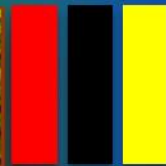


Genome Analysis and Resistance Breeding in Agriculture

JAMSARI

Presented at 2nd IGN-TTRC Seminar 20 August 2013-Surabaya



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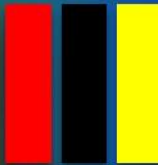


Agricultural Mission: Securing of food availability

- Food quantity
(developing countries)
- Food quality
(developed countries)



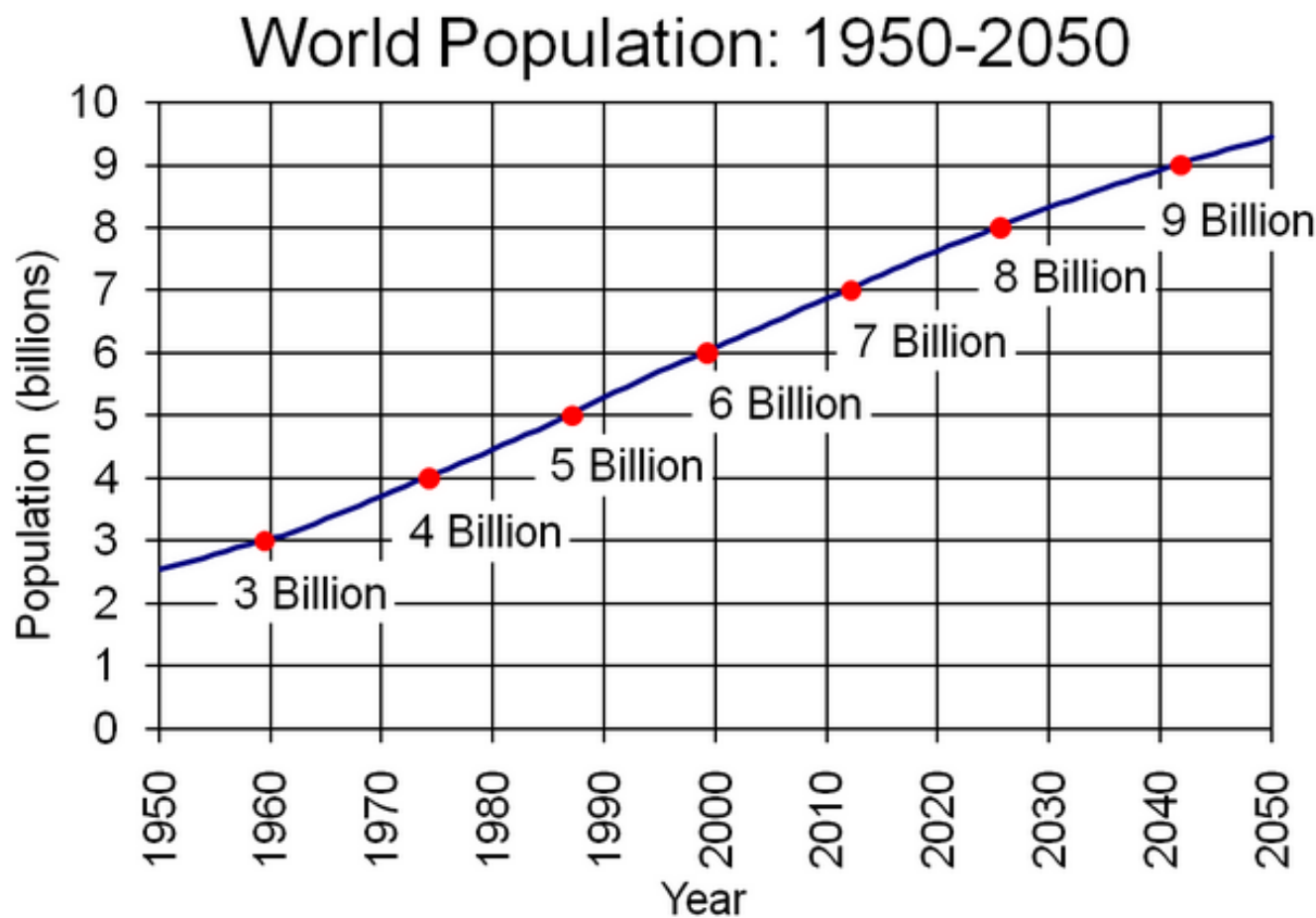
Introduction



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World Population Growth



Source: U.S. Census Bureau, International Data Base, June 2011 Update.

Introduction

2010



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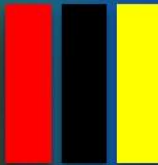


Growth Population VS Food Production

- Rising of world population from 6.8 billion (2010) to over 9 billion (By 2050)- 10 billion by 2100 (United Nations, 2011)
- meant: a 70% increase in food demand!

The increasing of food price !!! (ADB-2007)

Introduction

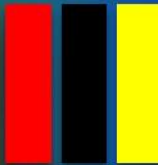


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Need for Increase of Crop Production

- From 1999 -2020 increase up to 80% (Pinstrup-Anderson and Lorch, 1997).
- from 590-600 MMT to 840 MMT by 2020 must be achieved.



Challenges!

Food production and food security faces several challenges

- **Land evolution for agricultural production to non agricultural activities:**
- **Global climate changes;**
- ❖ **Abiotic stresses: drought, heat shock, UV radiation, etc.**
- ❖ **Biotic stresses: pest and weeds attack**

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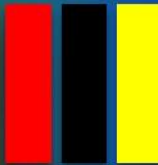
Challenges!

- Cultural techniques improvement is “deminishing return”.
- Genetic potency must be exploited via genetic based modification = breeding efforts

Role of Plant Breeding

- To enable stable yields with lower inputs of fertilizers, energy and water use,
- To produce safe and quality food,
- To meet the demand of a projected raise in human population and livestock production.

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Resistance Breeding, plant-pathogen interaction

- Improvement of crop production regarding pest and disease management.
- Pathogens cause huge yield losses in the agriculture every year with large economic losses and damage to ecosystems
 - Global yield loss of 16% (Oerke, 2006).
 - 26-29% for sugar beet, barley, soybean, wheat and cotton, to 31-40% for maize, potato and rice (Oerke, 2006).



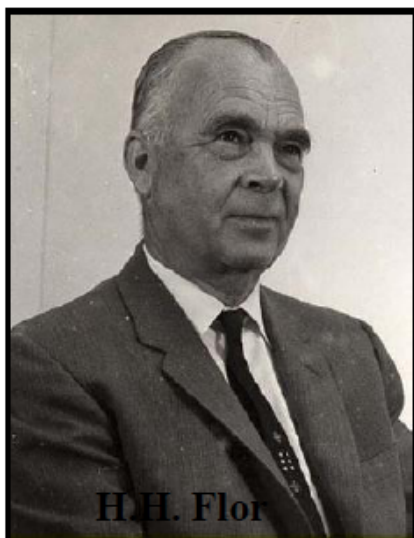
Genetic Modification Based Approach

- Since disease attack dealing with interaction between plant-pathogen, they must be in compatible manner:

Involving:

- plant genetic background
- pathogen genetic background

Gene for Gene Concept (Flor, 1942: Phytopathology)



“for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite”

Gene for Gene Concept

Pathogen genotype	Host genotype	
	R1	r1
<i>Avr1</i>	-	+
<i>avr1</i>	+	+

- = Incompatible reaction

+ = Compatible reaction

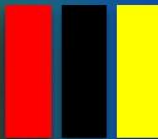
Outline

- **Introduction**
- **Molecular Plant-Microbe Interaction**
- **R-gene Evolution**
- **Concluding Remarks**

- **Molecular Plant-Microbe Interaction
(MPMI)**

Molecular Plant-Microbe Interaction (MPMI)

- Plants use different strategies and mechanisms to survive and reproduce successfully.
- Basal resistance is provided by pre-existing physical and chemical barriers in order to disable penetration of pathogen to the host-cell.
- Recognition of microbial surfaces by cell surface receptors that trigger immune response and offer broad-spectrum resistance pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) (Jones & Dangl, 2006).



Basal Principle in Plant-Pathogen Interaction

A

Interactions involved in
toxin-dependent compatibility

		Host plant genotype	
		<i>Rx</i>	<i>rr</i>
Pathogen genotype	<i>TOX</i>	I	C
	<i>Tox</i>	I	I

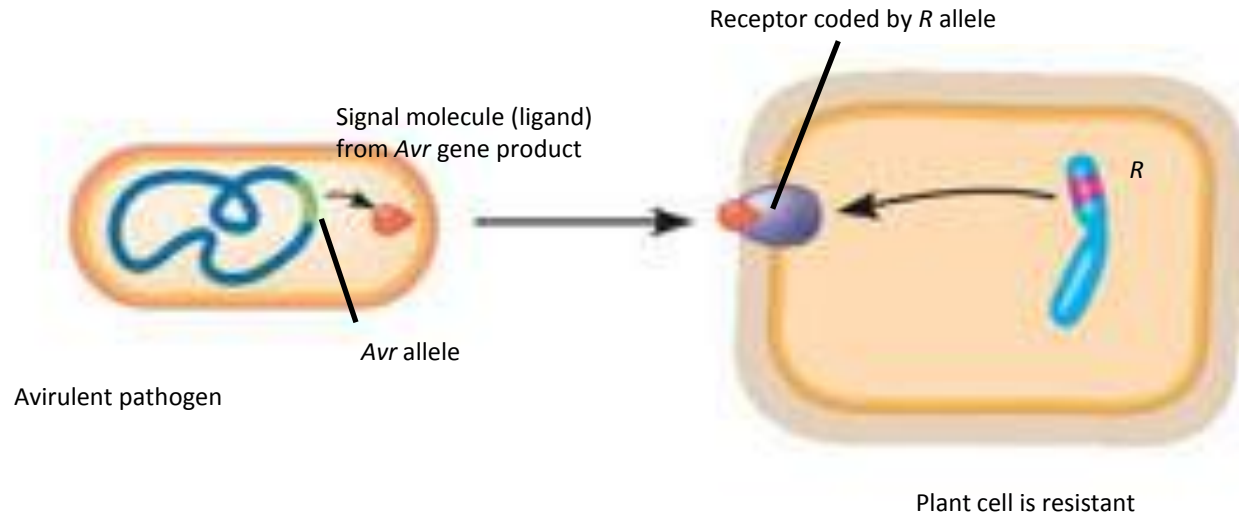
B

Interactions involved in
R gene -*Avr* gene incompatibility

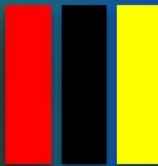
		Host plant genotype	
		<i>R1 r2</i>	<i>r1 R2</i>
Pathogen genotype	<i>Avr1, avr2</i>	I	C
	<i>avr1, Avr2</i>	C	I

**Gene-for-gene recognition is a widespread
form of plant disease resistance**

- A pathogen is avirulent
 - If it has a specific *Avr* gene corresponding to a particular *R* allele in the host plant



(a) If an *Avr* allele in the pathogen corresponds to an *R* allele in the host plant, the host plant will have resistance, making the pathogen avirulent. *R* alleles probably code for receptors in the plasma membranes of host plant cells. *Avr* alleles produce compounds that can act as ligands, binding to receptors in host plant cells.

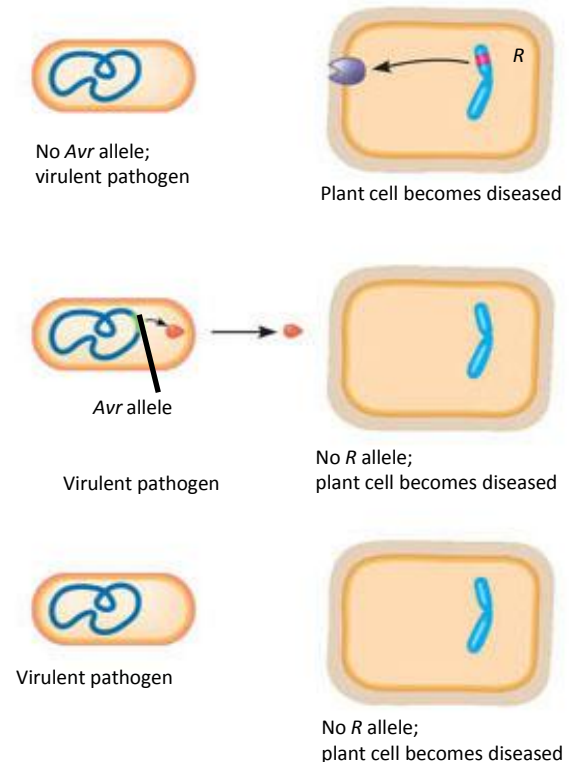


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- If the plant host lacks the *R* gene that counteracts the pathogen's *Avr* gene

Then the pathogen
can invade and kill the plant



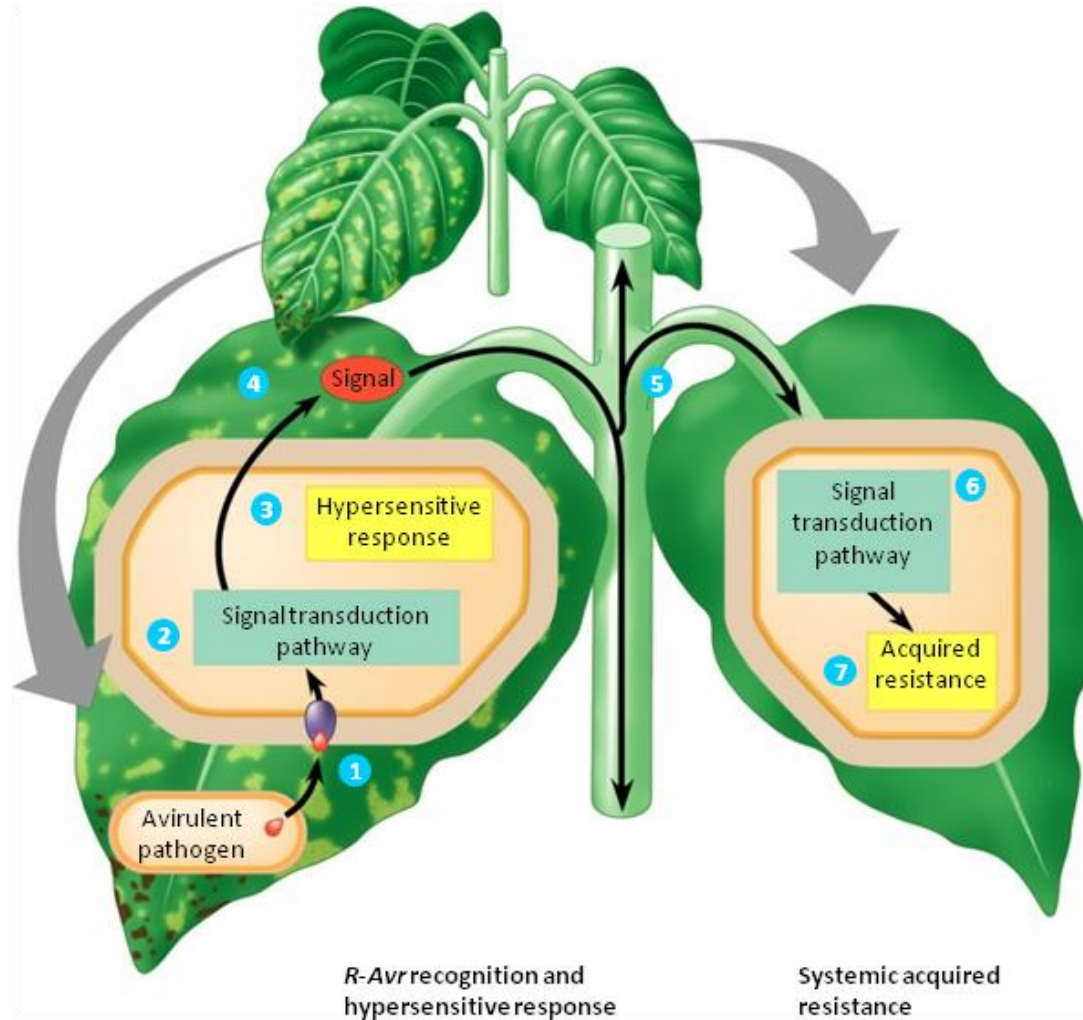
(b) If there is no gene-for-gene recognition because of one of the above three conditions, the pathogen will be virulent, causing disease to develop.



- **Complex Plant Responses to Pathogen
Invasions**

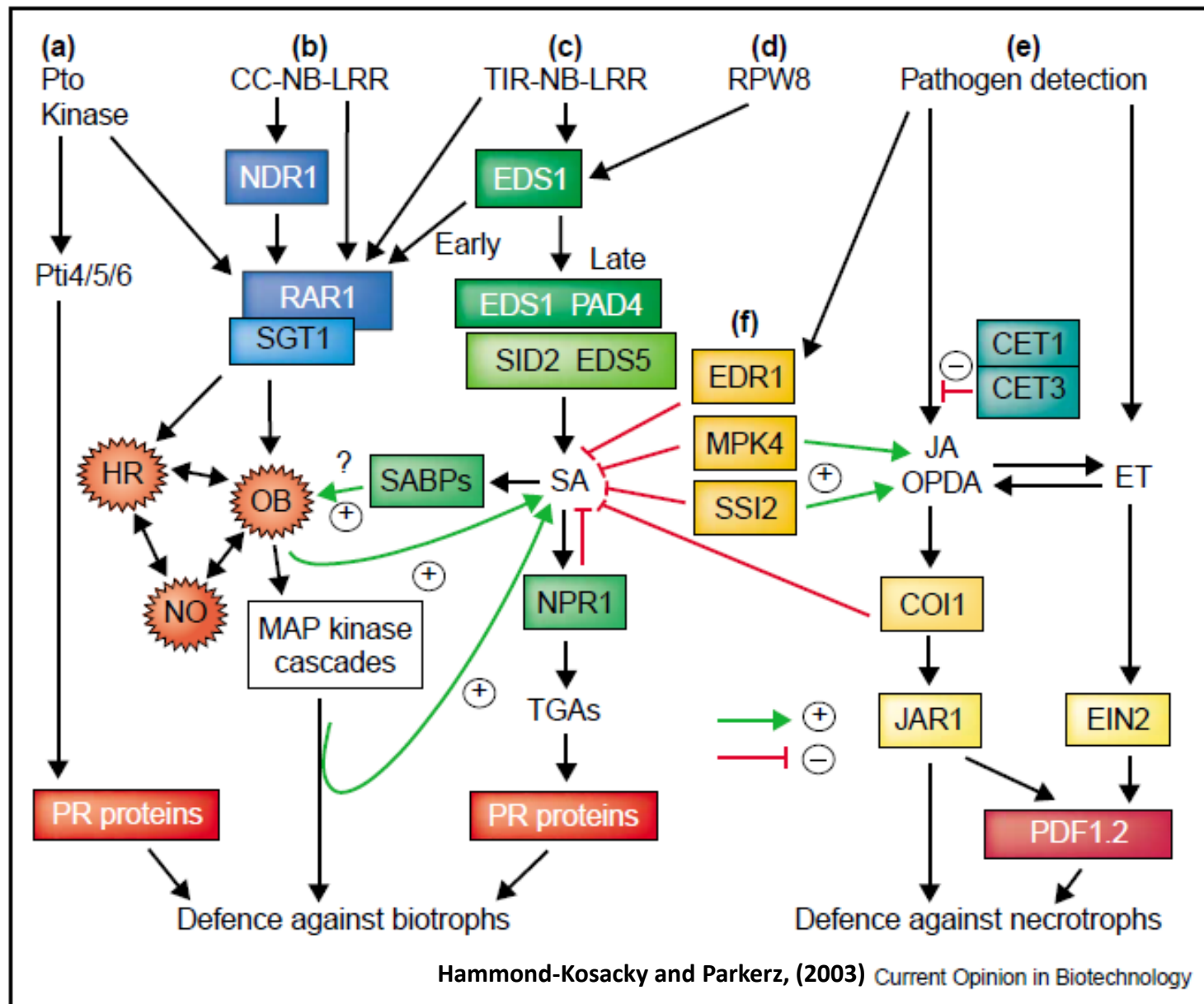
A hypersensitive response (HR): Seals off the infection and kills both pathogen and host cells in the region of the infection

- 1 Specific resistance is based on the binding of ligands from the pathogen to receptors in plant cells.
- 2 This identification step triggers a signal transduction pathway.
- 3 In a hypersensitive response (HR), plant cells produce anti-microbial molecules, seal off infected areas by modifying their walls, and then destroy themselves. This localized response produces lesions and protects other parts of an infected leaf.
- 4 Before they die, infected cells release a chemical signal, probably salicylic acid.



- 5 The signal is distributed to the rest of the plant.
- 6 In cells remote from the infection site, the chemical initiates a signal transduction pathway.
- 7 Systemic acquired resistance is activated: the production of molecules that help protect the cell against a diversity of pathogens for several days.

Local signalling networks controlling activation of local defence responses

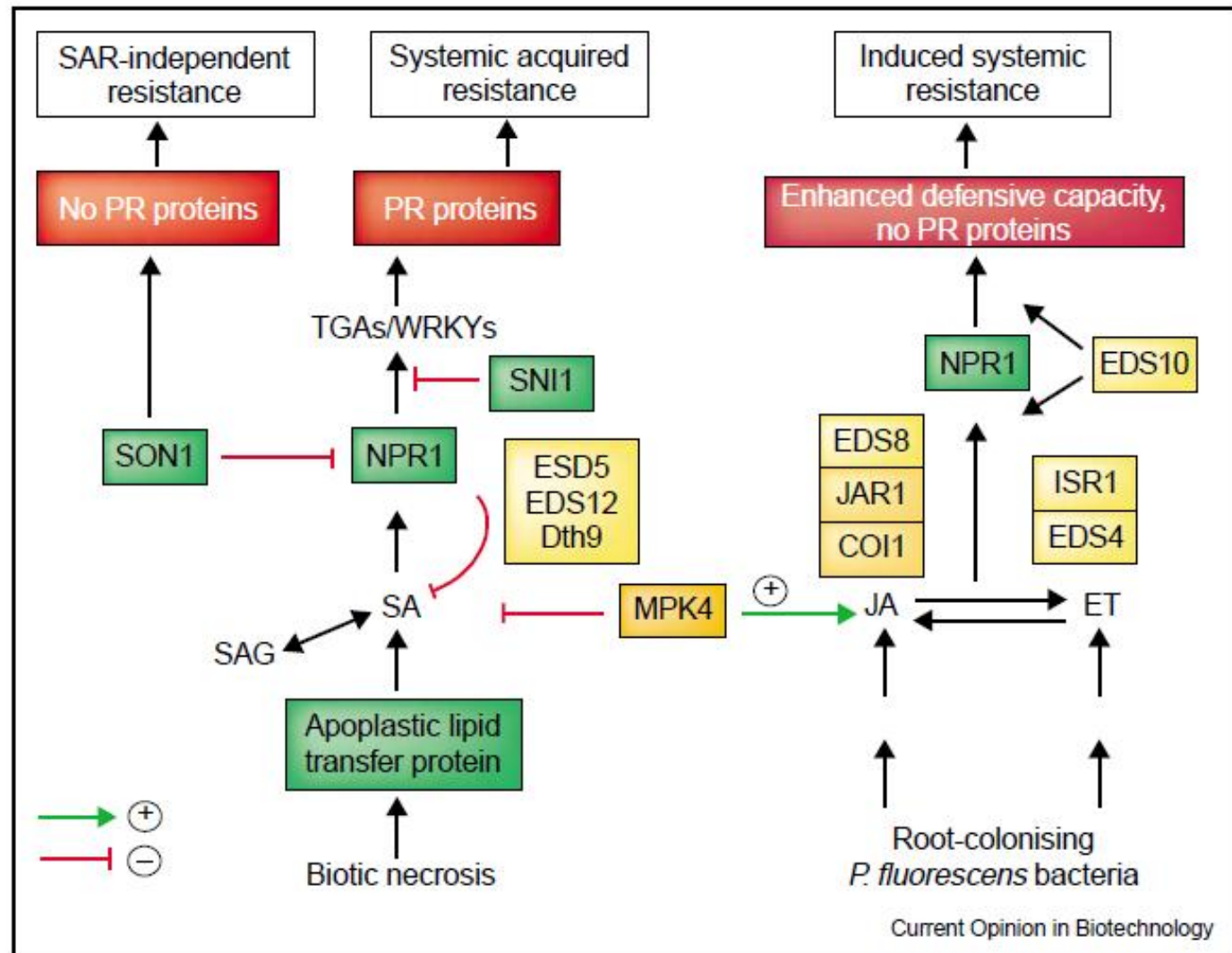


Plant Responses to Pathogen Invasions

- **Systemic acquired resistance (SAR)**
 - Is a set of generalized defense responses in organs distant from the original site of infection
 - Is triggered by the signal molecule salicylic acid



Systemic defence signalling networks



“Guarding” as one of plant response to pathogen

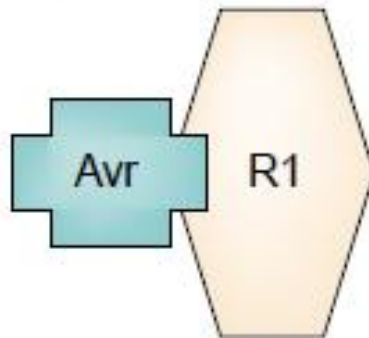
(a) Compatible interaction



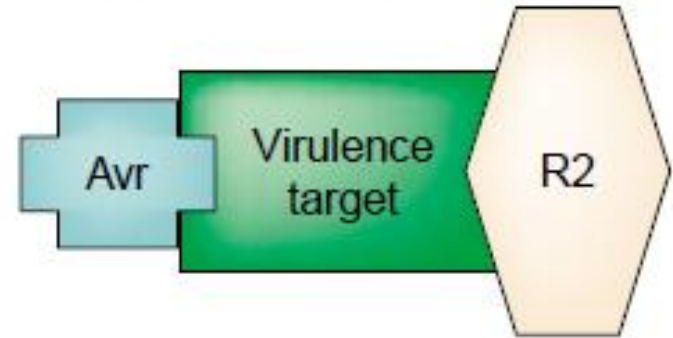
Susceptible response
to favour pathogen
growth and development

(b) Incompatible interaction

1. Resistance by direct interaction



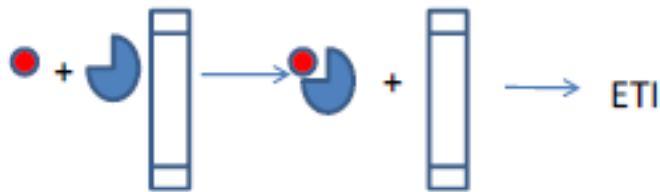
2. Resistance by guarding



Guard and Decoy Model

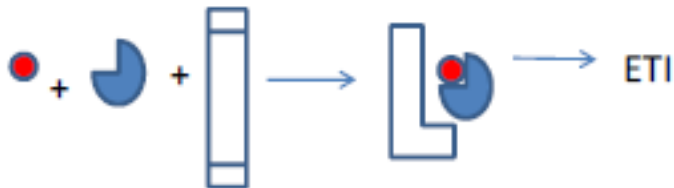
THE GUARD MODEL in the presence of R proteins

1. scenario



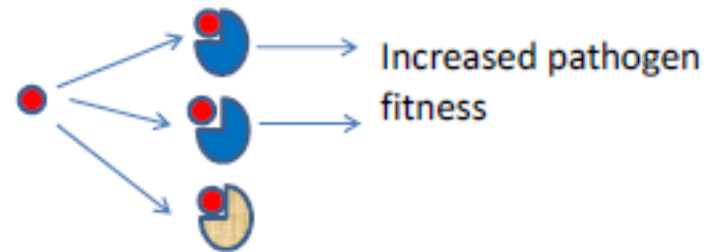
Binding of Avr molecules to target cause dissociation and activation of NBS-LRR protein

2. scenario



NBS-LRR protein is activated when binds with AVR and target molecules

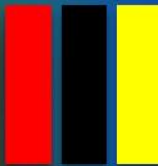
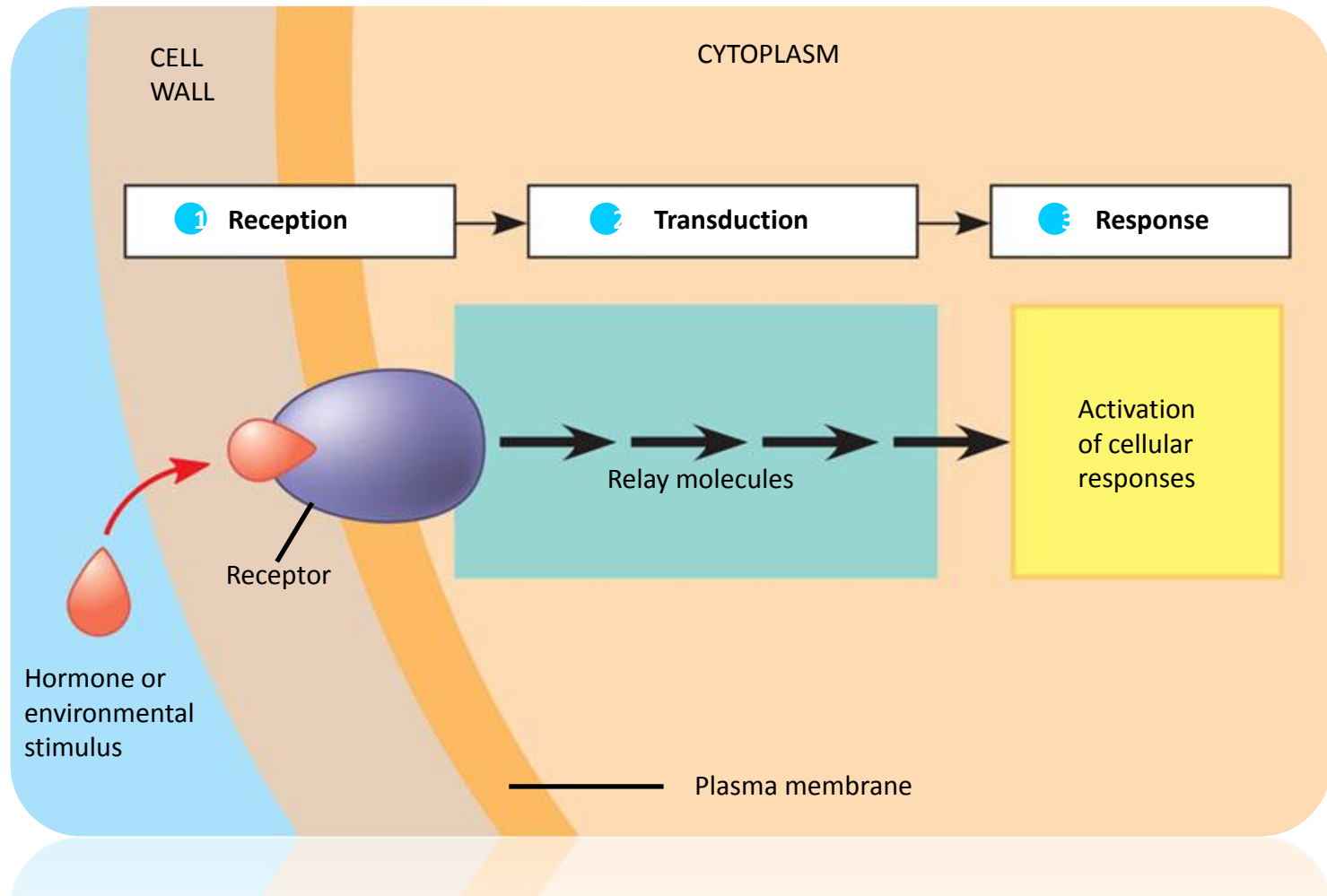
THE DECOY MODEL in the absence of R proteins



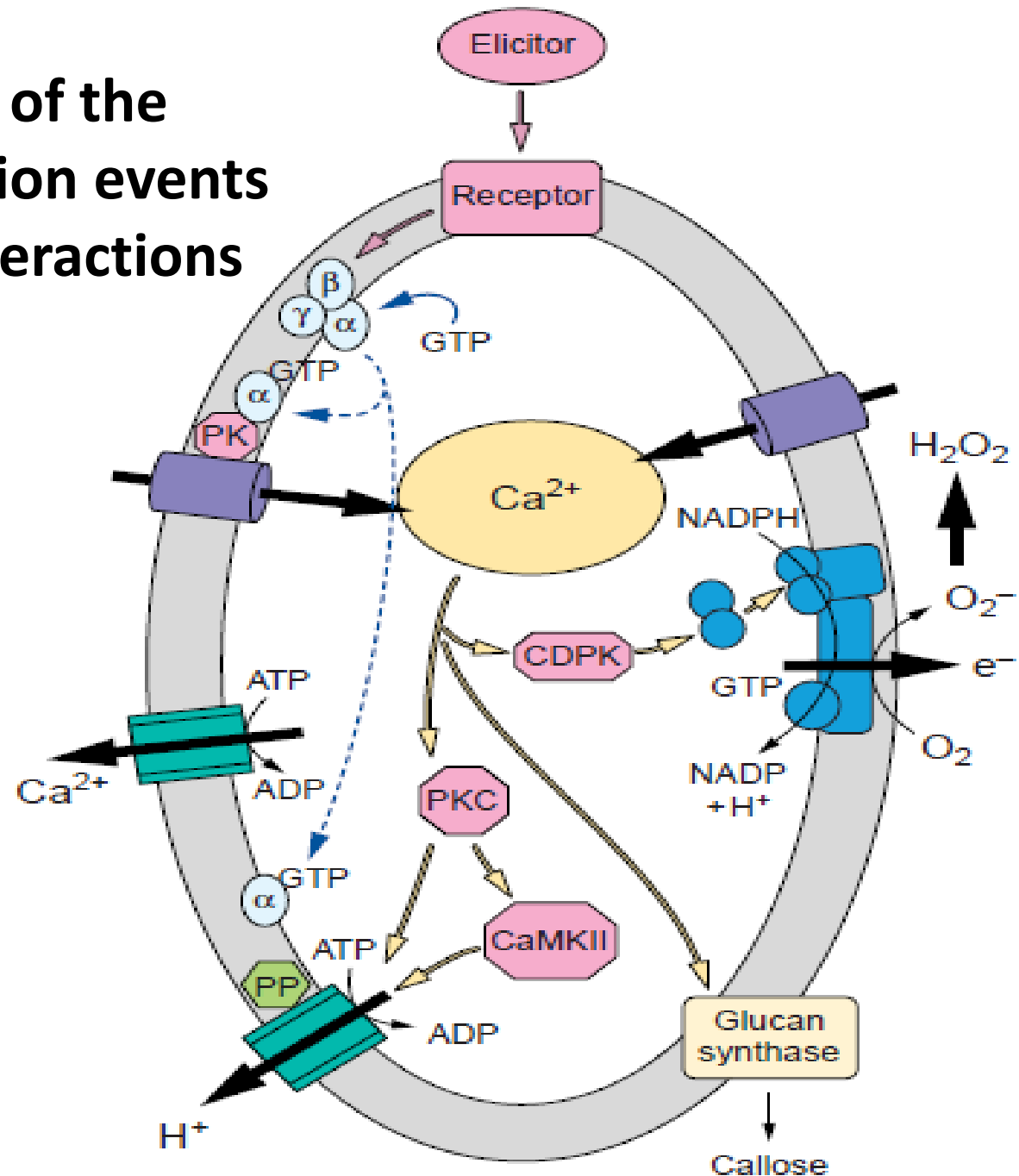
Legend:

- Effector
- Effector target – the guardee
- R protein (NBS-LRR) – the guard
- Decoy
- ETI Effector triggered immunity

- **General Plant's response to environmental stimulus**



A hypothetical model of the early signal transduction events in plant-pathogen interactions



Blumwald, et al., (1998)

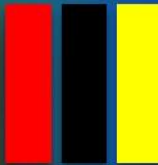
Essential pathogen components detectable by plants

Essential pathogen components detectable by plants*.				
Organism and component type	Protein or molecule	Example of signal type	Species	Potential plant receptor/interactor
Bacteria				
Structural components	Subunit of flagellum	Flagellin	All pathogenic bacteria	PM-bound FLS2
	Subunit of pilus	HrpA HrpY	<i>Pseudomonas syringae</i> <i>Ralstonia solanacearum</i>	?
	Subunit of T-pilus	VirB1, VirB2, VirB5, HcrC, HrpT (outer membrane proteins)	<i>Agrobacterium</i> spp.	?
Quorum sensing	Subunit of type III secretion apparatus		All pathogenic bacteria	?
	Secreted autoinducer	N-Acyl homoserine lactone	All Gram(−) pathogenic bacteria, <i>Erwinia</i> spp.,	?
	Secreted modified oligopeptide	3-Hydroxyl-palmitic acid-methylester	<i>Ralstonia</i> spp.	
Non-host specific toxins	Exopolysaccharides	Multiple related types	<i>R. solanacearum</i>	
	Lipopolysaccharides	Multiple related types		
	Non-proteinaceous	Coronatine	<i>P. syringae</i>	
Hrp and Hrc proteins	Delivered by type III secretion	Tabtoxin	<i>P. syringae</i> pv. <i>tabaci</i>	Glutamine synthase
		Phaseolotoxin	<i>P. syringae</i> pv. <i>Phaseolicola</i>	OCTase
		AvrBs2	<i>P. syringae</i> pv. <i>vesicatoria</i> <i>P. syringae</i>	?
Hormones	Regulator of <i>Hrc/H</i> gene expression	HrpD, HrpO, HrpP AvrRXv (YopJ family) PrhA (OM siderophore receptor)	<i>R. solanacearum</i>	MAPK kinases Siderophores/cell wall factor
	Auxin	Indole-3-acetic acid	Gall forming <i>Pseudomonas</i> sp. <i>Agrobacterium</i> sp.	IAA receptor
		Indole-3-acetic acid		
Fungi and Oomycetes				
Structural components	Cell-wall chitin	Nascent synthesis at hyphal tip	All fungal species	Soluble chitinases extracellular
	Appressorium	Cell-wall melanin MPG1 hydrophobin	Many species <i>Magnaporthe grisea</i>	? ?
	Cell wall glucans (β1–3, β1–4 type)	Nascent synthesis at hyphal tip	All <i>Oomycete</i> species	Soluble glucanases
Degradation of plant polymers	Cell walls	pelA and pel D, pectate lyase	<i>Fusarium solani</i> f. sp. <i>pisi</i>	
Overcoming plant defences	Cuticle	AcpG1 endopolygalacturonase	<i>Alternaria citri</i>	PGIPs
	Tomatinase	Cut A cutinase	<i>Fusarium solani</i> f. sp. <i>pisi</i> <i>Septoria lycopersici</i>	?
	Membrane transporter	ABC1, secretion of plant xenobiotics	<i>M. grisea</i>	?
Nutrition	Oxalic acid	Oxalic acid or low pH	<i>Sclerotinia sclerotiorum</i>	Apoplastic oxalate oxidase
	Amino acid transporters	Influx of essential amino acids	Many fungal species	Amino acid sensor
	Proteinaceous, host-specific	ToxA for HC toxin	<i>Cochliobolus carbonum</i>	Membrane channels?
Secreted toxins	Non-proteinaceous	Ptr toxin	<i>Pyrenophora tritici-repentis</i>	?
		AAL toxin	<i>Alternaria alternata</i> f.sp. <i>lycopersici</i> <i>Gibberella zeae</i> <i>Cladosporium fulvum</i>	TM helix-LAG1 motif
			<i>Phytophthora infestans</i> <i>Phytophthora sojae</i> <i>C. gloeosporioides</i> <i>Magnaporthe grisea</i>	Peptidyl transferase Cf-ECP2
Secreted peptides		DON		?
		ECP2		PM receptor
		INF1		
Hormones	Gibberellins	Pep13		
		cgDN3		
		Avr-Pita (zinc protease) (essential?)		Pi-ta protein
Hormones		PLW2	<i>Magnaporthe grisea</i>	PM-receptor?
		Elicitins (essential?)	Many <i>Phytophthora</i> species	PM receptor for endocytosis
		GA3	<i>Gibberella fujikuroi</i>	GA receptor

Molecular aspects of Plant-Pathogen Interaction

- Two Central Molecules in Plant-Microbe Interaction in Successful Disease Initiation are

R-Protein and AVR-Protein



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Role of “R-protein” in Plant-Pathogen Interaction

“R protein” plays a central role in plant defence responses

- Programmed cell death (apoptosis) during hypersensitive reaction (HR).
- It plays either directly involved in the recognition of pathogen effectors or act as a guardian for the modification of plant proteins.



Evolution of “R-Gene”

R gene Features

- ***R* genes encode at least five diverse classes of proteins (R proteins).**
- **The largest class of R proteins contain a nucleotide binding site and leucine-rich repeat domains (NBS-LRR proteins).**
- **NBS-LRR proteins may recognize the presence of the pathogen directly or indirectly.**

Classes of *R* genes

Class	Gene	Plant	Pathogen	Infection type/ organ attacked	Predicted Features of R protein	Reference
1.	<i>Hm1</i>	Maize	<i>Helminthosporium maydis</i> (race 1)	Fungal necrotroph / leaf	Detoxifying enzyme HC-toxin reductase	[35]
2.	<i>Pto</i>	Tomato	<i>Pseudomonas syringae</i> p.v. <i>tomato</i> (<i>avrPto</i>)	Extracellular bacteria / leaf	Intracellular serine/ threonine protein kinase	[59]
3a	<i>RPS2</i>	Arabidopsis	<i>Pseudomonas syringae</i> p.v. <i>tomato</i> (<i>avrRpt2</i>)	Extracellular bacteria / leaf	L. Zip / NBS / LRR	[5] [65]
	<i>RPM1</i>	Arabidopsis	<i>Pseudomonas syringae</i> p.v. <i>maculicola</i> (<i>avrRpm1/avrB</i>)	Extracellular bacteria / leaf	Intracellular protein with amino terminal leucine zipper domain, and nucleotide binding site (NBS) and leucine rich repeat (LRR) domains	[23]
	<i>l₂</i>	Tomato	<i>Fusarium oxysporium</i> f.sp. <i>lycopersicon</i>	Necrotrophic fungus/root and vascular tissue		[A]
	[B]					
3b	<i>N</i>	Tobacco	Mosaic virus	Intracellular virus / leaf and phloem	Toll / NBS / LRR	[105]
	<i>L6</i> <i>M</i>	Flax	<i>Melampsora lini</i> (<i>AL6, AM</i>)	Biotrophic fungal rust with haustoria / leaf	Intracellular protein with amino terminal domain homology with <i>Drosophila</i> Toll protein, and NBS and LRR domains	[53] [C]
	<i>RPP5</i>	Arabidopsis	<i>Peronospora parasitica</i>	Biotrophic downy mildew fungus with haustoria / leaf		[D]
4	<i>Cf-9</i> , <i>Cf-2</i> , <i>Cf-4</i> <i>Cf-5</i>	Tomato	<i>Cladosporium fulvum</i> (<i>Avr9</i> , <i>Avr2</i> , <i>Avr4</i> <i>Avr5</i>)	Biotrophic extracellular fungus without haustoria / leaf	Extracellular LRR protein with single membrane spanning region and short cytoplasmic carboxyl terminus	[38] [14] [41] [E]
5.	<i>Xa-21</i>	Rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (all races)	Extracellular bacteria / leaf	Extracellular LRR protein with single membrane spanning region and cytoplasmic kinase domain	[86]

Hammond-Kosack and Jones, 1996

[A] G. Simons and R. Fluhr, pers. comm.; [B] a very tightly linked marker to the wheat *Cre3* gene that confers resistance to the root invading cereal cyst nematode *Heterodera avenae* is highly homologous to this *R* gene class, E. Lagudah and S. Anderson, pers. comm.; [C] P. Anderson, G. Lawrence and J. Ellis, pers. comm.; [D] J. Parker, M. Coleman, V. Szabo, M. Daniels and J. Jones, unpublished; [E] M. Dixon, K. Hatzixanthos and J. Jones, unpublished.

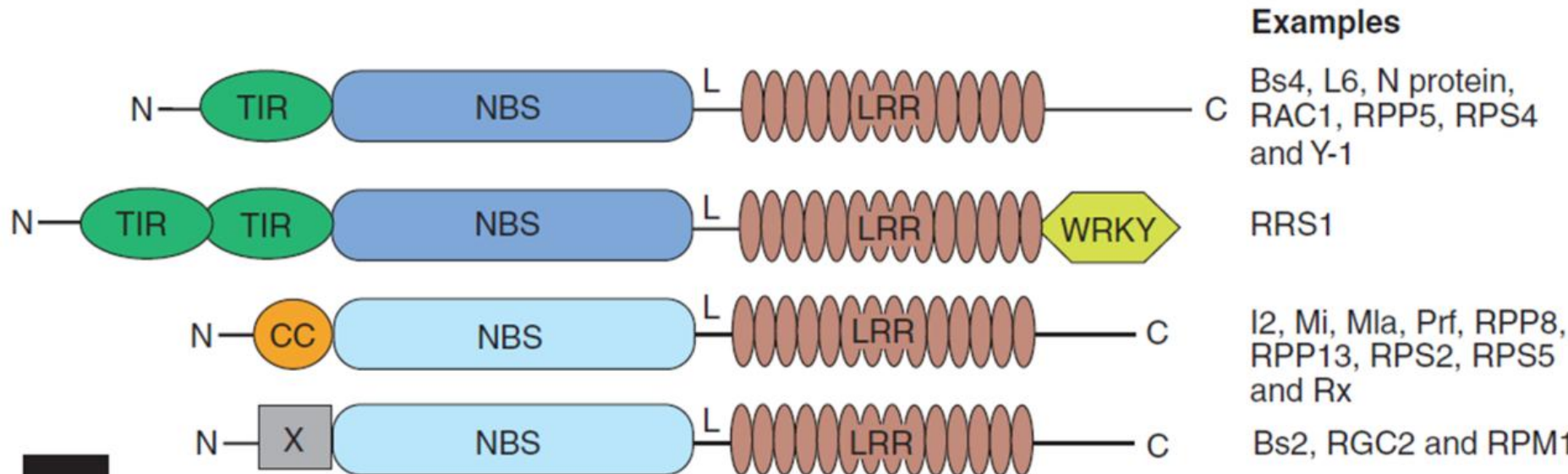
The major classes of cloned plant disease resistance genes*.

Class	Gene	Plant	Pathogen	Infection type/organ attacked	Predicted features of R protein	Race-specific	Year isolated	References
1	<i>Hm1</i>	Maize	<i>Helminthosporium maydis</i> (race 1)	Fungal necrotroph/leaf	Detoxifying enzyme HC toxin reductase	Yes	1992	†
2	<i>Asc-1</i>	Tomato	<i>Alternaria alternata</i> f.sp. <i>lycopersici</i> (AAL toxin)	Fungal necrotroph/leaf	TM helix-LAG1 motif	No	2000	[66]
3A	<i>Pto</i>	Tomato	<i>Pseudomonas syringae</i> p.v. tomato (<i>avrPto</i>)	Extracellular bacteria/leaf	Intracellular serine/threonine protein kinase	Yes	1993	†
3B	<i>PSB1</i>	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> p.v. <i>phaseolicola</i> (<i>avrPphB</i>)	Extracellular bacteria/leaf	Different subfamily	Yes	2001	[27]
4A	<i>RPS2</i>	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> p.v. <i>maculicola</i> (<i>avrRpt2</i>)	Extracellular bacteria/leaf	CC-NB-LRR Intracellular protein	Yes	1994	†
	<i>Mla1/Mla6</i>	Barley	<i>Blumeria graminis</i> f.sp. <i>hordei</i> (resp. race 1, race 6)	Biotrophic intracellular fungus with haustoria/leaf		Yes	2001	[58]
	<i>R1</i>	Potato	<i>Phytophthora infestans</i> (race 1)	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf and tuber		Yes	2001 2002	[67] [68]
	<i>RPP8</i>	<i>Arabidopsis</i>	<i>Peronospora parasitica</i>	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf		Yes	1998	†
4B	<i>N</i>	Tobacco	Mosaic virus	Intracellular virus/leaf and phloem	TIR-NB-LRR Intracellular protein	Yes	1994	†
	<i>RPP4</i>	<i>Arabidopsis</i>	<i>Peronospora parasitica</i>	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf		Yes	2002	[69]
4C	<i>Bs2</i>	Pepper	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (<i>avrBs2</i>)	Extracellular bacteria/leaf	NB-LRR Intracellular protein	Yes	1999	†
	<i>Dm3</i>	Lettuce	<i>Bremia lactuca</i>	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf		Yes	2002	[70]
4D	<i>RRS-1</i>	<i>Arabidopsis</i>	<i>Ralstonia solanacearum</i> (race 1)	Extracellular bacteria/leaf	TIR-NB-LRR-NLS-WRKY	Yes	2002	[5]
4E	<i>Pi-ta</i>	Rice	<i>Magnaporthe grisea</i> (<i>avrPita</i>)	Hemibiotrophic intracellular fungus without haustoria/leaf	NB-LRD	Yes	2000	†
5A	<i>Cf-9</i>	Tomato	<i>Cladosporium fulvum</i> (<i>Avr9</i>)	Biotrophic extracellular fungus without haustoria/leaf	eLRR-TM-sCT Extracellular protein with single membrane-spanning region and short cytoplasmic C terminus	Yes	1994	†
5B	<i>Ve1</i> <i>Ve2</i>	Tomato	<i>Verticillium albo-atrum</i>	Extracellular vascular wilt fungus without haustoria/root and stem	CC-eLRR-TM-ECS eLRR-TM-PEST-ECS	Yes	2001	[6]
6	<i>Xa-21</i>	Rice	<i>Xanthomonas oryzae</i> p.v. <i>oryzae</i> (all races)	Extracellular bacteria/leaf	eLRR-TM-kinase	Yes	1995	†
	<i>FLS2</i>	<i>Arabidopsis</i>	Multiple bacteria (flagellin)	Extracellular bacteria/leaf		No	2000	†
7	<i>RPW8.1</i> <i>RPW8.2</i>	<i>Arabidopsis</i>	Multiple powdery mildew species	Biotrophic intracellular fungus with haustoria/leaf	Small, probable membrane protein with CC domain	No	2001	[18]
8	<i>Rpg1</i>	Barley	<i>Puccinia graminis</i> f.sp. <i>tritici</i>	Biotrophic intracellular fungus with haustoria/stem	Receptor kinase-like protein with 2 tandem kinase domains	No	2002	[19]

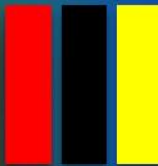
Classes of *R* genes

Hammond-Kosacky and Parkerz, (2003)

Sequence Homology among R-Proteins



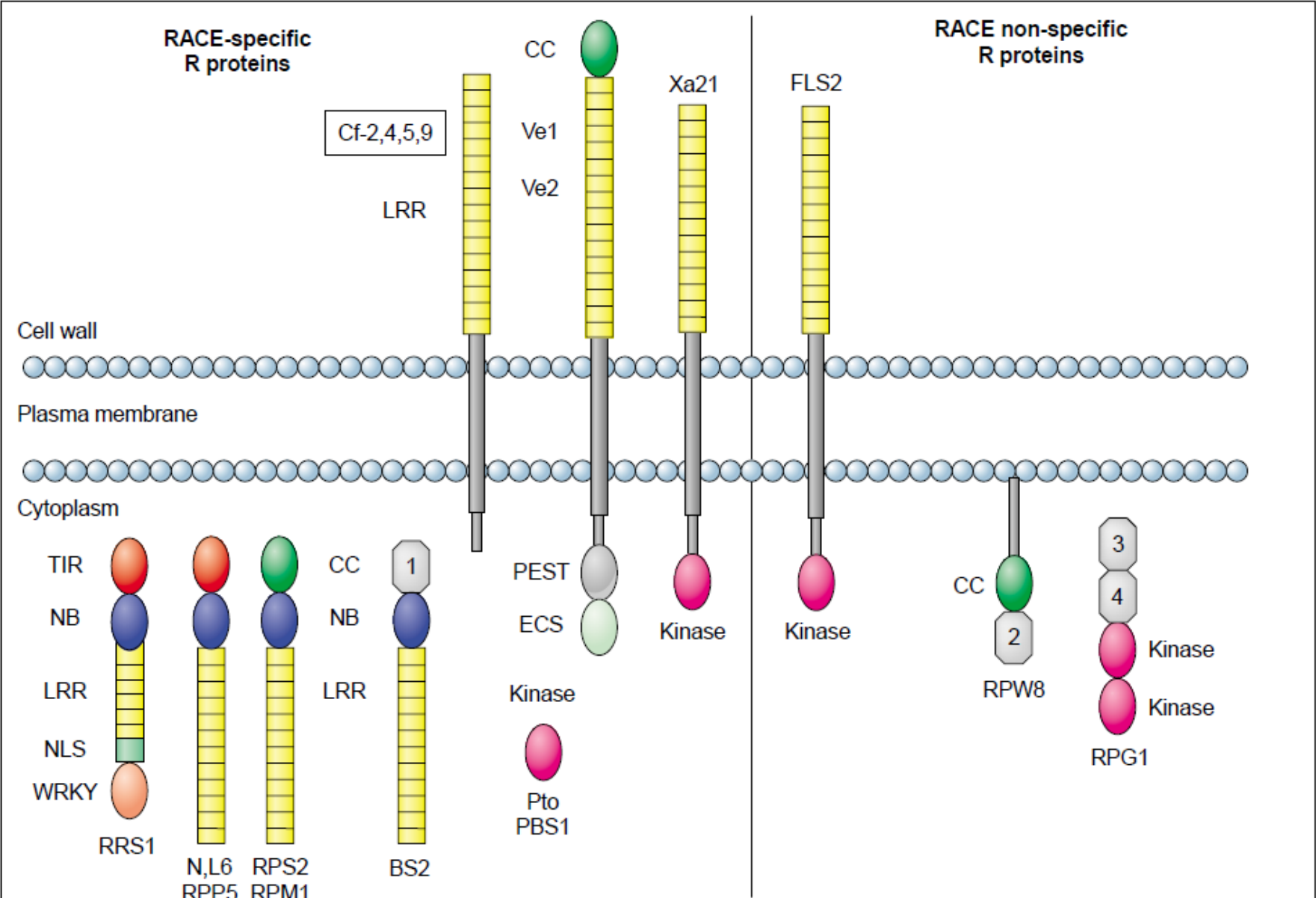
GB.c



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R proteins race-specific mostly located in both cell wall and cytoplasm.



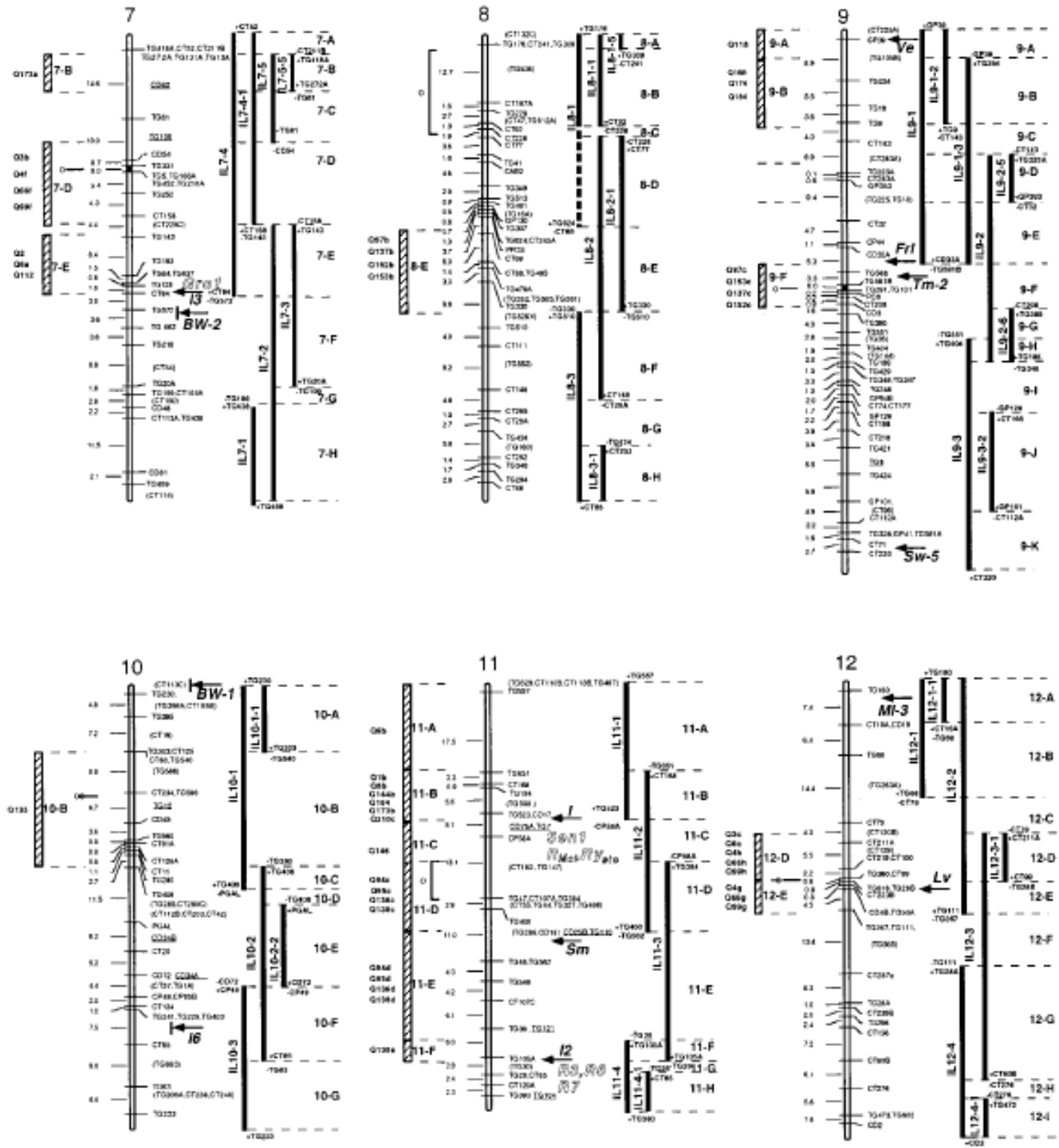
Numbers of Arabidopsis Genes That Encode Domains Similar to Plant R Proteins

Predicted Protein Domains ^a	Letter Code	Previous No. ^b	Full Manual ^c
CC-NBS-LRR	CNL	48	51
NBS _{CC} -LRR	NL	2	4
TIR-NBS-LRR	TNL	82	83
NBS _{TIR} -LRR	NL	2	2
TIR-NBS-LRR-X	TNLX	5	5
TIR-NBS-TIR-NBS-LRR	TNTNL	2	2
TIR-TIR-NBS-LRR	TTNL	0	2
Total with LRRs		141	149
TIR-NBS	TN	14	21
TIR-X	TX	23	30
X-TIR-NBS-X	XTNX	0	2
CC-NBS	CN	4	4
CC-NBS-X	CNX	1	1
CC (related to CNL)	C	0	1
NBS _{CC}	N	1	1
Total without LRRs		43	58

Table updated from Meyers et al. (2002).

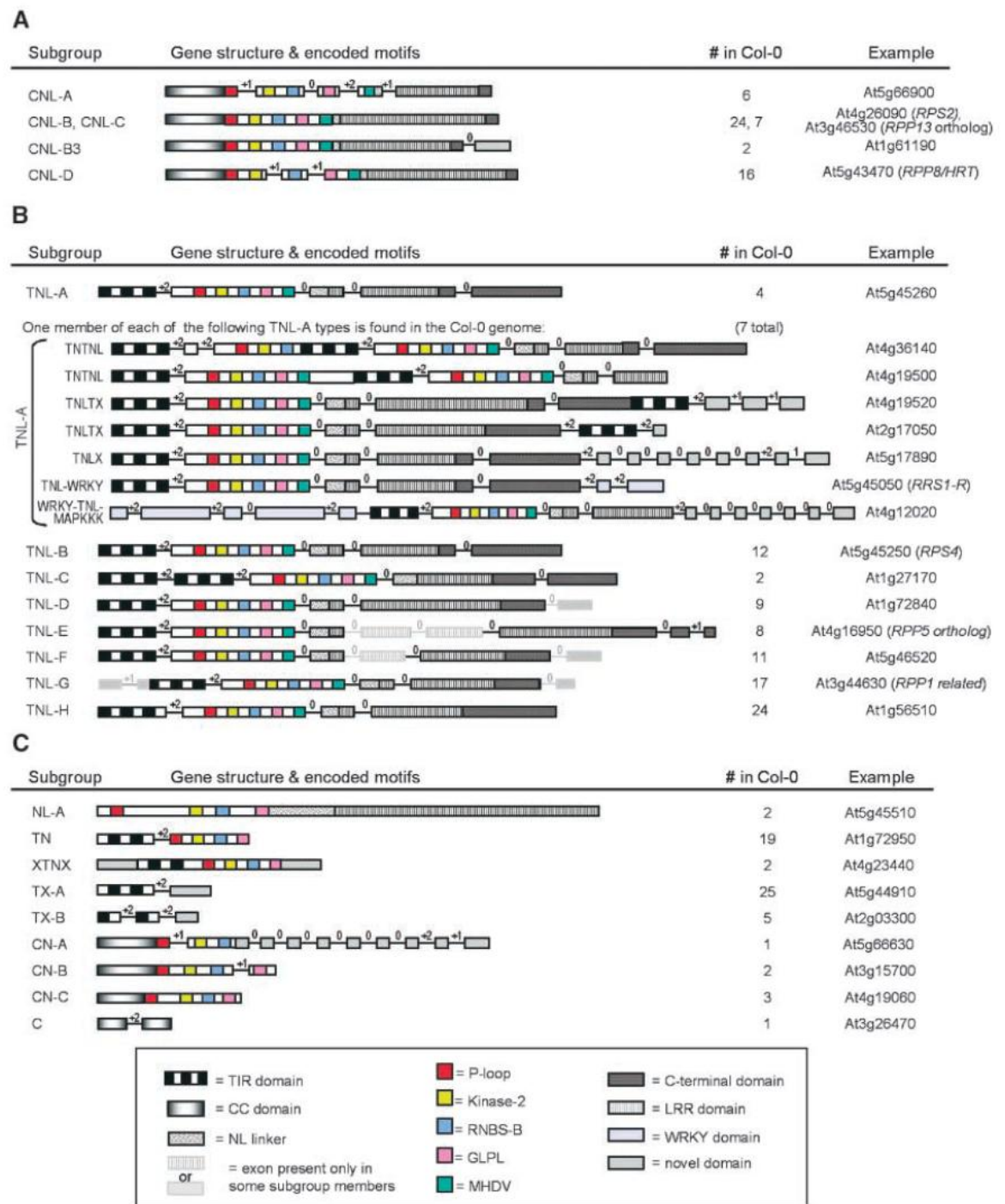
~ 200 R-gene

Comparison of R gene position between tomato and potato



Intron/Exon Configurations and Protein Motifs of NBS-LRR-Encoding Genes in Arabidopsis.

Meyers, et al., 2003



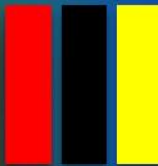
- **Evolution of “R-Gene”**

Evolution of “R-Gene”

- Natural selection drives the pathogen to avoid resistance either by evolving the existent effector gene or by acquiring additional effectors.
- The new effector put the selection pressure on host plant to evolve new *R gene alleles*. *The co-evolution of plant defence and pathogen attacks* are the result of constant selection pressure that occur across spatial and temporal scales (Ravensdale et al., 2011).
- In PTI immunity system there is an evidence of molecular evolutionary conservation in structure and functions across kingdoms borders (Medzhitov & Janeway, 1997; Imler & Hoffmann; 2001),



Leaf abnormalities caused by PepYLCV



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Agressivity Testing

8 days after inoculation



TD -21-Isolate
(non aggressive)

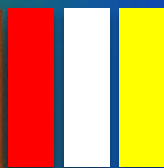


TD-21

PSS-14-Isolate
(agressive)



PSS141



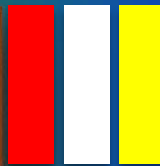
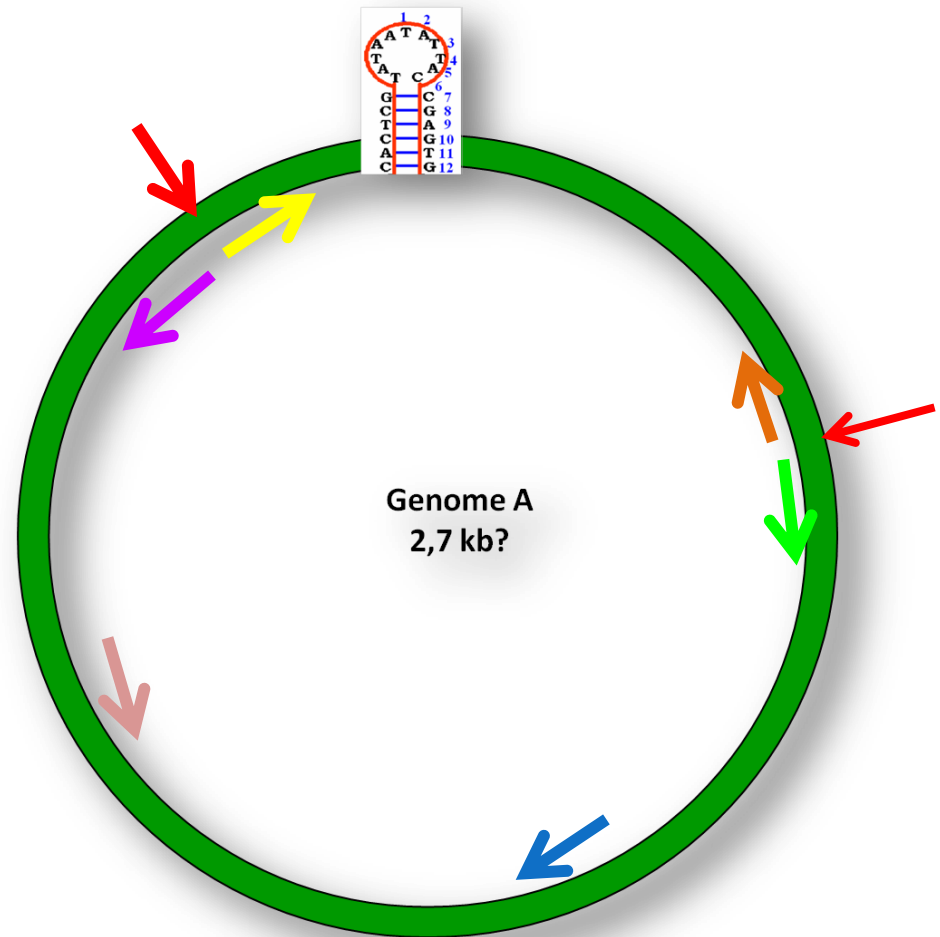
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Primer walking for whole genome sequencing.

Primer:

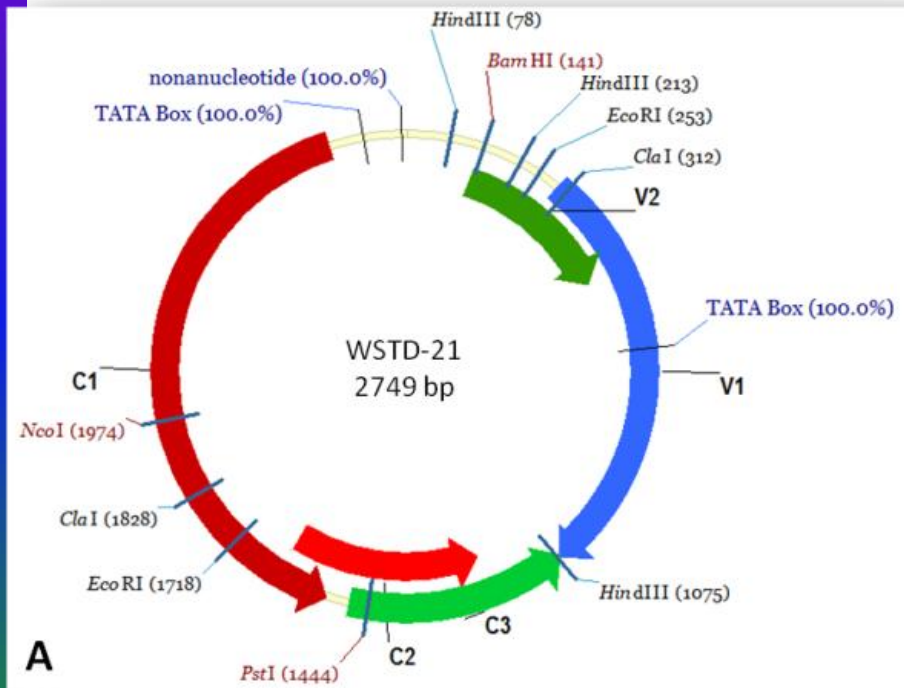
PAL1v1978 / PAR1c715



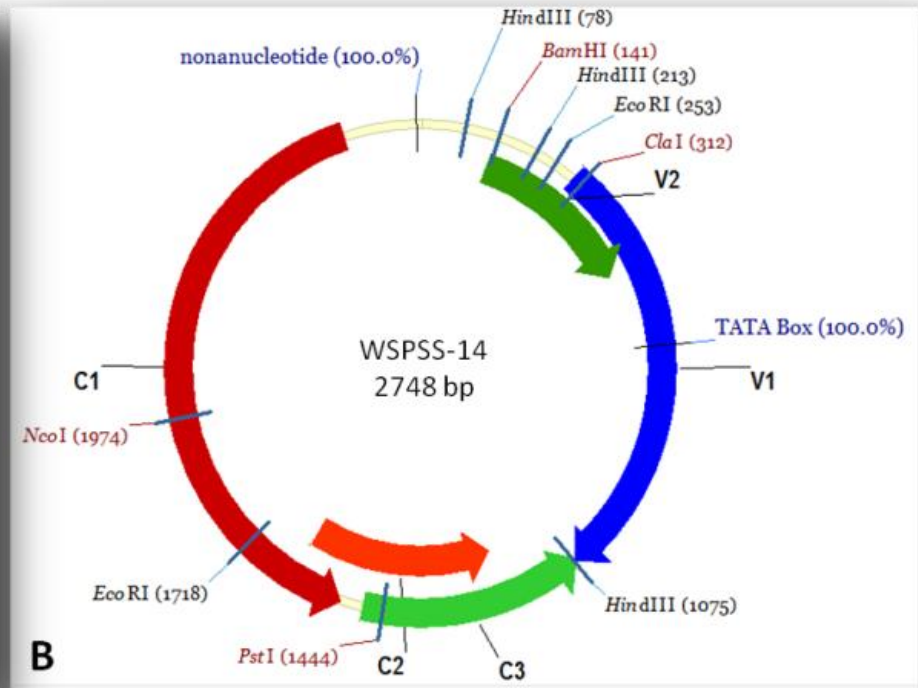
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Genome A of TD-21 and PSS-14 isolate



TD-21 ; 2749 bs



PSS-14; 2748 bs



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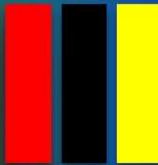
Genome A size of TD-21 and PSS-14 isolates

TD21 : 2749 nts

PSS14: 2748 nts

Gene/ORF	TD21	PSS14
V1 (sense)	135-488	135-488
V2 (sense)	295-1071	295-1071
C1 (non sense)	1520-2608	1520-2608
C2 (non sense)	1213-1630	1213-1630
C3 (non sense)	1068-1478	1068-1478

1 InDel event in Common region (CR)



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**16 points;
96% similarity**

5 points different
96% similarity



Coat protein (CP) /V2 gene is conserved

```
PSS14-V1      MPKRSIDTVSSLPMSITRRRLNYASQYSLPAAAPTAFGMSYKRRRAWVNRPMNRKPRFYRG 60
TD21-V1      MPKRSIDTVSSLPMSITRRRLNYASQYSLPAAAPTAFGMSYKRRRAWVNRPMNRKPRFYRG 60
*****

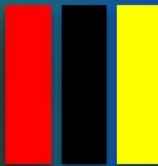
PSS14-V1      RRTSDVPRGCEGPKVQSFEQRHDIHTGKVLCSVDVTRGGGITHRVGKRFCVKSVYIIG 120
TD21-V1      RRTSDVPRGCEGPKVQSFEQRHDIHTGKVLCSVDVTRGGGITHRVGKRFCVKSVYIIG 120
**:*:*****:*:*****:*:*****:*:*****

PSS14-V1      KVWMDENIKSKNHTNNVMFWLVDRRPVITPYGFGELFNMYDNEPSTATIKNDLRDRVQV 180
TD21-V1      KVWMDENIKSKNHTNNVMFWLVDRRPVITPYGFGELFNMYDNEPSTATIKNDLRDRVQV 180
*****

PSS14-V1      LHRFSATVTGGQYASKEQAIVKRFFRVNNYVVYNHQEAAKYENHTENALLLYMACTHASN 240
TD21-V1      LHRFSATVTGGQYASKEQAIVKRFFRVNNYVVYNHQEAAKYENHTENALLLYMACTHASN 240
*****

PSS14-V1      PVYATLKIRIYFYDNVTN 258
TD21-V1      PVYATLKIRIYFYDNVTN 258
*****
```

4 points; Similarity: 98,5%



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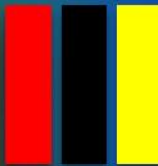


Rep (C1) Gene Showed High Level of Polymorphisms

PSS14-V1	MERSYSFQVKAKNIFLTYFKCPIPKKEEALILLNIIQCESDKLFIIRVAQEMHSDGSLHLHV	60
TD21-V1	MEPPRRFKLQSKNYFLTYFHCSLTKEEALQLKSTINIFVNKLFVKICRELHEDGSEHLHV	60
	** . * : : : : * * : : : : * : : : : * * : : : * : : : : * * : : : * * : : : *	
PSS14-V1	LIQFKGAQFRNNHFDITHTNTSTQFHPNFQGAKSSSDVKSYIEKDGDDVDWGFQIDG	120
TD21-V1	LIQFEGKYVCTNNRFFDIVSETRSAHFHPNIQGAKSSSDVKAMMDKDGDTTEWGEFQIDG	120
	***** * : : : : * * : : : * : : : : * : : : : * : : : : * : : : : * : : : *	
PSS14-V1	RSARGGQQTANDAAPEALNAGSKQAMATIREKLPHETIFQFHNNANLDRIFAPELEVF	180
TD21-V1	RSARGGPHAVNDVYPQALNCGSKSDAIRLILKELAPHQIVLQYHNLSVNEFKIFAKEVDTF	180
	***** : : : * * : : : * * : : : * : : : : * : : : : * : : : : * : : : *	
PSS14-V1	VCHFSSSSFDQVPEELQAWAENVRDAAARPWRPISIVIEGESRTGKTMWARSGLHNYL	240
TD21-V1	VSEYPSSSFDQVPEELRWAAENVMDAAARPWRPISIVIEGESRTGKTMWARSGLGFHNYL	240
	* : : : * : : : : * : : : : * : : : : * : : : : * : : : : * : : : : * : : : *	
PSS14-V1	CGHLDLSPKVYNDAWYNVIDDVPHYLKHFKEFMGAQRNWQSNKYGKPIQIKGGIPTI	300
TD21-V1	CGHLDLSPKVYNDAWYNVIDDVPHYLKHFKEFMGAQRNWQSNKYGKPIQIKGGIPTI	300
	***** : : : * : : : : * : : : : * : : : : * : : : : * : : : : * : : : *	
PSS14-V1	FLCNPGPSTSSYKEYLDEDKNNALKSWALKNATFVTINGPLYSSSTEDTAPNCEEENNPE	360
TD21-V1	FLCNPGPSTSSYKEYLDEDKNNALKSWALKNATFVTINGPLYSSSTEDTAPNCEEENNPE	360
	***** : : : * : : : : * : : : : * : : : : * : : : : * : : : : * : : : *	
PSS14-V1	TY	362
TD21-V1	TY	362
	**	

Dissimilarity = 23%
Similarity = 77%

Big question: does C1 gene play important role in the aggression of PepYLCV?



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Coat Protein Sequence Variation of 47 GV Isolates

Position: 1

669 bp

		120	130	140	150	160	170	180	190	200	210	220	
► Translate		GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-ARCAAGCTCTTTAATCA-ATCGAAG---AGCWTCCgacTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 2-1.ab1(47>687)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAGAGCA--TCGGAC-TTACTTCCACAATTTAGGGCCTGCGC-											
► TD 1-1.ab1(48>677)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 3-5.ab1(37>653)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► AG 3-5.ab1(61>576)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► TD 2-1.ab1(1>639)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAGAGCA--TCGGAC-TTACTTCCACAATTTAGGGCCTGCGC-											
► TD 2-4.ab1(32>638)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAGAGCATCGGAC---TTACTTCCACAATTTAGGGCCTGCGC-											
► TD 1-4.ab1(54>592)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► TD 3-5.ab1(33>642)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACGTAGTCTTTTGG-AGCAAGCTCTTTAATCA-ATTGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTCGCG-											
► SO 1-3.ab1(48>538)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► PY 1.SEQ(47>623)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACGTAGTCTTTTGG-AGCAAGCTCTTTAATCA-ATTGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 2-5.ab1(28>660)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAGAGCA--TCGGAC-TTACTTCCACAATTTAGGGCCTGCGC-											
► AG 2-1.SEQ(42>697)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAGAGCA--TCGGAC-TTACTTCCACAATTTAGGGCCTGCGC-											
► AG 2-4.ab1(61>678)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► AG 1-4.ab1(44>709)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► PSS 2-4.ab1(48>590)	●→	GAAATTAACAGTTAAATTGTGGTACTGTAGAACGTAGTCTTTTGG-AGCAAGCTCTTTAATCA-ATTGAAG---AGCATTTGACTTACTTCCACTATTTAGGGCCTGCGCA											
► AG 1-3.ab1(62>548)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 1-6.ab1(1>894)	●→	TAAATTAGAATTTAAATTATGAAATTTGAAAAATATAATCTTTTGG-GAGTTTTTCCCTTATTA-TACTAAGGGCTGCTTTT---TTGAACCTGCGTTTAGTGCCCTCTGC-											
► PSS 1-4.ab1(1>920)	●→	TAAATTAGCATTTAAATTATGAAATTTGAAAAATATAATCTTTGGG-AAGTTTTTCCCTTATTA-TTGCCATTGCTGCTGT---TTCGAACCTGCGTTCAAGGCTTCAGCA											
► PSBT 1-4.ab1(1>897)	●→	TAAATTAGAATTTAAATTATGAAATTTGAAAAATATAATCTTTTGG-GAGTTTTTCCCTTATTA-TACTAAGGGCTGCTTCT---TTGAACCTGCGTTTAGTGCCCTCTGC-											
► PY 3.ab1(1>921)	●→	TAAATTAGCATTTAAATTATGAAATTTGAAAAATATAATCTTTGGG-AAGTTTTTCCCTTATTA-TTGCCATTGCTGCTGT---TTCGAACCTGCGTTCAAGGCTTCAGCA											
► PSS 1-2.ab1(1>928)	●→	TAAATTAGCATTTAAATTATGAAATTTGAAAAATATAATCTTTGGG-AAGTTTTTCCCTTATTA-TTGCCATTGCTGCTGT---TTCGAACCTGCGTTCAAGGCTTCAGCA											
► PSBT 1-2.ab1(23>410)	●→	TAAATTAGAATTTAAATTATGAAATTTGAAAAATATAATCTTTTGG-GAGTTTTTCCCTTATTA-TACTAAGGGCTGCTTCT---TTGAACCTGCGTTTAGTGCCCTCTGC-											
► PSBT 2-2.ab1(203>56)	●→	GAAATTAGCACTTAAATTATGAAATTTGAAAAATATAATCTTTAGG-AGCTAGCTCTTTAATCA-ATTGAAG---AGCCTCGGACTTACTGCGCTGTTAAGTGCCTT-GGG											
► PSS 3-4.ab1(1>890)	●→	TAAATTAGAATTTAAATTATGAAATTTGAAAAATATAATCTTTTGG-GAGTTTTTCCCTTATTA-TACTAAGGGCTGCTTCT---TTGAACCTGCGTTTAGTGCCCTCTGC-											
► PSS 1-3.ab1(1>997)	●→	TAAATTAGCATTTAAATTATGAAATTTGAAAAATATAATCTTTGGG-AAGTTTTTCCCTTATTA-TTGCCATTGCTGCTGT---TTCGAACCTGCGTTCAAGGCTTCAGCA											
► PSS 1-5.ab1(54>492)	●→	GAAATTAGCACTTAAATTATGAAATTTGTAACAAAATCTTTAGG-AGCTAGCTCTTTAATCA-ATTGAAGAGAC-CTCCGAC-TTACTGCCGCTGTTAAGTGCCTTGGC-											
► PSBT 1-3.ab1(51>542)	●→	AAAATTAGCATTTAAATTATGAAATTTGTAACAAAATCTTTAGGAGCTAGCTCCTTAATTACATTCAGAGAC-CTCCGAC-TTACTGCCGCAGTTAAGTGCCTTGGC-											
► PSBT 2-1.ab1(51>516)	●→	TAAATTAATAATTTAAATTATGAAATTTGAAAAATATAATCTTTTGGGAGCAAGCTCTTTAATCA-ATTCAAGAGCA--TTCTTC-TTACTTCCACAATTTAGGGCCTGCGC-											
► PSBT 3-1.ab1(1>929)	●→	TAAATTAGAATTTAAATTATGAAATTTGAAAAATATAATCTTTTGG-GAGTTTTTCCCTTATTA-TACTAAGGGCTGCTTCT---TTGAACCTGCGTTTAGTGCCCTCTGC-											
► PSBT 2-3.ab1(85>621)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 1-4.ab1(91>240)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAAGACTAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► PSBT 3-4.ab1(76>648)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTTGG-AGCAAGCTCTTTAATCA-ATTGAAG---AGCGTCGGACTTACTTCCACAATTTAGGGCCTGCGCA											
► PSS 2-3.ab1(1>624)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 1-2.ab1(1>907)	●→	TAAATTAGCATTTAAATTATGAAATTTGAAAAATATAATCTTTGGG-AAGTTTTTCCCTTATTA-TTGCCATTGCTGCTGT---TTCGAACCTGCGTTCAAGGCTTCAGCA											
► TD 2-5.ab1(63>563)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATTGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► TD 3-2.ab1(87>538)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 3-3.ab1(119>634)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 3-4.ab1(33>573)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► TD 3-3.ab1(93>634)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► SO 2-2.ab1(61>495)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											
► TD 1-5.ab1(99>593)	●→	GAAATTAACACTTAAATTGTGGTACTGTAGAACATAGTCTTTCCGG-AGCAAGCTCTTTAATCA-ATCGAAG---AGCATCGGACTTACTTCCACAATTTAGGGCCTGCGC-											

● = Low land

● = Medium Land

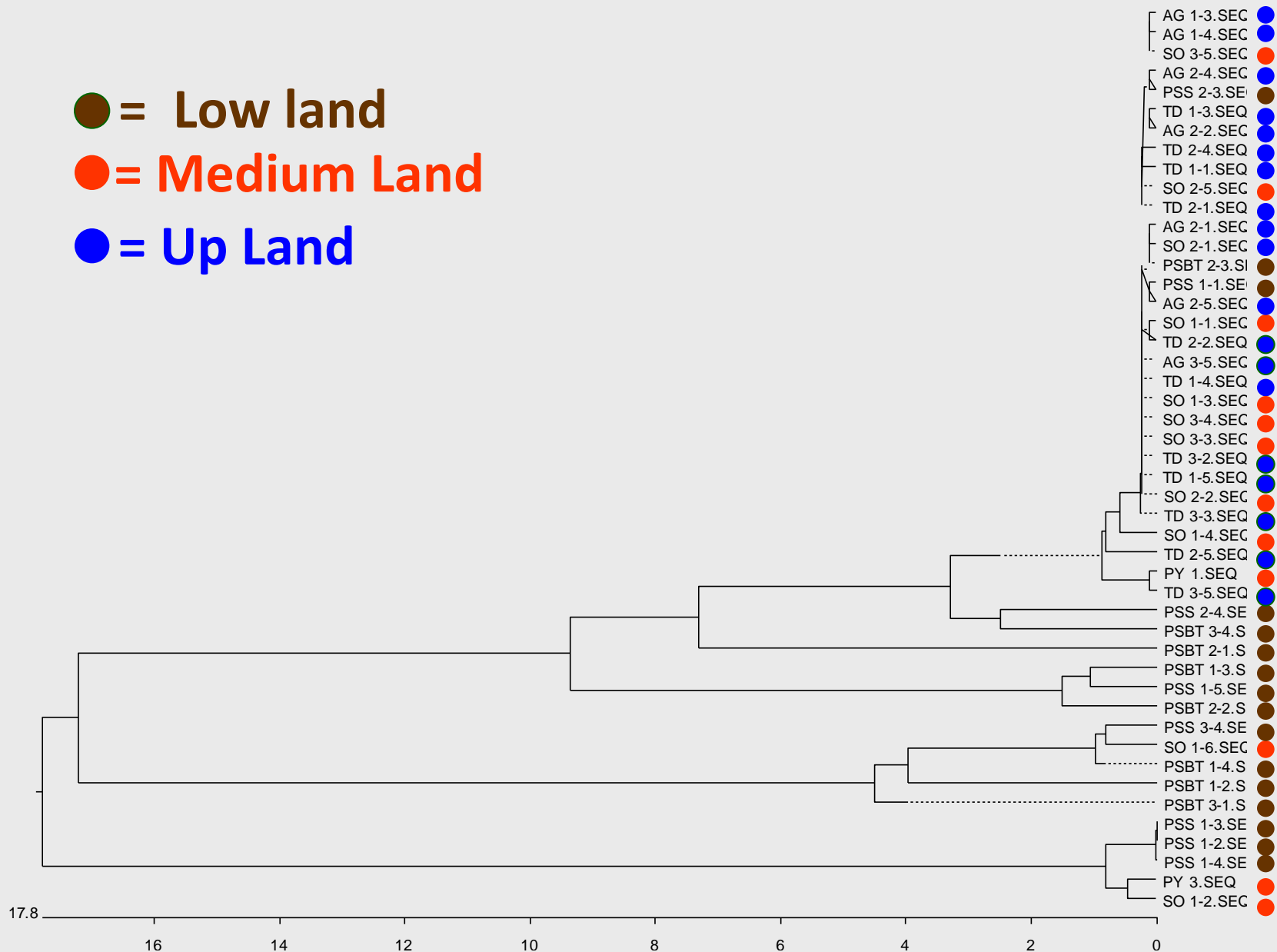
● = Up Land

Protein Gene in GV



Pylogeny of 47 GV Isolates

● = Low land
● = Medium Land
● = Up Land

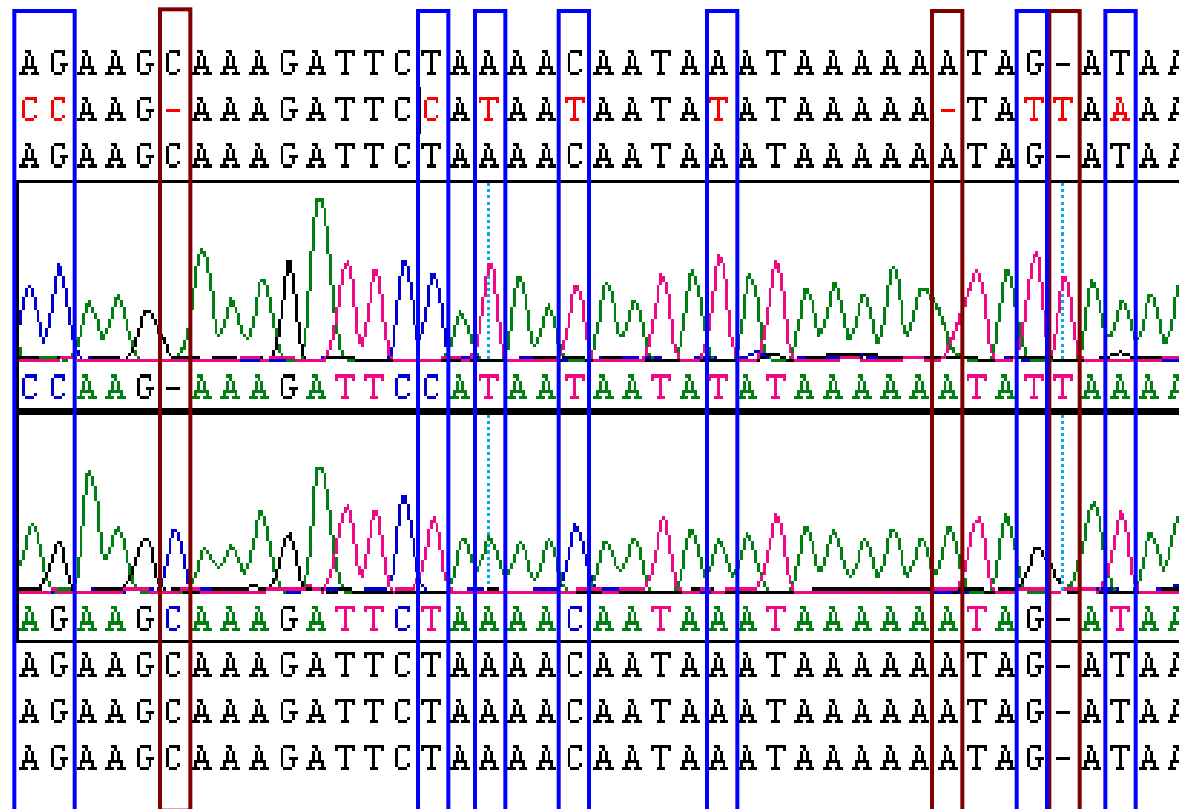


Type of sequence Variation of *COI* Gene in *B. tabacci*

► Bt-T-PDG-3-COIRV.ab1 (8>699) →
 ► Bt-50K-1b.ab1 (28>693) →
 ► Bt PSS-2b.ab1 (73>564) →
 ▼ Bt TD-3I.ab1 (12>581) →

▼ Bt PSS-2a.ab1 (9>553) →

► Bt 50K-2c.ab1 (29>563) →
 ► Bt AG-2a.ab1 (25>560) →
 ► Bt 50K-2b.ab1 (24>782) →

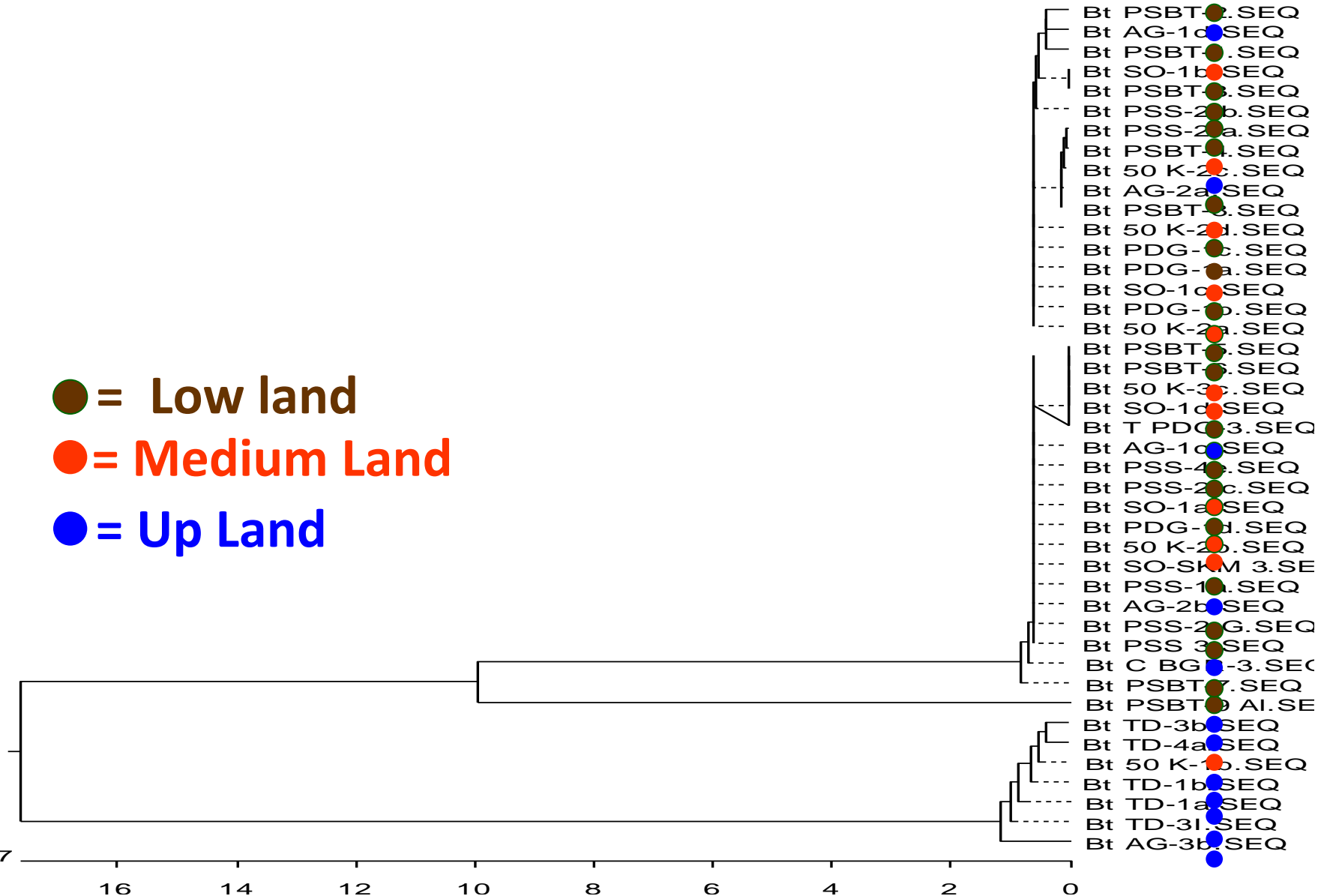


Substitusi

Insersi-delesi

Pylogeny of *B. tabacci* from 7 districts

● = Low land
● = Medium Land
● = Up Land



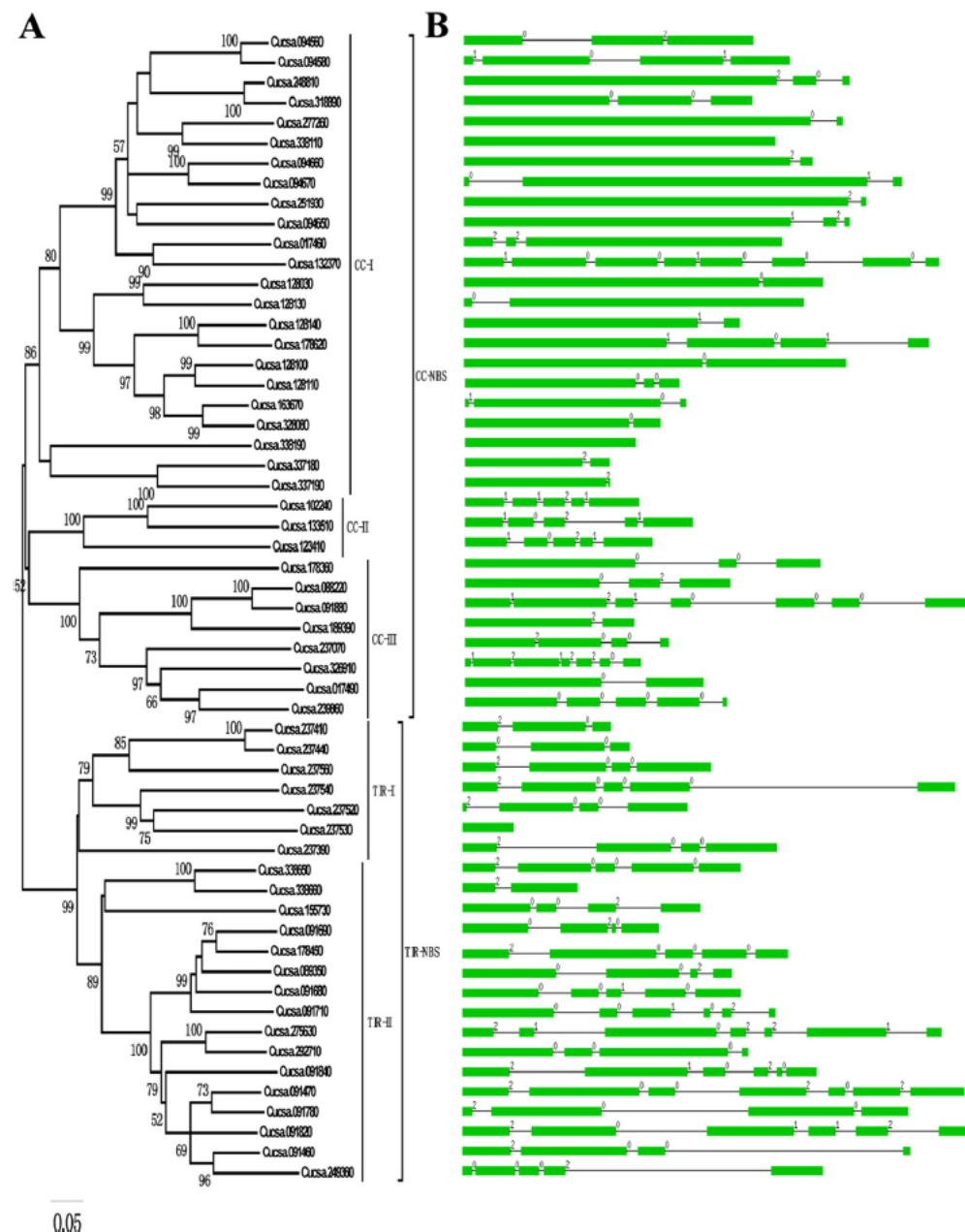


Figure 1 Phylogenetic analysis and intron/exon configurations of NBS-encoding genes in cucumbers. A phylogenetic tree of NBS-encoding genes was constructed using MEGA 5.0. Introns and exons are drawn to scale with the full encoding regions of their respective genes. Boxes indicate the exon, and lines indicate the intron. 0=intron phrase 0; 1=intron phrase 1; 2=intron phrase 2.

Distribution of Three Multigene Families That Encode NBS-LRR, Cytochrome P450, and LRR Kinase Proteins in the Arabidopsis Col-0 Genome Relative to Segmental Duplications

Class	Gene Family		
	NBS-LRR	Cytochrome P450	LRR Kinase
No. of pairs of segmental duplications	81	81	81
No. of pairs with gene(s) in either or both segments	34	47	52
No. of pairs with gene(s) in only one segment	25	19	24
No. of pairs with gene(s) in both segments	9	28	28
No. of pairs with simple duplication of a gene ^a	4	15	21
Total genes in family	149	245	206
No. (%) of genes residing in segmental duplications	124 (83%)	199 (81%)	163 (79%)
No. (%) of genes in simple segmental duplications ^a	14 (9%)	81 (33%)	66 (32%)

^a See text. Each pair of genes had to have at least 40% identity, and their element on the diagonal plot is located along the duplication diagonal (see supplemental data online).

Duplication in the NBS-encoding R genes among three plants

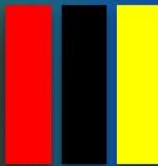
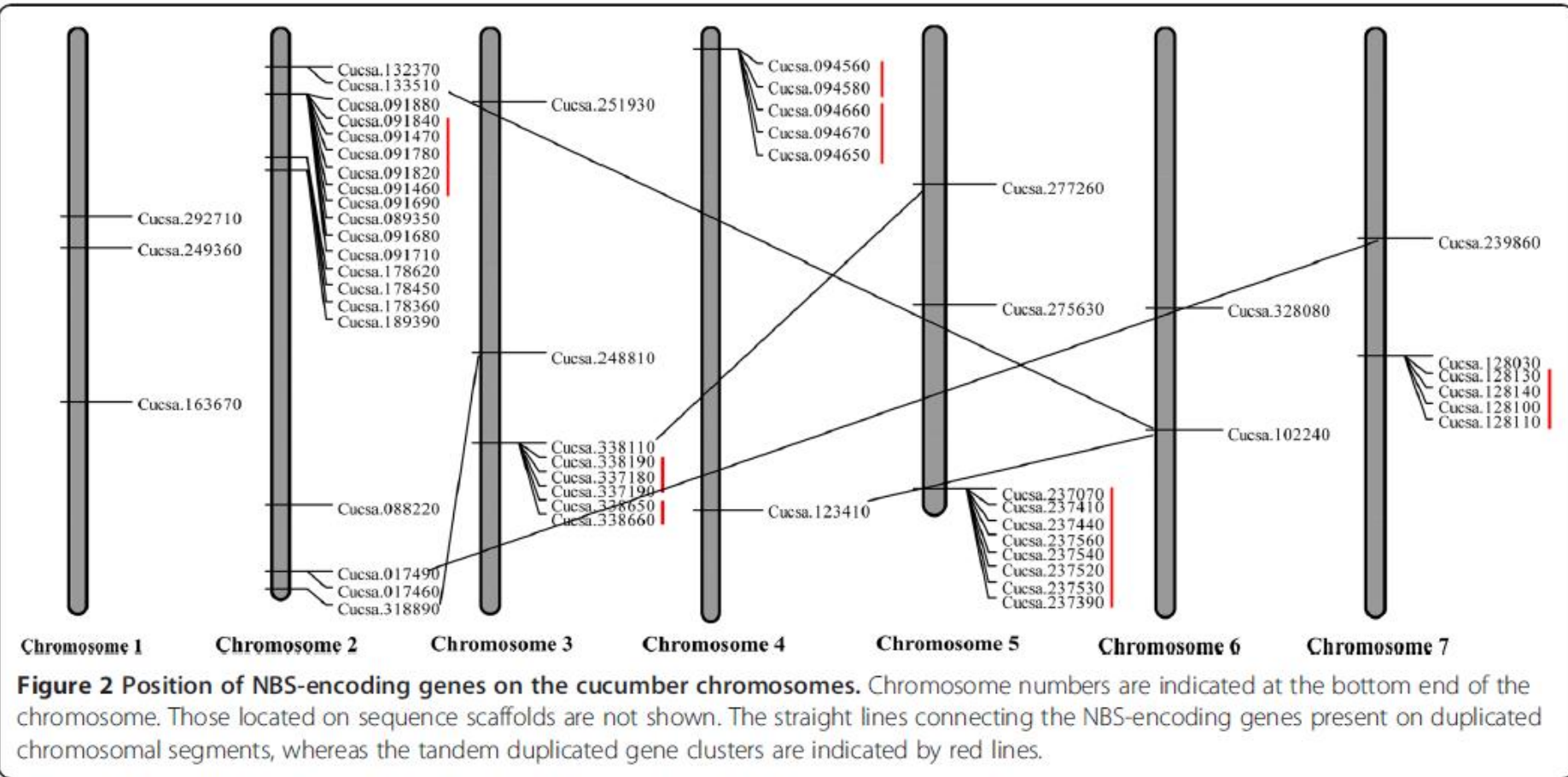
TABLE 3: Comparison of duplications in the NBS-encoding R genes from the three plant genomes.

Organization	<i>B.distachyon</i>	<i>Arabidopsis</i> ^b	Rice ^a
Single-genes	77	93	216
Multigenes	49	81	248
Number of family members	20	25	93
Maximal family members	7	7	10
Average members per family	2.45	3.24	2.67
Multigenes/single-gene families	0.64	0.87	1.14
Percentage of multigene families	38.9%	46.6%	53.4

Note: ^aData from Zhou et al. [11].

^bData from Meyers et al. [10].

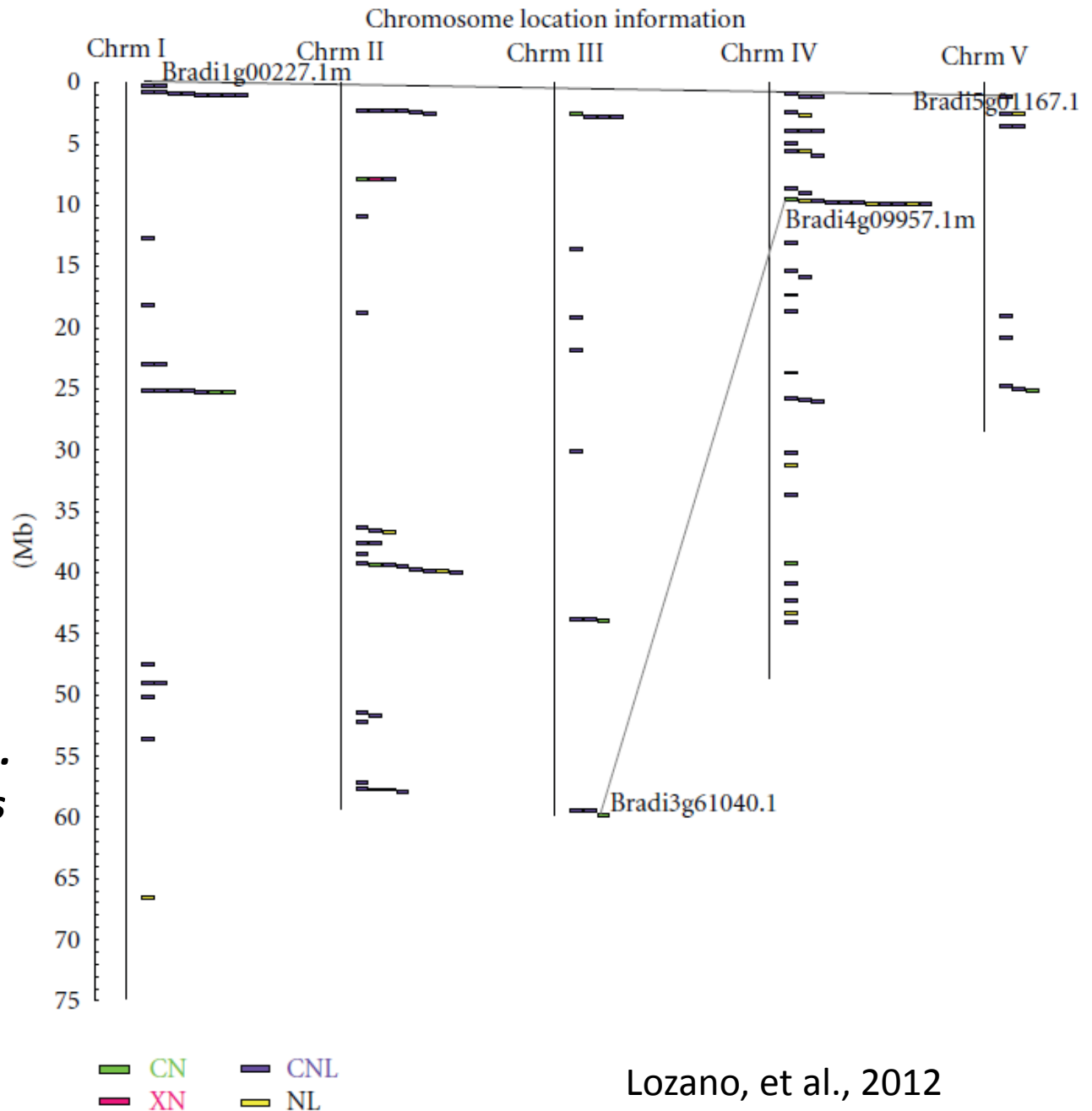
Clustering of R-gene



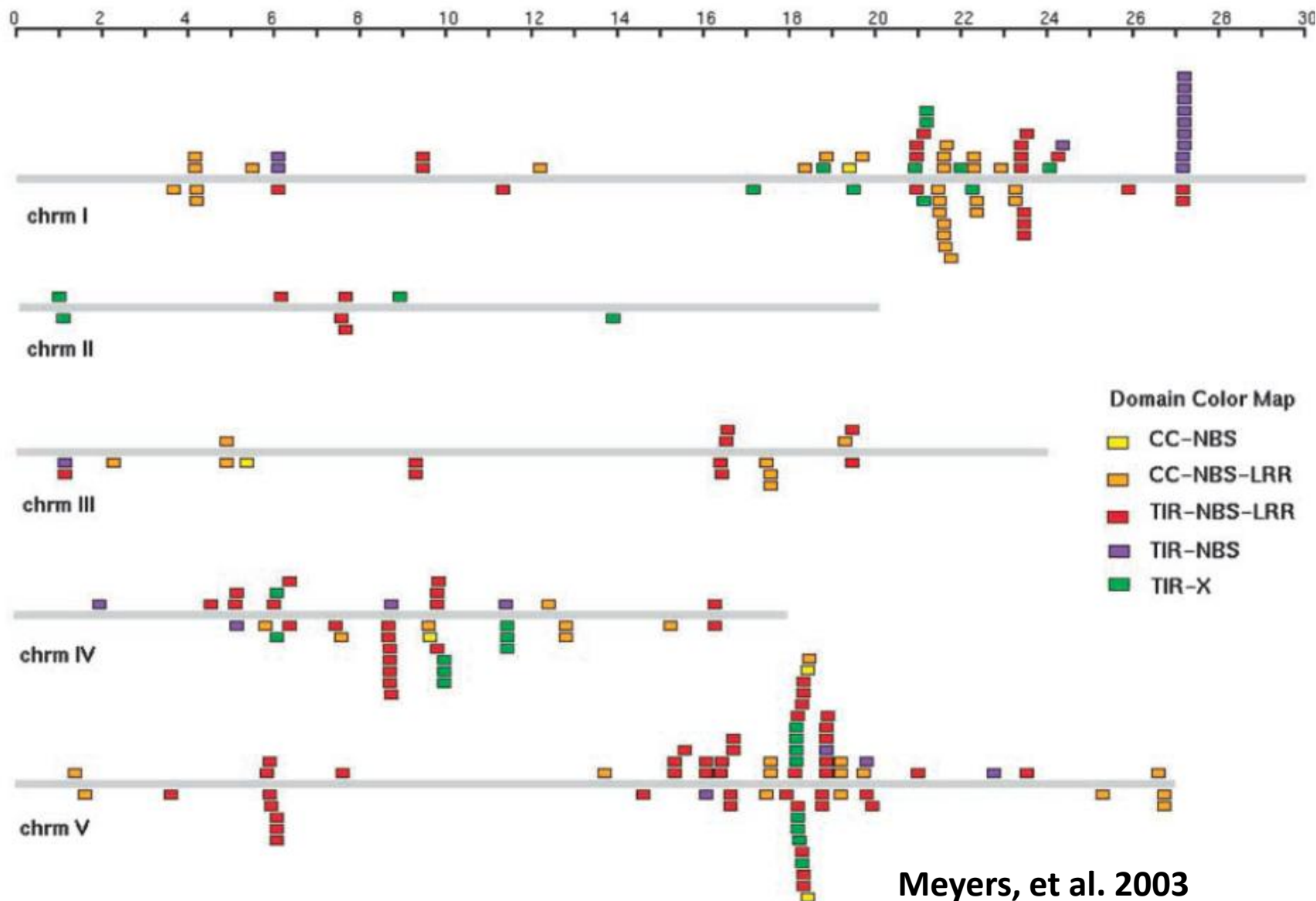
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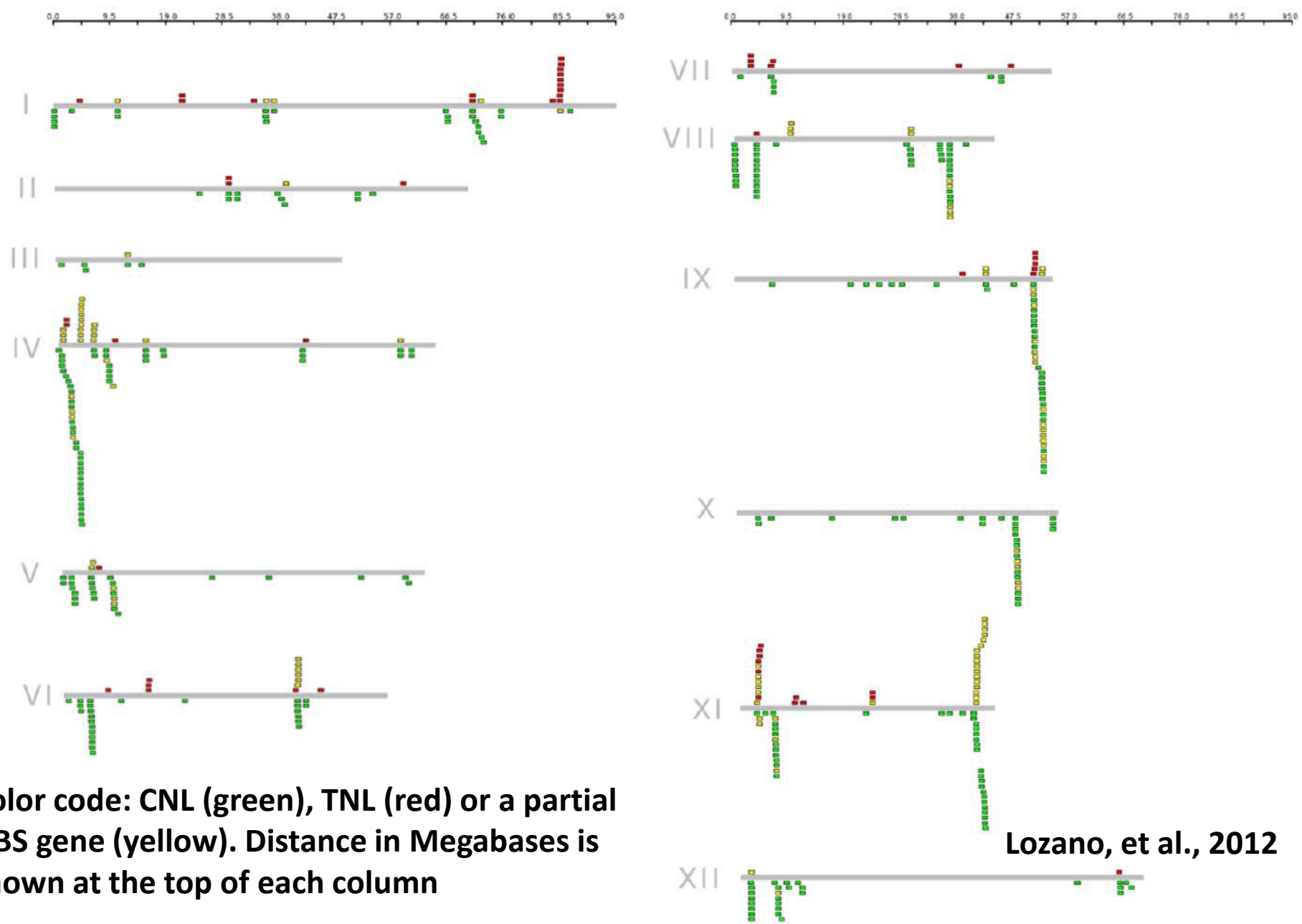
Distribution of the regular NBS encoding genes on the *B. distachyon* chromosomes. The scale is in megabases (Mb). Grey straight line connects the NBS genes present on duplicate chromosomal segments.



Physical Locations of Arabidopsis Sequences that Encode NBS Proteins Similar to Plant *R Genes*.

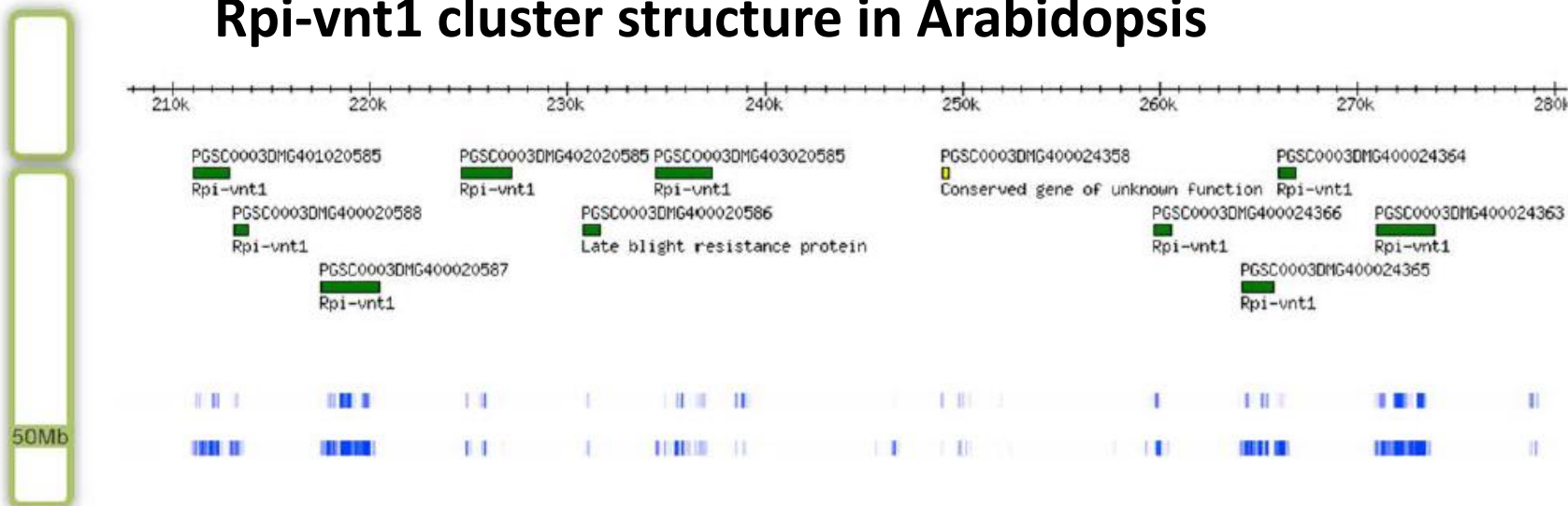


Distribution of *Solanum tuberosum* group phureja sequences that are predicted to encode NBS resistance proteins

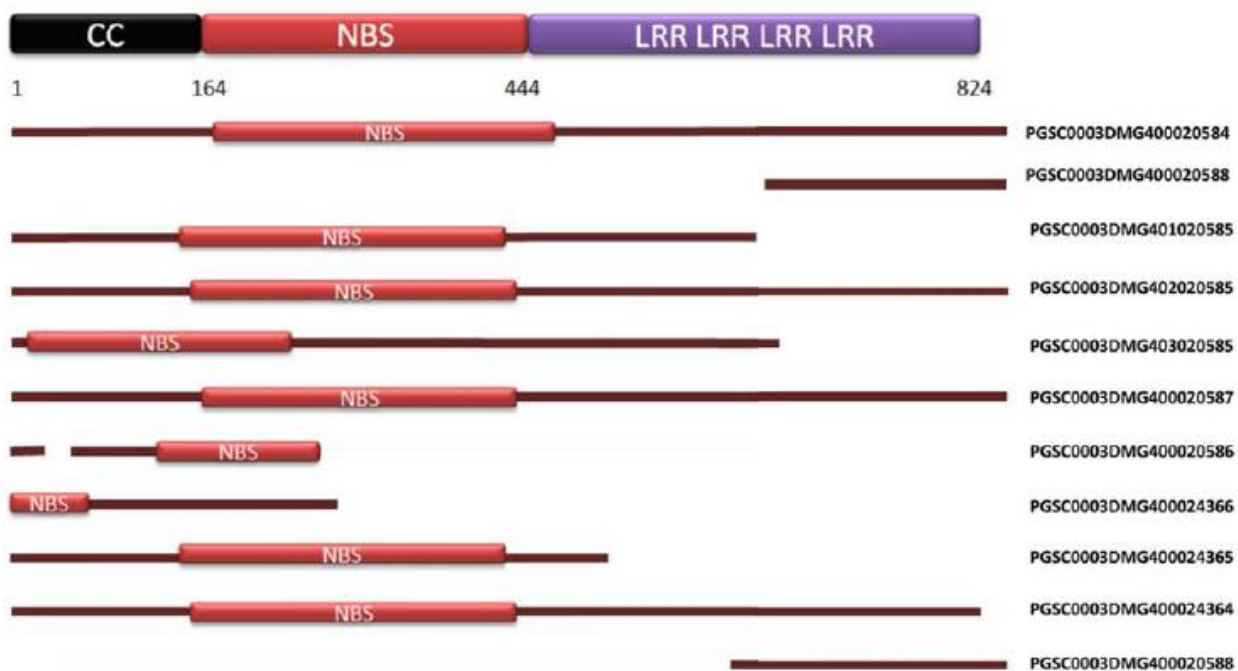


a Chr. 09

Rpi-vnt1 cluster structure in Arabidopsis

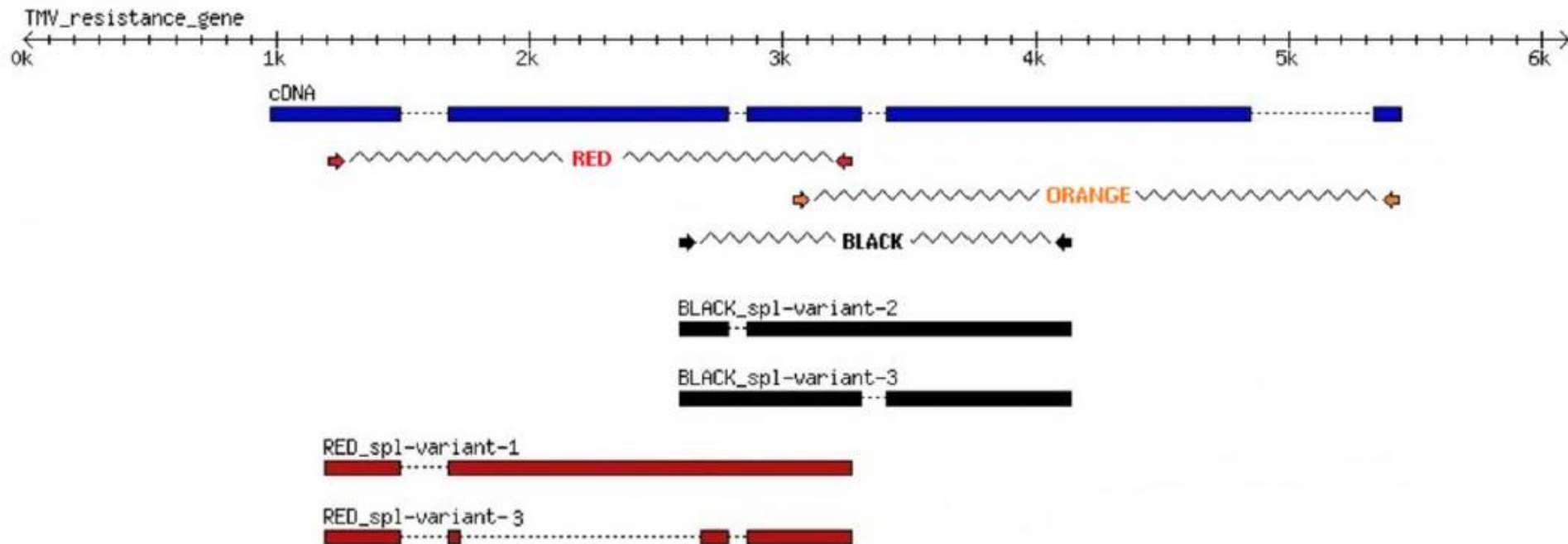


b



Meyers, et al. 2003

Alternative splicing of a TIR-NBS-LRR resistance gene



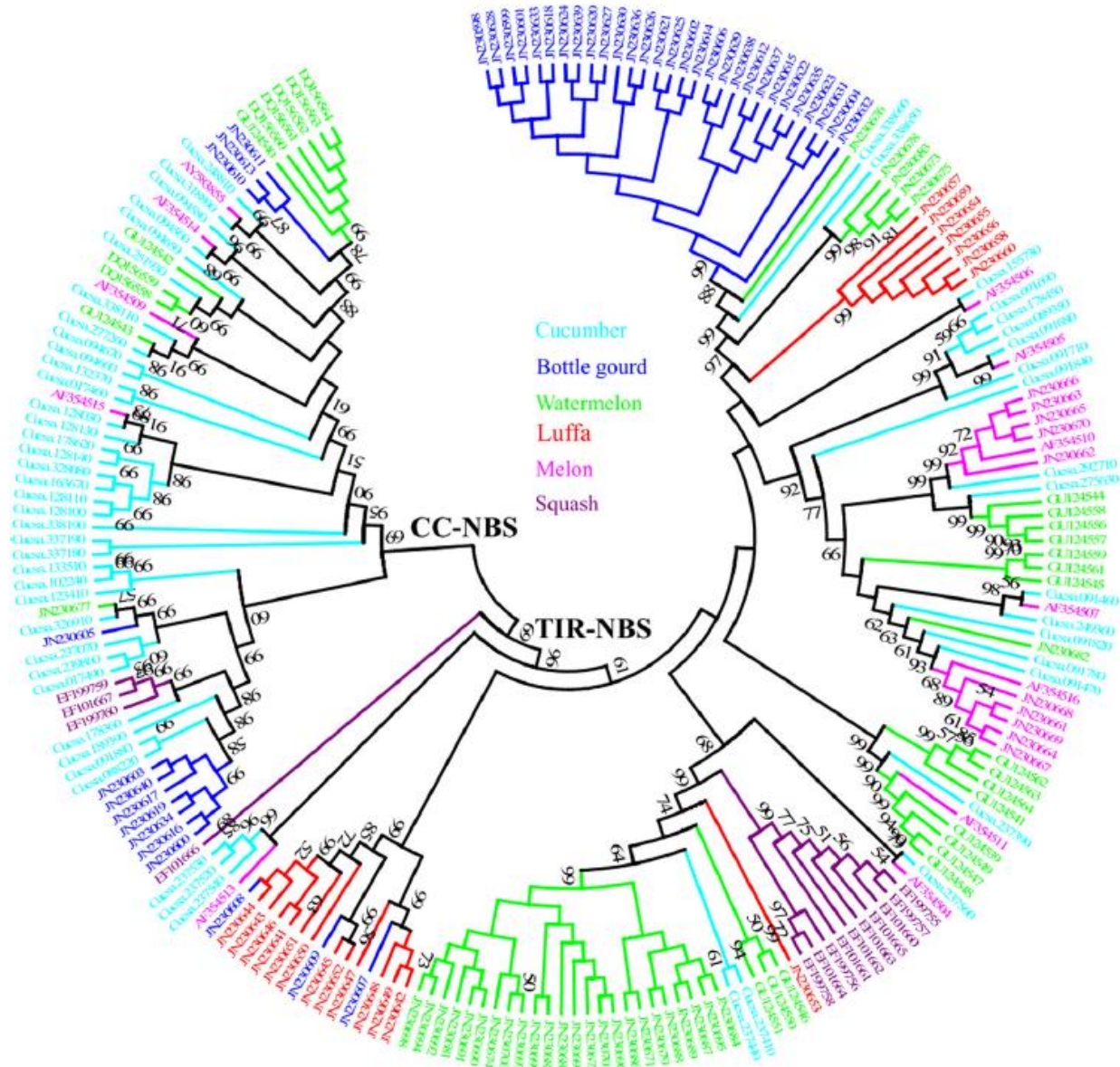


Figure 3 Phylogenetic comparison of Cucurbitaceae NBS-encoding genes and RGHS. TIR-NBS and CC-NBS families are distinguished. The scale represents the average number of substitutions per site. The numbers on the branches indicate the percentage of 1000 bootstrap replicates that support the node with only values > 50% reported. The detailed phylogenetic tree is shown in Additional file 8.

Concluding Remarks

- **Number and Diversity of R proteins represent a major part of the spectrum of recognition molecules available in an individual plant genotype to detect diverse pathogens.**
- **Although other types of proteins may play important roles in pathogen recognition, the majority of the R genes cloned to date encode CNL and TNL proteins.**
- **Highly conserved of NBS-LRR region characteristic among R-gene in diversity of plants enable R-gene isolation from many type of plants via PCR-based cloning**



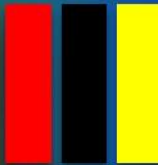
Concluding Remarks

- Fresh insights into the activities of R and Avr proteins, defence signalling networks and microbial pathogenicity factors should provide a range of materials to design new and effective disease control strategies.
- A key challenge now is to harness their collective potential.
- It is also important to elucidate the molecular processes underlying pathogen adaptation to new plant germplasms or chemical applications.
- Monitoring the losses to pathogen fitness associated with their resistance breaking ability will undoubtedly contribute to achieving durable disease resistance Agriculture sector.



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