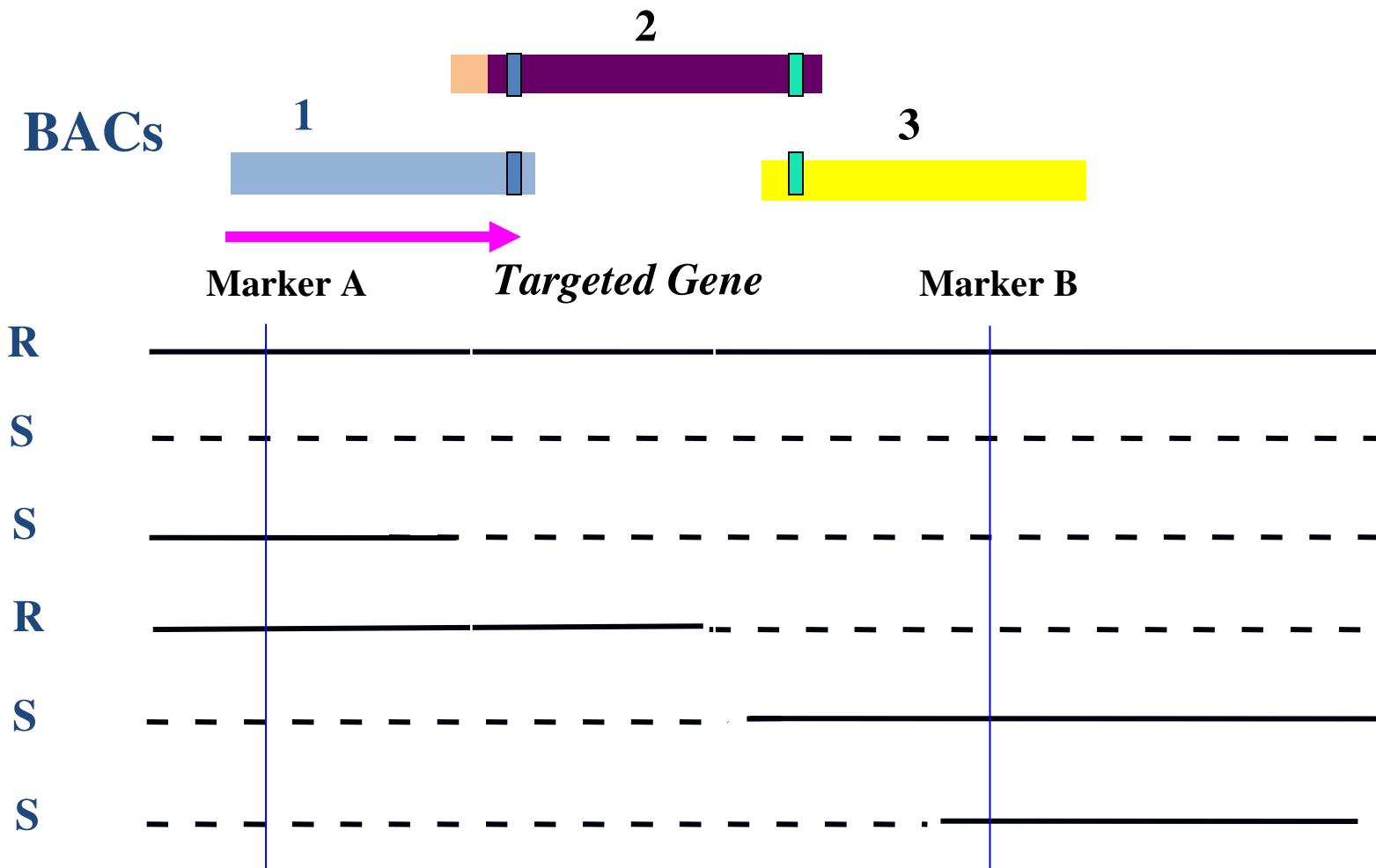


# Procedure for fine-mapping in *Arabidopsis*

High –resolution map



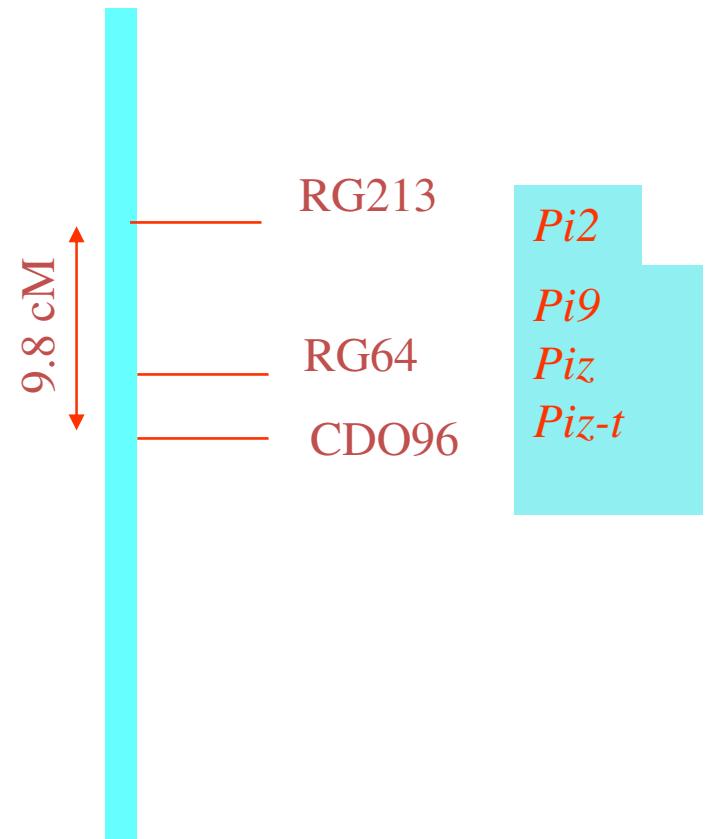
# Chromosome Walking



## Four broad-spectrum resistance genes are genetically linked on chromosome six



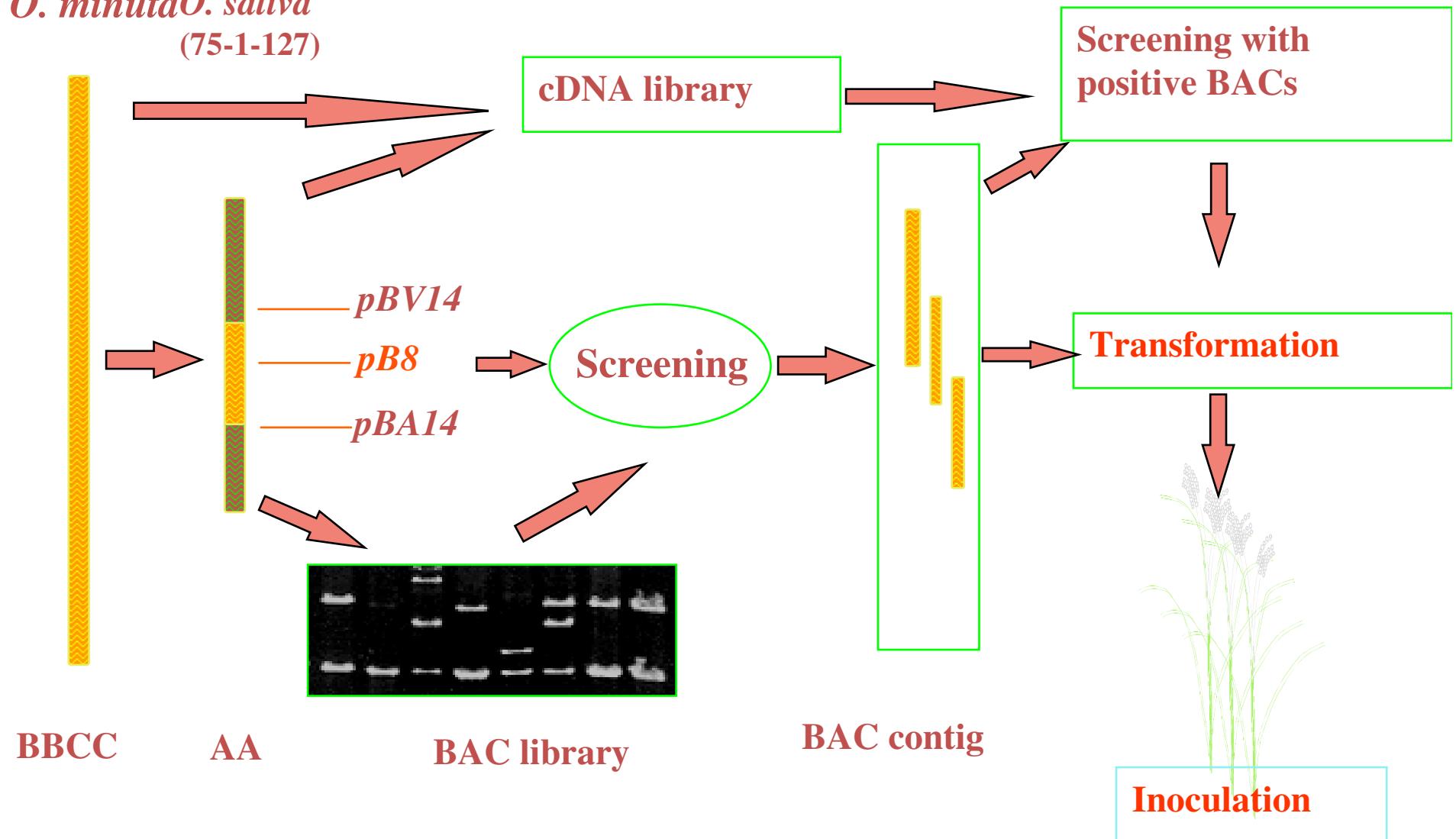
43 isolates from  
14 countries tested:  
*Pi9*: R to all  
*Pi2*: R to 36



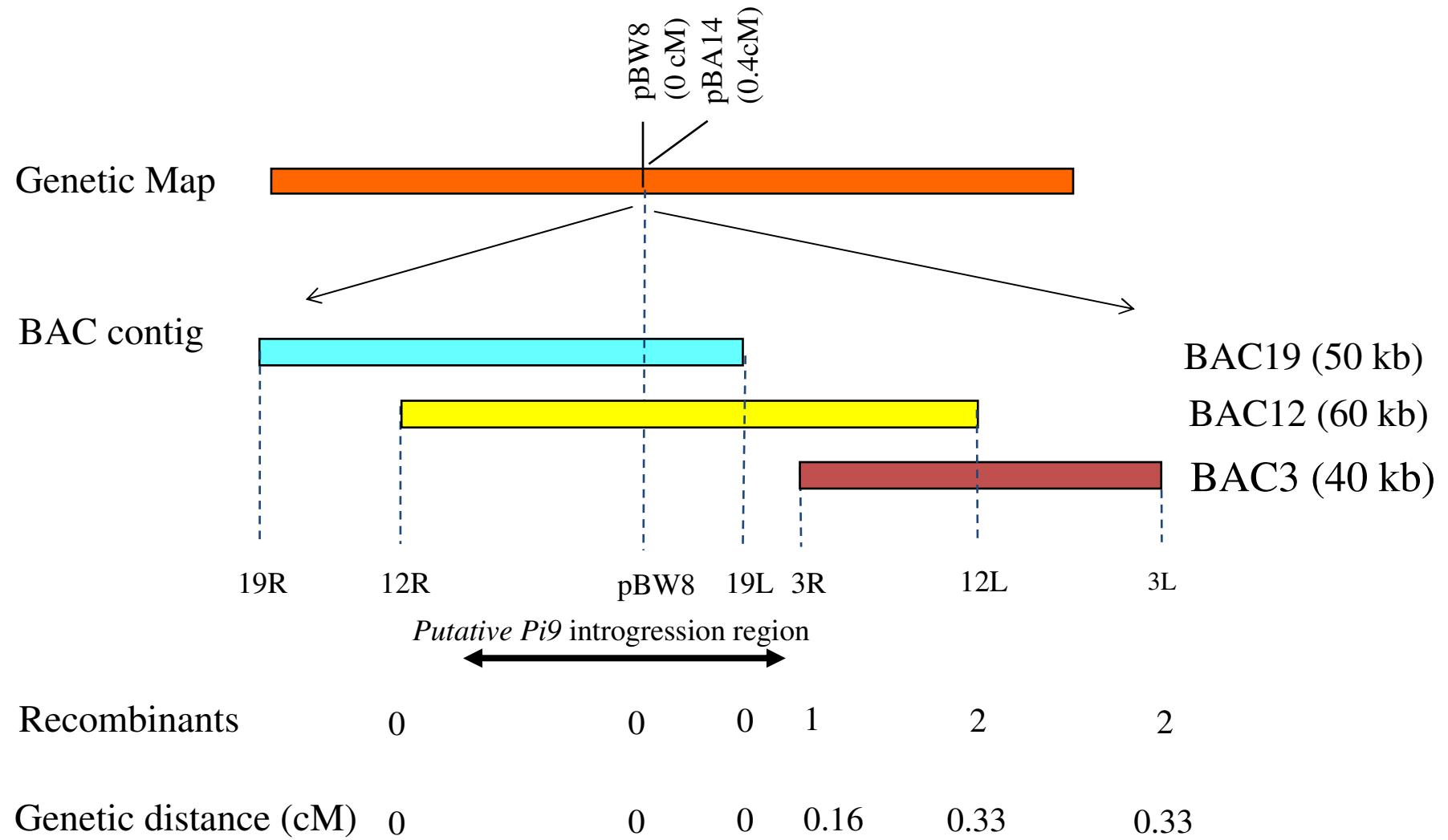
Liu et al. MGG, 2002

# Map-based cloning of the *Pi9* gene

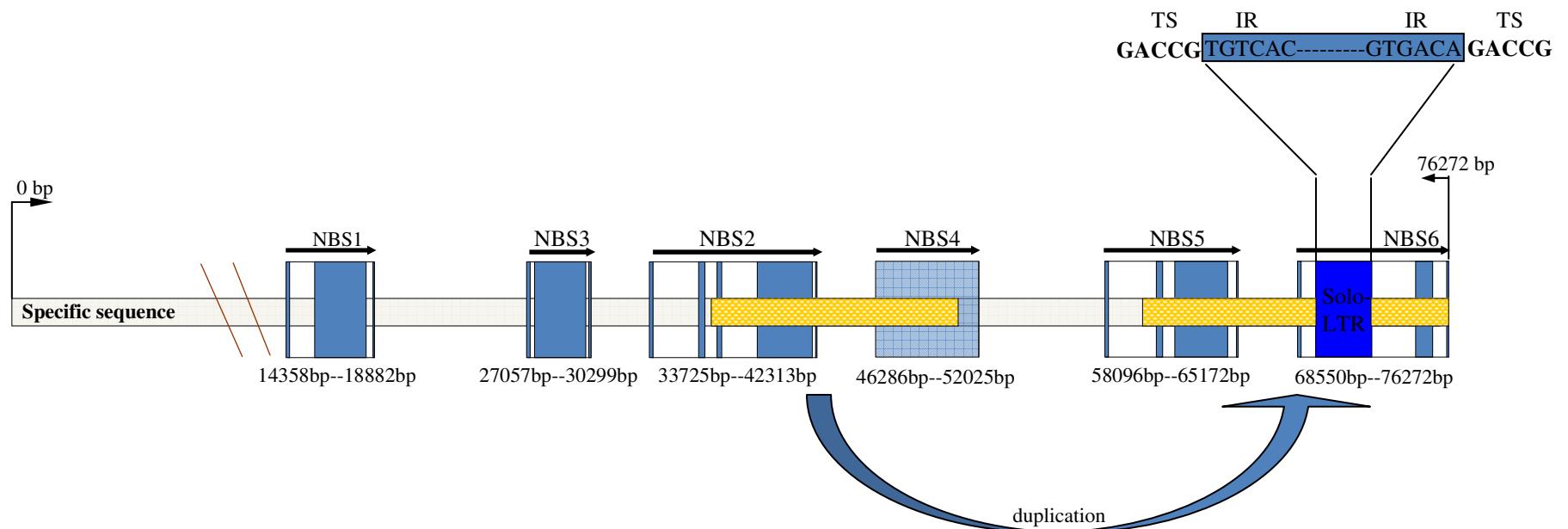
*O. minuta* *O. sativa*  
(75-1-127)



# Genetic and physical mapping of the *Pi9* region



# Structure of the *Pi9* locus



# Disease Reaction of the *Pi9* (*Nbs2-Pi9*) Transgenic Plants



# *Pi9* encodes an NBS/LRR protein

(a)

1 MAETVLSMAR SLVGS AISKA ASAAANETS L LLGVEKDIWY IKDELKTMQA FLRAAEVMKK KDELLKVWAE  
71 QIRDLSYDIE DSLDEF KVHI ESQT IAI RIHNLKSRVE EVSSRNTRYN LVEPISSGTE  
141 DDMDSYAEDI RNQSAR NVDE AELV DTN ANDGPAKVIC VVGMGGLGKT ALSRKIFESE  
211 EDIRKNFPCI AWITVSQSFH RIEL LDQ LLQELQGKVV VQVHLSEYL IEELKEKRYF  
281 VILDDLWILH DWNWINEIAF PKNN DLA EKCATASLVY HLDFLQMND A ITLLLRTNK  
351 NHEDMESNKN MQKMVERIVN KCGRLPLAIL TIGAVLATKH VSEWEKFYEQ LPSELEINPS LEALRRMVTL  
421 GYNHLPShLK PCFLYLSIFP EDFEIKRNRL VGRWIAEGFV RPKVGMTKD VGESYFNELI NRSM IQRSRV  
491 GIAGKIKTCR IHDIIRDITV SISROENEVI I PMGDGS DLV QENTRHIAFH GMSCKTGLD WSIIRSLAIF  
561 GDRPKSLAHA VCLDQLRMLR VLDL RIA LLCHLKYLSI GYSSSIYSLP RSIGKLQGLQ  
631 TLNMLRTYIA ALPSEISKLQ CLHT LN NH PMKCITNTIC LPKVFTPLVS RDDRAKQIAE  
701 LHMATKSCWS ESFGVKVPKG IGKL RTSS RAIKE LGHLS KLRKLGVITK GSTKEKCKIL  
771 YAAIEKLSSL QSLYVNAALL SDIE PLLR TLGL NGSLEE MPNWIEQLTH LKKIYLLRSK  
841 LKEGKTMIL GALPNLMVLY LYWNAYLGEK LVFKTGAFPN LRTLRIYELD QLREMRFEDG SSPLEKIEI  
911 SCCRLESGII GIIHLPRLKE ISLEYKSKVA RLGQLEGEVN THPNRPVLRM DSDRRDHDLG AEAEGSSIEV  
981 QTADPVDAE GSVTVAVEAT DPLPEQEGES SQSQVITLT NDSE EIGTAQ AG\*

NBS

LRR

(b)

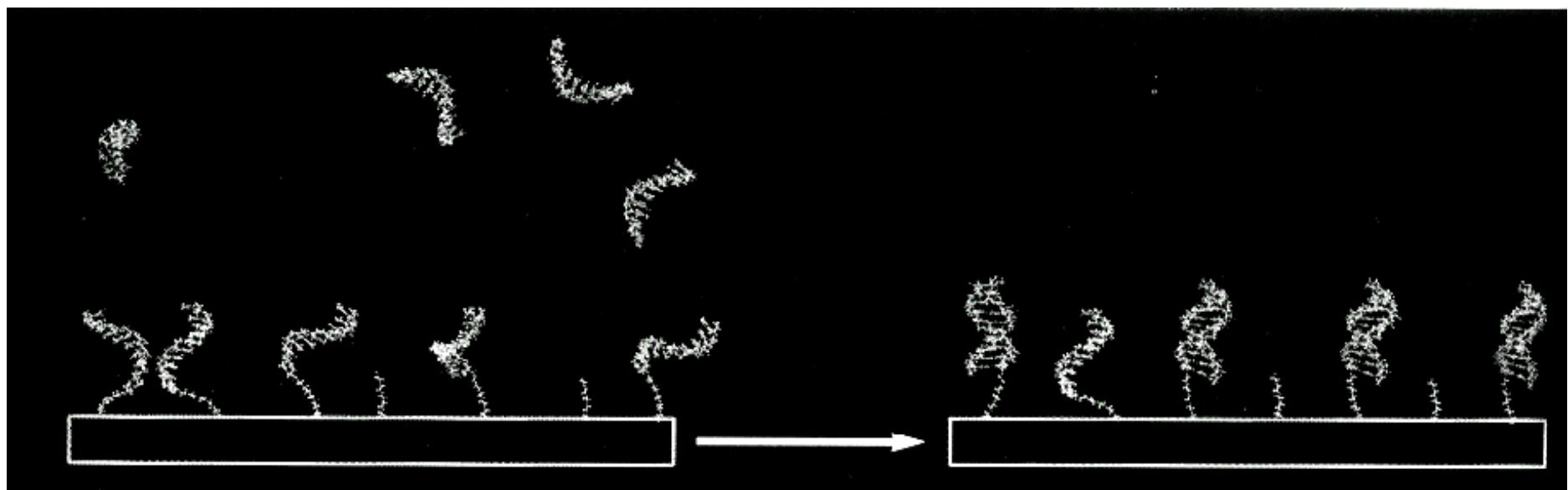
LRR1 LDQLRMLRVLD-LEDVTFLITQKDFDR  
LRR2 IALLCHLKYLS-IGYSSSIYSLPRS  
LRR3 IGKLQGLQTLNMLRTYIAALPSE  
LRR4 ISKLQCLHTLCSRKFVYDNFSLNHP  
LRR5 MKCITNTICLPKVFTPLVSRDDRAKQ  
LRR6 IAELH-MATKSCWSESFGVKVPKG  
LRR7 IGKL RD LQVLEYVDIRRTSSRAIKE  
LRR8 LGHLSKLRKLGVIITKGSTKEKCKILYAA  
LRR9 IEK LSS LQSLY-VNAAL  
LRR10 LSDIETLECLDSISSPPPL  
LRR11 LRTLGLNGSLEEMPNW  
LRR12 IEQLTHLKKIYLLRSKLKEGKMLI  
LRR13 LGALPNLMVLYLYWNAYLGEKLVFKTGAFPNLRTL  
LRR14 IYELDQ LREMRFEDGSSPLLEKIEISCCRLESG  
LRR15 IIIGIIHLPRLKEISLEYKSK  
LRR16 VARLGQLEGEVNTHPNRP  
LRR17 VLRM DSDRRDHDLGAEEAEG  
Consensus IXXLXXXLXXL

# **Microarray-based mutation detection and gene cloning strategy**

Problems in traditional map-based cloning:

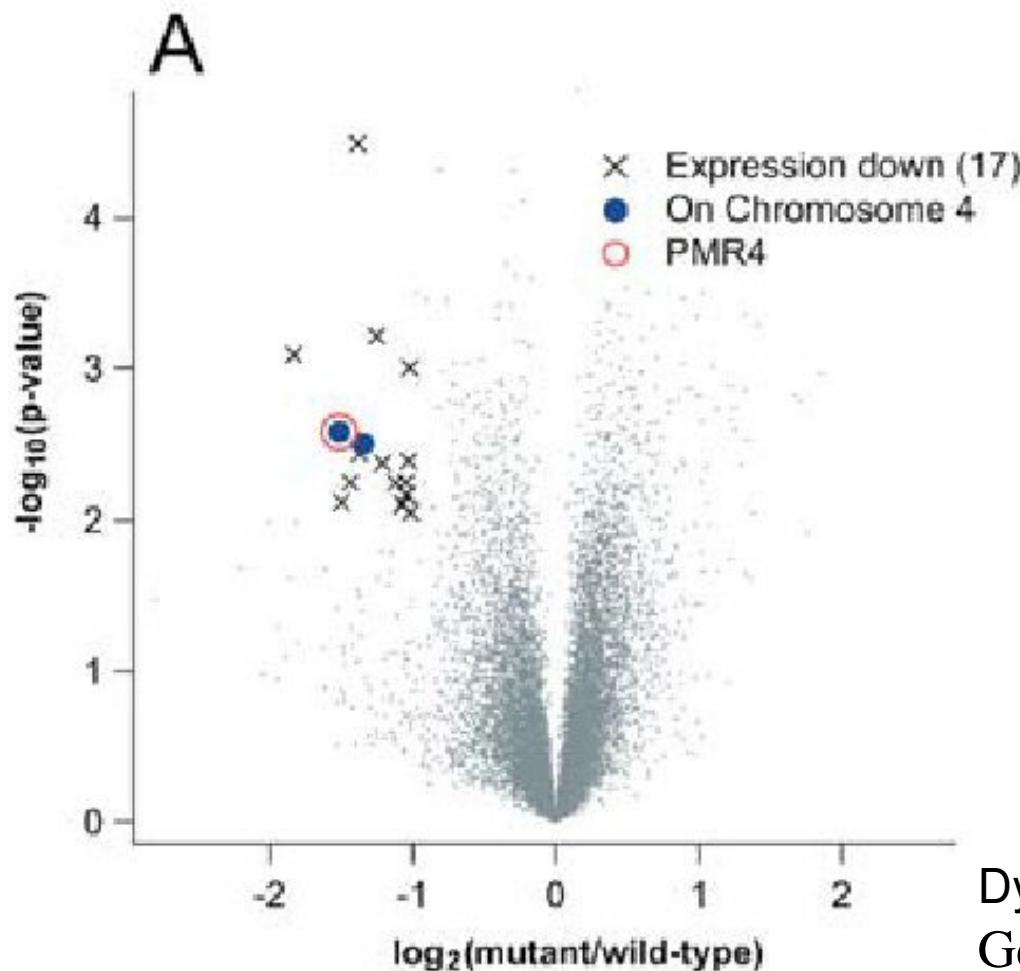
1. Phenotyping of individuals in the mapping population is time- and labor-intensive.
2. Fine mapping with tightly linked markers is difficult for some genomic regions.
3. The parental lines used to generate F2 mapping populations may show a low level of DNA polymorphism

# Chip-based gene detection



# Using microarray to facilitate deletion detection

## Arabidopsis volcano plot



Dybbs et al. 2004 Plos  
Genetics, 2005 1:6-16

# Mapping and cloning of avirulence genes

- Isolation of avirulence genes in bacteria
- Isolation of avirulence genes in fungi

# Isolation of avirulence genes in bacteria

Avirulence genes are dominant (Flor's experiment)

Plant	Pathogen	Avr	avr
R			
r			

# Isolation of avirulence genes in bacteria

## Staskawicz and Keen Labs

- Prepare genomic library from one race
- Complement in the another race
- Observe avirulence (if avirulence is dominant!)

**Table I.** *Bacterial disease reactions on soybean cultivars*

<i>P. syringae glycinea</i> Race	Harosoy Cultivar	Norchief Cultivar
<i>Psg</i> race 6	HR	Susceptible
<i>Psg</i> race 5	Susceptible	HR
<i>Psg</i> race 5 ( <i>avrA</i> )	HR	HR

*Plant Physiology*, January 2001, Vol. 125, pp. 73–76,

Add another slides to illustrate the steps for isolating avrulence genes from bacteria

# Isolation of avirulence genes in bacteria

Identification of Hrp genes (Hypersensitive response) from bacteria (Labs: Collmer, Bonas, Beer, Boucher)

Hrp Genes: 23-25kb clusters containing several operons

Further sequencing of Hrp regions in phytopathogenic bacteria revealed that the region has high homology to bacterial typeIII secretion system (T3SS)

As T3SS delivers avirulence proteins, subsequently by analyzing this sequence from many plant pathogenic bacteria, a number of avirulence genes were identified.

# Isolation of avirulence genes in bacteria

Hrp Box promoter pattern searches

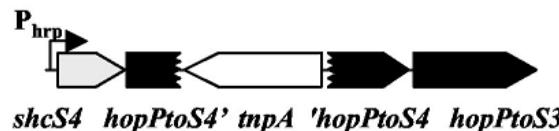


Candidates



Assays: Knock-out,  
Translocation and  
expression in the plants

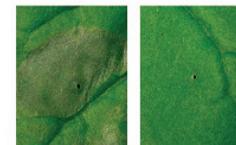
pEDV system  
(*Pseudomonas* or  
*Burkholderia* based)



Translocation and expression in plants

*P. fluorescens* 55  
+ pLN18 + pCPP3297

HopPtoK-Cya



HopPtoQ-Cya



HopPtoT1-Cya

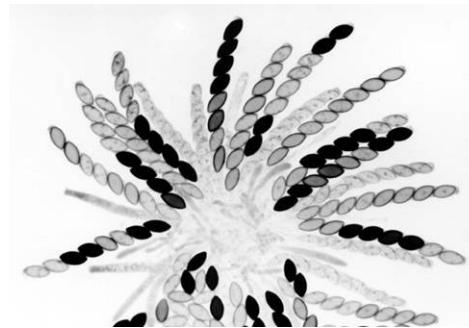


# Cloning of the *AvrPiz-t* effector from *M. oryzae*

avirulent isolate 81278ZB15  
(MAT1-1)

X

virulent isolate GUY11  
(MAT1-2)

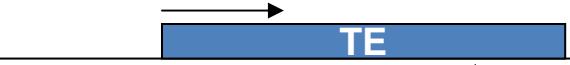


Of 52 single ascospores tested  
29 avirulent and 23 virulent

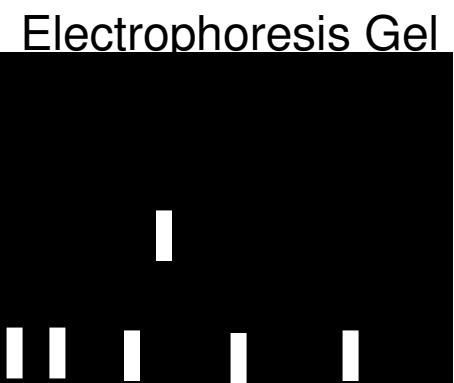
Presence or absence of a transposable element (PATE) markers

81278ZB15

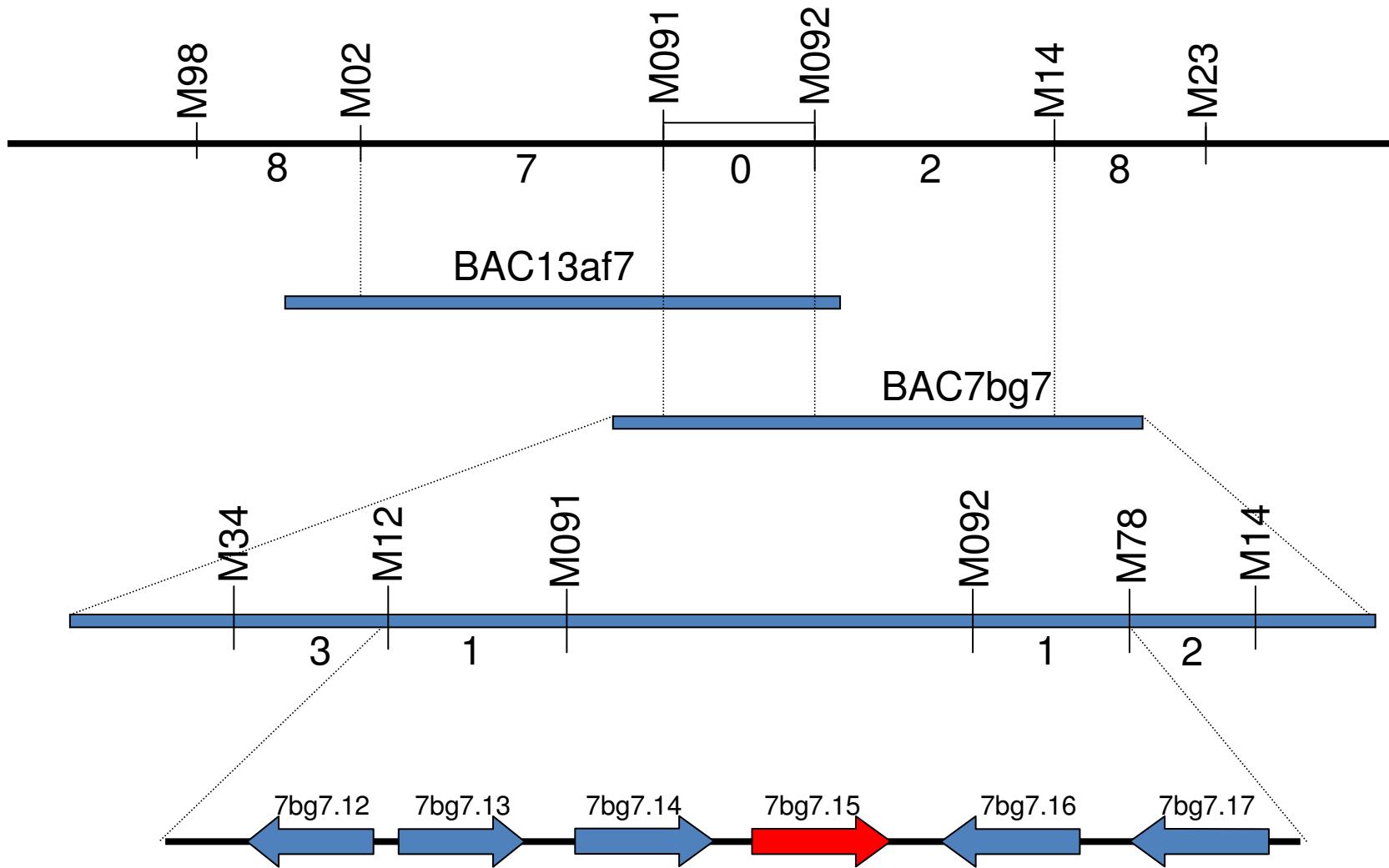
GUY11



Example of polymorphic marker



# Cloning of the *AvrPiz-t* effector from *M. oryzae*



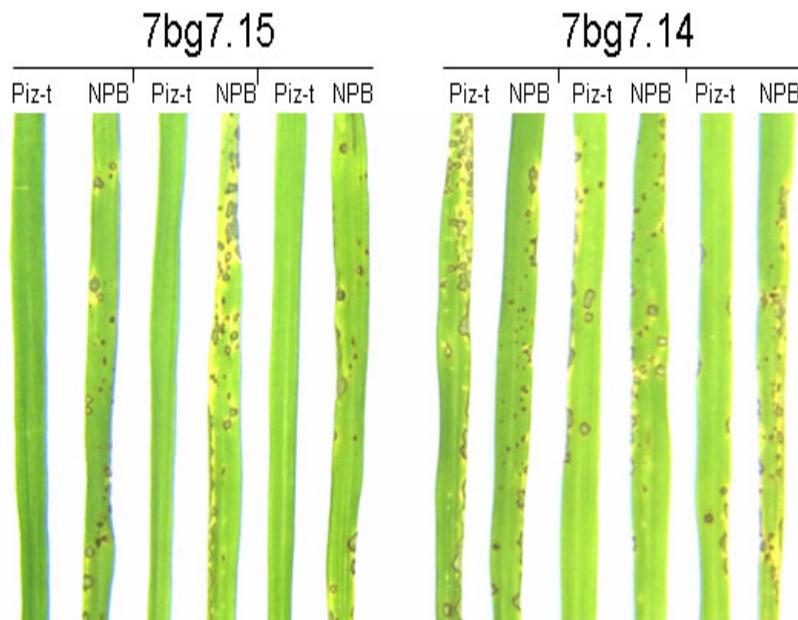
Collaboration with Drs. Bo Zhou and Zonghua Wang

Li et al. MPMI, 2009

# Prediction of six candidate genes

<b>Gene</b>	<b>Polymorphism</b>	<b>Classification</b>
7bg7.12	No	Non-secretory protein
7bg7.13	Single amino acid	Endoplasm protein
7bg7.14	No, but a transposon upstream of ATG	Non-secretory protein
7bg7.15	No, but a transposon 462bp upstream of ATG	Secretory protein
7bg7.16	Single amino acid	Mitochondria protein
7bg7.17	No	Nucleus protein

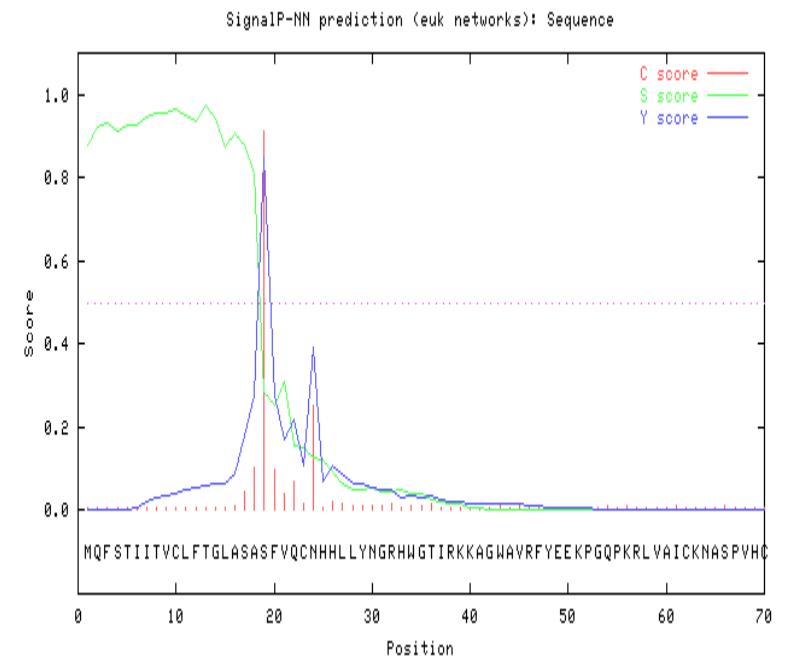
# *7bg7.15* complemented the *AvrPiz-t* function in virulent isolates



	NPB (Piz-t)	NPB
7bg7.13	∨	∨
7bg7.14	∨	∨
7bg7.15	A	∨
7bg7.16	∨	∨

# *AvrPiz-t* encodes a small secreted protein

atgcagttctcaaccatcatcaccgtgtgcctttcacccggctcgccctccgcccagcttc  
M Q F S T I I T V C L F T G L A S A S F  
gtacaatgcaatcatcatctcctgtacaatggcagacactggggcacgataaggaagaag  
V Q C N H H L L Y N G R H W G T I R K K  
gcgggttgggcccgttagattttacgaagaaaaaccaggggcagccaaagaggctggtcgcg  
A G W A V R F Y E E K P G Q P K R L V A  
atttgcaaaaacgcgtcaccctgtacactgcaactatctgaaatgcaccaattggcagca  
I C K N A S P V H C N Y L K C T N L A A  
ggcttctcggcagggacgtccactgtatgttctctccggcaccgttggctcgattggg  
G F S A G T S T D V L S S G T V G S I G  
aatgaccctcaggctcagcgccaatag  
N D P Q A Q R Q -



## Reverse genetic approaches

1. In Silico mutant identification
2. Targeted mutagenesis of candidate genes

# *In silico* Mutant Screen

1. Isolate flanking sequences from all mutants
2. Establish a flanking sequence database
3. *In silico* search for a mutant of interest
4. Request mutants from the mutant collection center

A total of 47203 total lines were sent to ABRC, representing 26596 individual genes.  
<http://signal.salk.edu>

# Salk T-DNA Mutant Update

## 4/2011

Target genes: 28633  
Genes with T-DNA alleles: 25762  
T-DNA alleles to be genotyped: 82703  
Genes with T-DNA alleles in pipeline : 23821  
T-DNA alleles in pipeline: 63437

# Targeted gene mutagenesis

1. Sense or anti-sense expression
2. Homologous recombination
3. Virus induced gene silencing (VIGS)
4. RNA interference (RNAi)
5. Artificial miRNA
6. Zinc-Finger Nucleases (ZFNs)
7. TAL effector nucleases (TALENs)

Gene Silencing

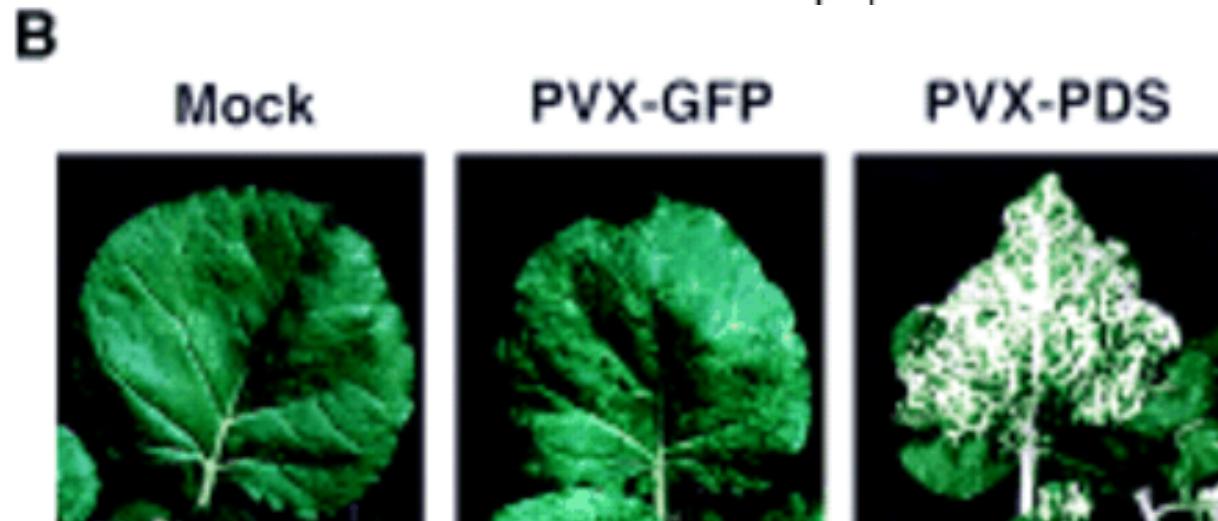
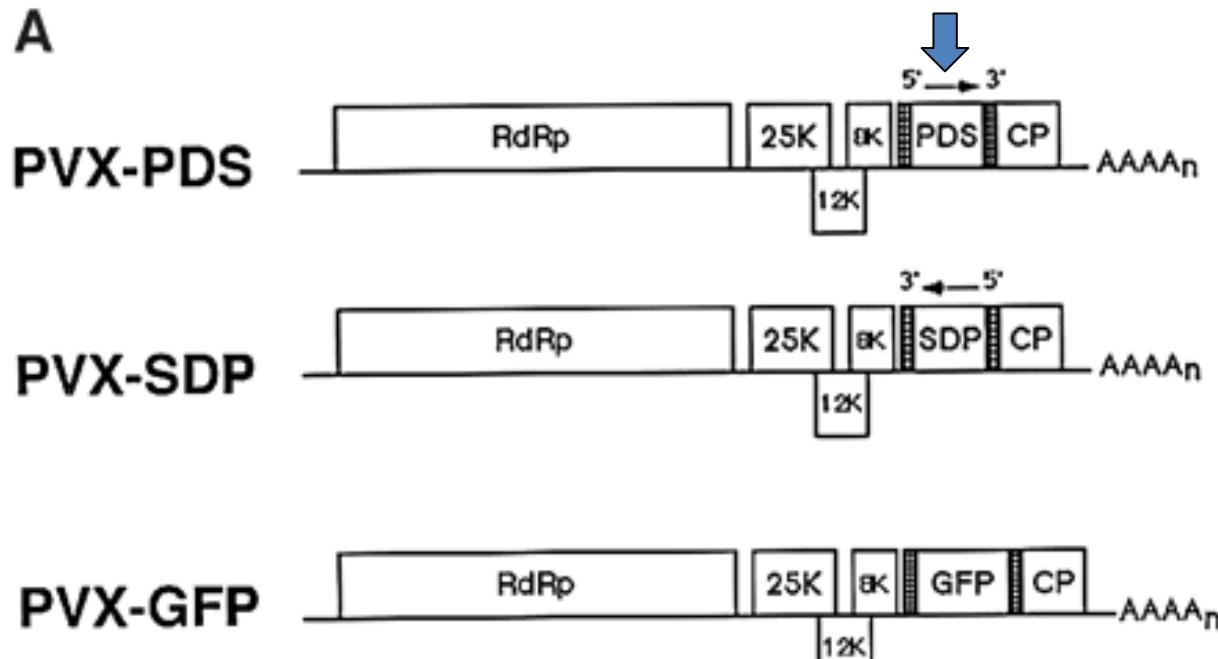
Complete or partial reduction of gene expression

## Virus-induced gene silencing (VIGS) in plants

1. Uses virus vectors carrying DNA elements from the exons of plant host genes to suppress the expression of endogenous genes.
2. Ruiz et al. (Plant Cell, 1998. 10:937-946) cloned a fragment of phytoene desaturase (PDS) into the PVX vector and found that the upper leaves of the infected plants developed a spectacular bleached effect due to the decline in expression of the endogenous PDS mRNA.

# VIGS of PDS gene using PVX vector

(due to low expression of PDS and loss of protection against photobleaching)



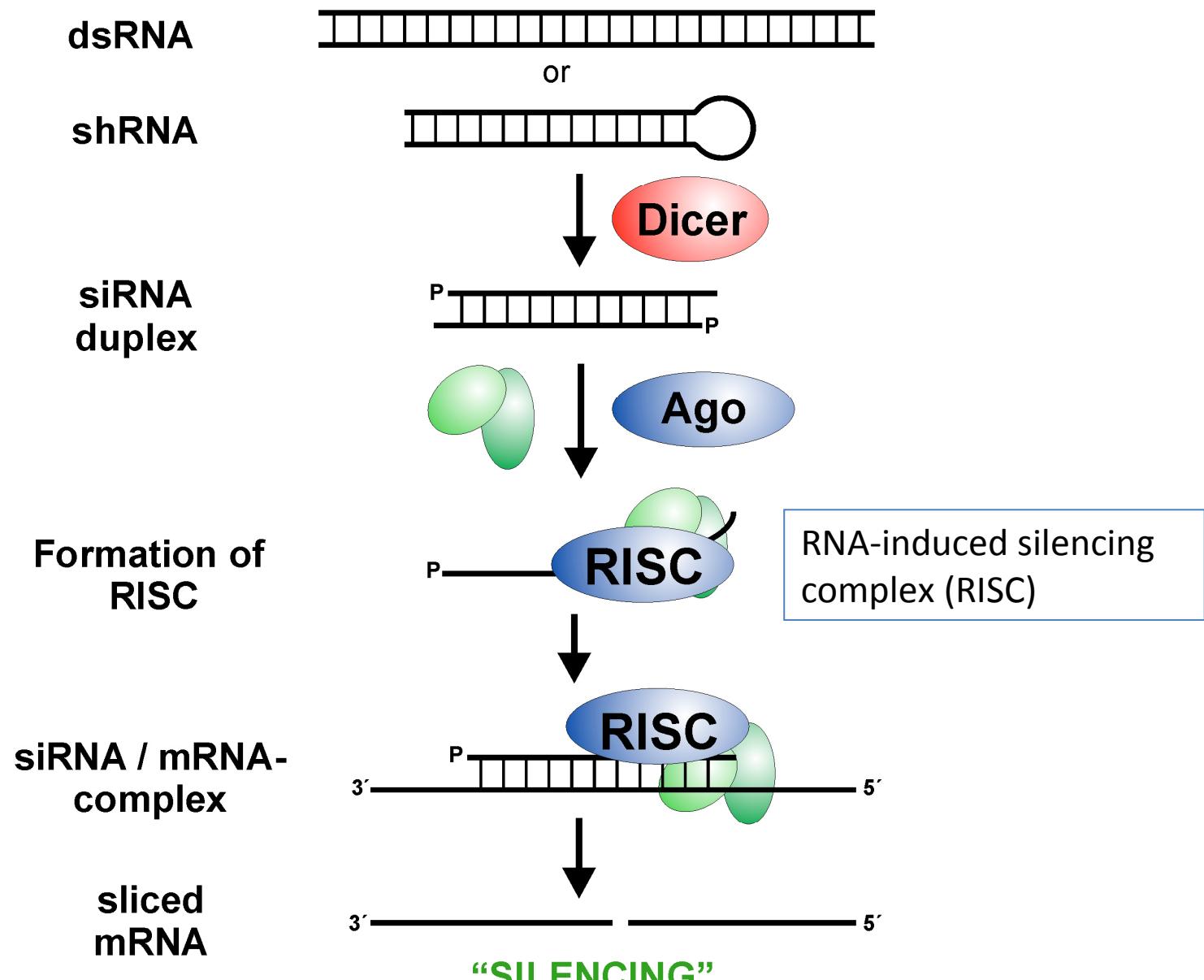
The advantages of VIGS over other knockout methods such as antisense or homology recombination are:

- 1) Lethal genes can be identified since VIGS is applied in mature plants and suppression may be partial
- 2) Multigene families with functional redundancy can be targeted or one can target not only the precise homologue but also suppress a partial homologue of the gene (80-90% homology).

# RNA interference (RNAi)-mediated gene silencing

When double-stranded RNA that corresponds to a particular gene is introduced into an organism, a specific degradation of the targeted mRNA was observed and led to suppression of the gene expression (Meister and Tuschl, 2004, Nature 431:343-349).

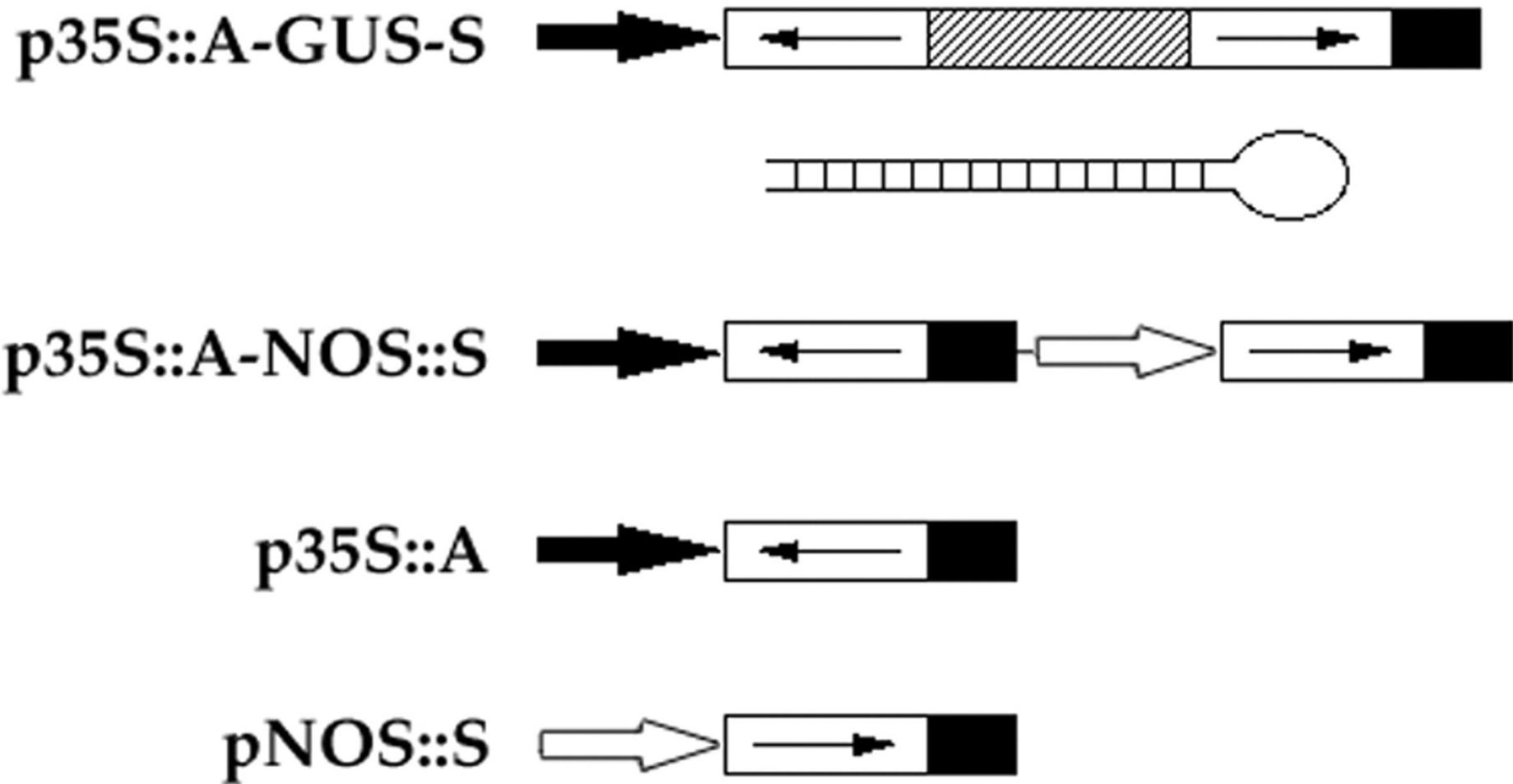
# A simplified model for the RNA interference (RNAi) Pathway



# Application of RNAi for gene silencing in plants

1. Chuang and Meyerowitz investigated the potential of double-stranded RNA interference (RNAi) with gene activity in *Arabidopsis thaliana* (2000, PNAS 97:4985-4990).
2. When introduced into the genome of *A. thaliana* by *Agrobacterium*-mediated transformation, double-stranded RNA-expressing constructs corresponding to four genes, *AGAMOUS* (AG), *CLAVATA3*, *APETALA1*, and *PERIANTHIA*, caused specific and heritable genetic interference.
3. RNAi effects of different constructs on phenotype were compared.

## Gene constructs used for RNAi analysis in *Arabidopsis*



# Effect of sense, antisense and dsRNAs on transgenic plants

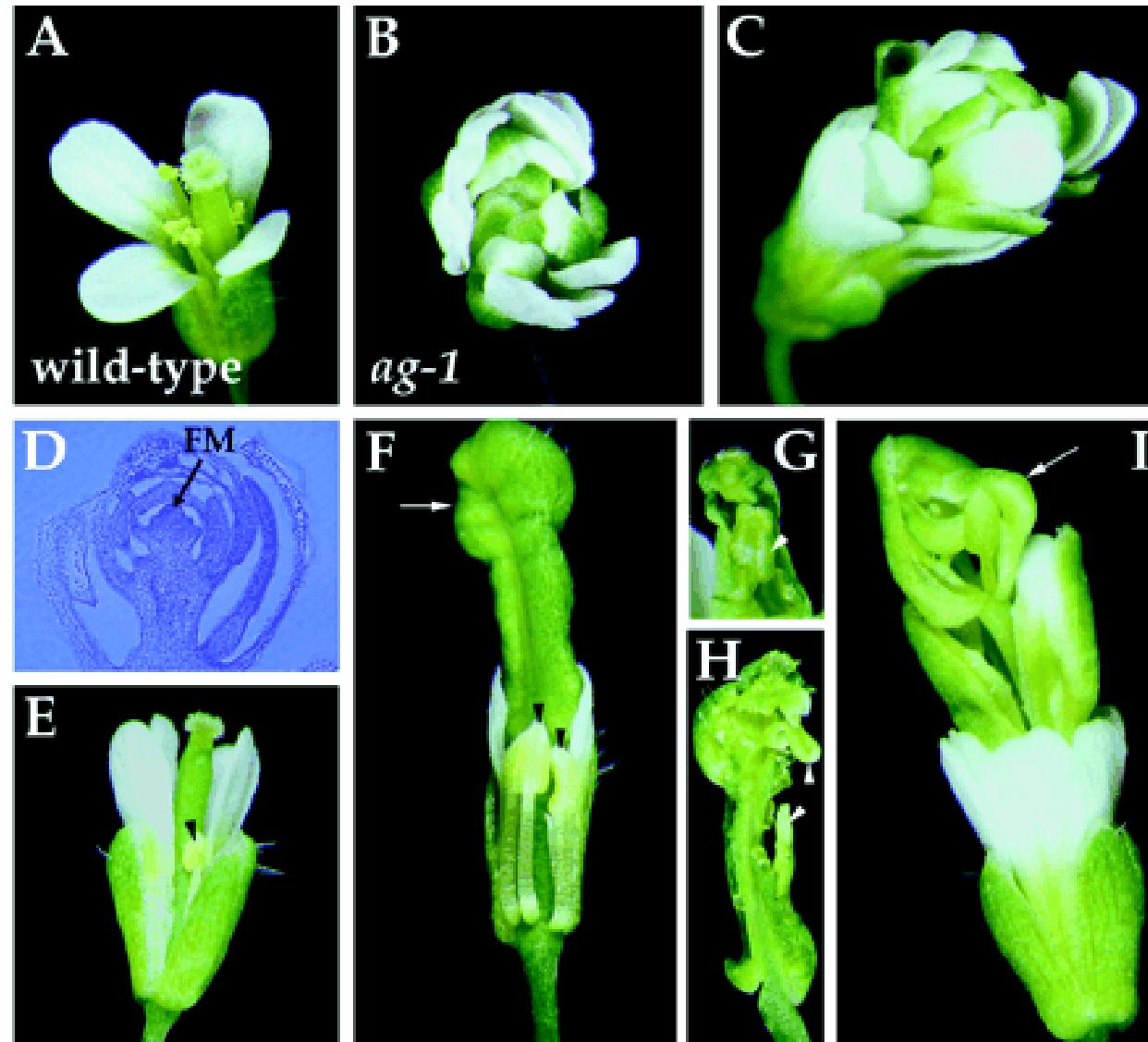
Gene	Transformed background	Transformed construct	RNAi mutants/total	%	
AG	Ws*	p35S::A-GUS-S	235/236	99.6	↙
		pNOS::A-GUS-S	2/32	6	
		p35S::A-NOS::S	3/124	2	
		p35S::A	0/111	0	↙
		pNOS::S	0/95	0	↙
CLV3	Ws	p35S::A-GUS-S	121/137	88	
		p35S::A-NOS::S	2/176	1	
		p35S::A	0/273	0	
		pNOS::S	ND†	ND	
AP1	L-er‡	p35S::A-GUS-S	249/260	96	
		p35S::A	8/140	6	
		pNOS::S	0/62	0	
PAN	crc-1	p35S::A-GUS-S	110/126	87	
		p35S::A-NOS::S	18/66	27	
		p35S::A	42/76	55	
		pNOS::S	2/6	33	

\*Ws, Wassilewskija.

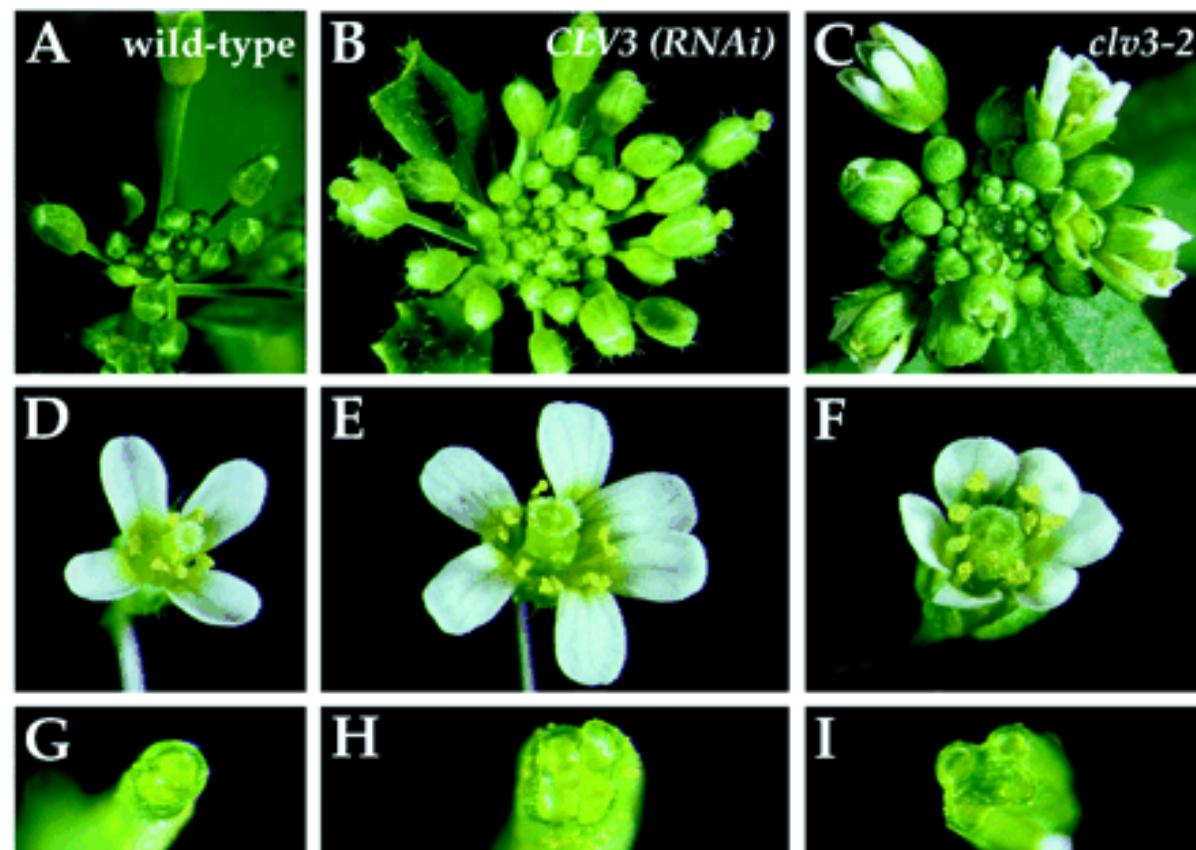
†ND, not determined.

‡L-er, Landsberg erecta.

## Flower phenotype of wild-type, *ag-1* and AG (RNAi) plants



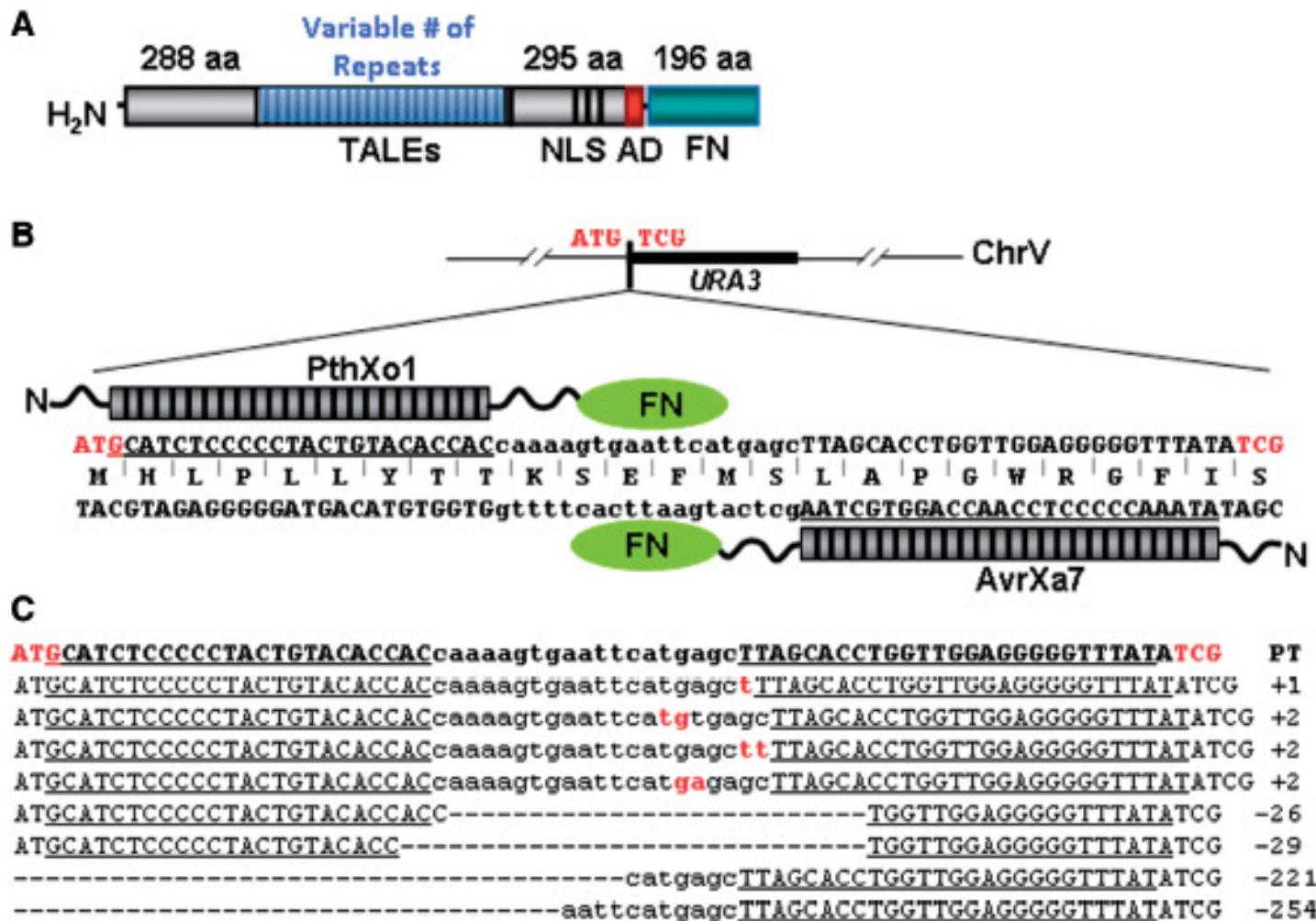
## Phenotype of wild-type, *CLV3* (RNAi) and *CLV-2* plants



## **Effector-Target Genome Modifications by TALEN**

1. DNA recognition domain of transcription activator-like (TAL) effectors can be combined with the nuclease domain of FokI restriction enzyme to produce TAL effector nucleases (TALENs)
2. TALENs bind adjacent DNA target sites and produce double-strand breaks between the target sequences, stimulating non-homologous end-joining and homologous recombination.

# Modification of host genes TALENs

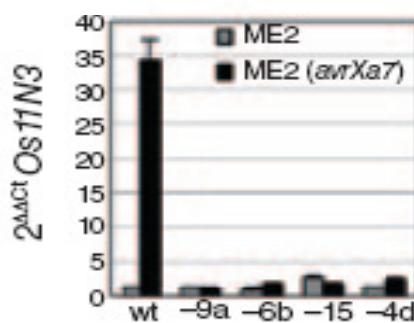


# Modification of host genes TALENs

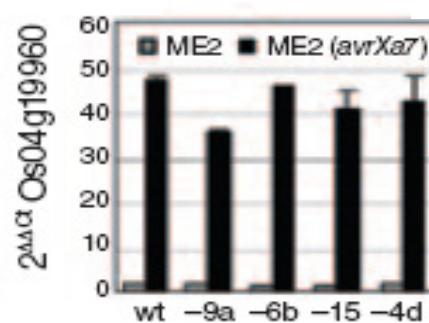
A

	WT
CTTCCTTCCTAGCACTATATAAAccccctccaaaccagggtgcTAAGCTCATCAAGCCTTCAAGC	-55a
-----gtgcTAAGCTCATCAAGCCTTCAAGC	-7/+3
CTTCCTTCCTAGCACTATATAAAccccctc-AAA- gtgcTAAGCTCATCAAGCCTTCAAGC	-32a
CTTCCTTCCTA ----- AGCTCATCAAGCCTTCAAGC	-18
CTTCCTTCCTAGCACTATATAAAccccct ----- CATCAAGCCTTCAAGC	-22/+5
CTTCCTTCCTAGCACTATATAAA-----GGAC----- CTCTCATCAAGCCTTCAAGC	-9a
CTTCCTTCCTAGCACTATATAAAccc ----- aggtgcTAAGCTCATCAAGCCTTCAAGC	-5b
CTTCCTTCCTAGCACTATATAAAccc ----- aaccagggtgcTAAGCTCATCAAGCCTTCAAGC	-4b
CTTCCTTCCTAGCACTATATAAAccccctccaa ----- gtgcTAAGCTCATCAAGCCTTCAAGC	-3a
CTTCCTTCCTAGCACTATATAAAccccctcc --- caggtgcTAAGCTCATCAAGCCTTCAAGC	+9
CTTCCTTCCTAGCACTATATAAAccccctccaaaccaggGTGTCATTAgtgcTAAGCTCATCAAGCCTTCAAGC	

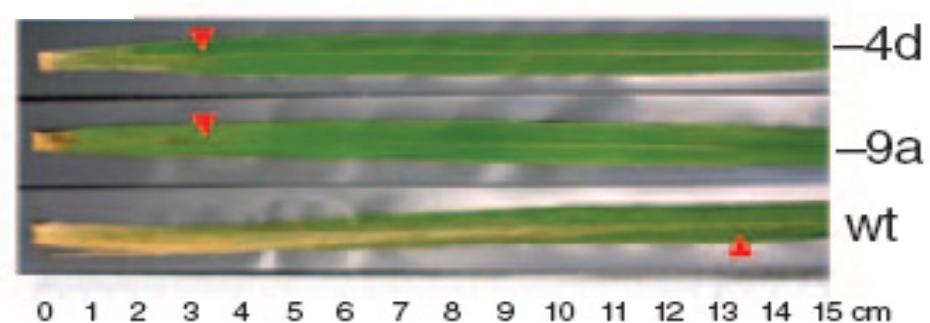
B



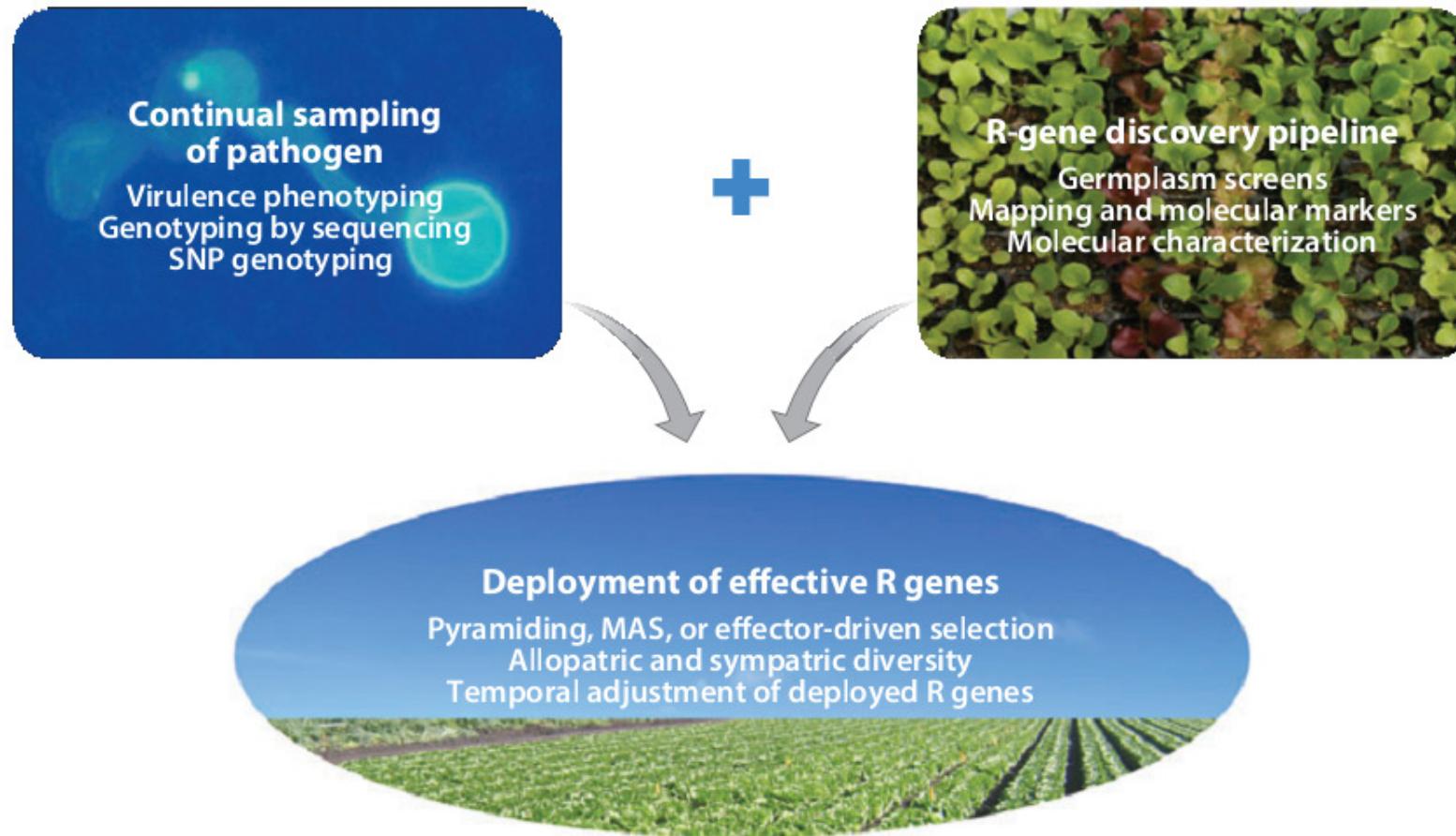
C



D



# Utilization of Genetics



# Summary

1. Plant host resistance is being explored by tools developed for molecular genetics, molecular biology and evolutionary biology
2. Pathogen virulence/avirulence is also getting equal attention in these fields
3. The knowledge generated together can be utilized by
  1. Epidemiologist for targeted deployment of R genes
  2. Molecular plant pathologist to engineer new recognition specificities for pathogens
  3. Plant biotechnologist/breeders : new genome editing tools to engineer crop plants with durable resistance.

# Additional reading

1. E. Guy *et al.*, Natural Genetic Variation of *Xanthomonas campestris* pv. *campestris* Pathogenicity on *Arabidopsis* Revealed by Association and Reverse Genetics. *Mbio* **4**, (May-Jun, 2013).
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3. R. W. Michelmore, M. Christopoulou, K. S. Caldwell, Impacts of resistance gene genetics, function, and evolution on a durable future. *Annu Rev Phytopathol* **51**, 291 (Aug 4, 2013).
4. B. J. Staskawicz, Genetics of plant-pathogen interactions specifying plant disease resistance. *Plant Physiol* **125**, 73 (Jan, 2001).
5. V. G. A. A. Vleeshouwers *et al.*, Understanding and Exploiting Late Blight Resistance in the Age of Effectors. *Annu Rev Phytopathol* **49**, 507 (2011).
6. K. Yoshida *et al.*, Association Genetics Reveals Three Novel Avirulence Genes from the Rice Blast Fungal Pathogen *Magnaporthe oryzae*. *Plant Cell* **21**, 1573 (May, 2009).