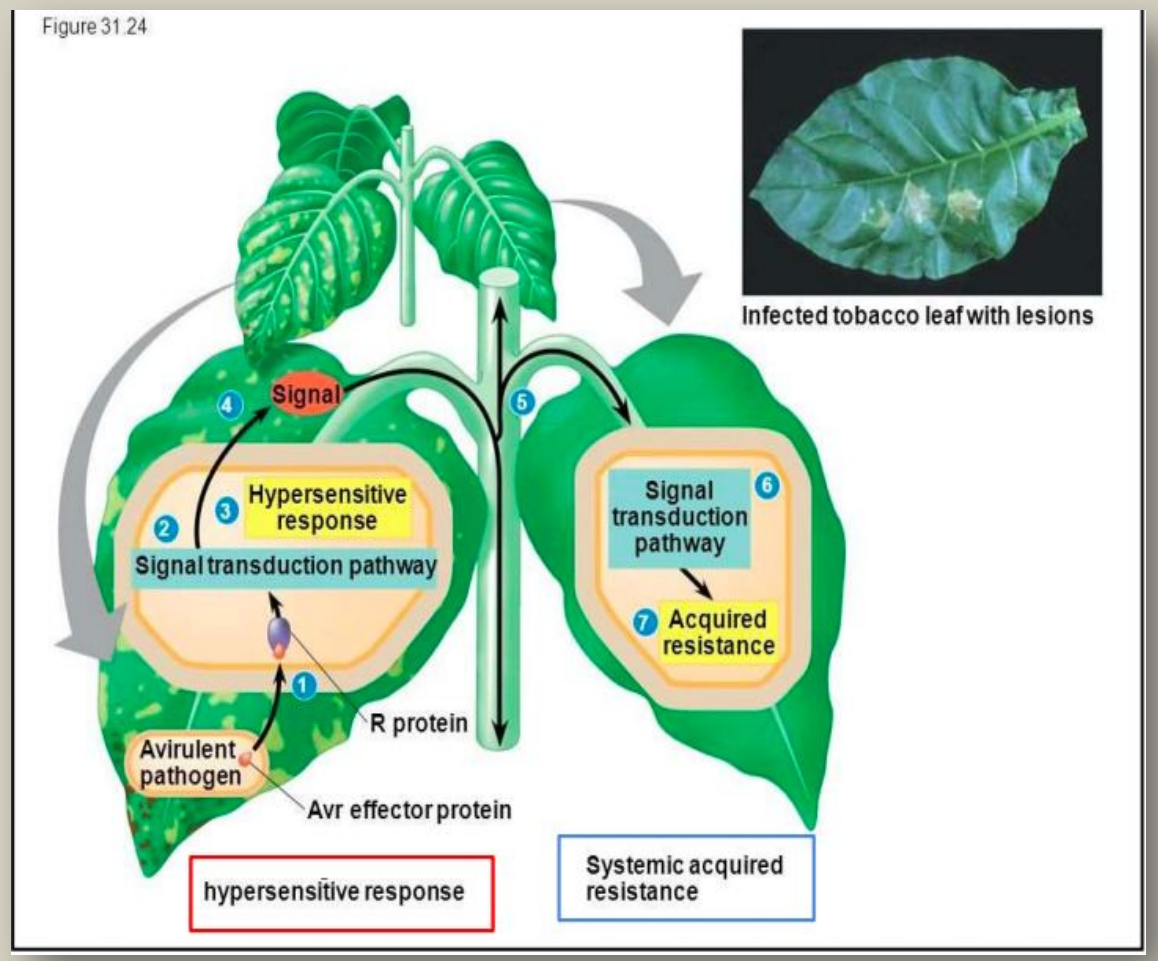
PLANT IMMUNITY AGAINST PATHOGEN

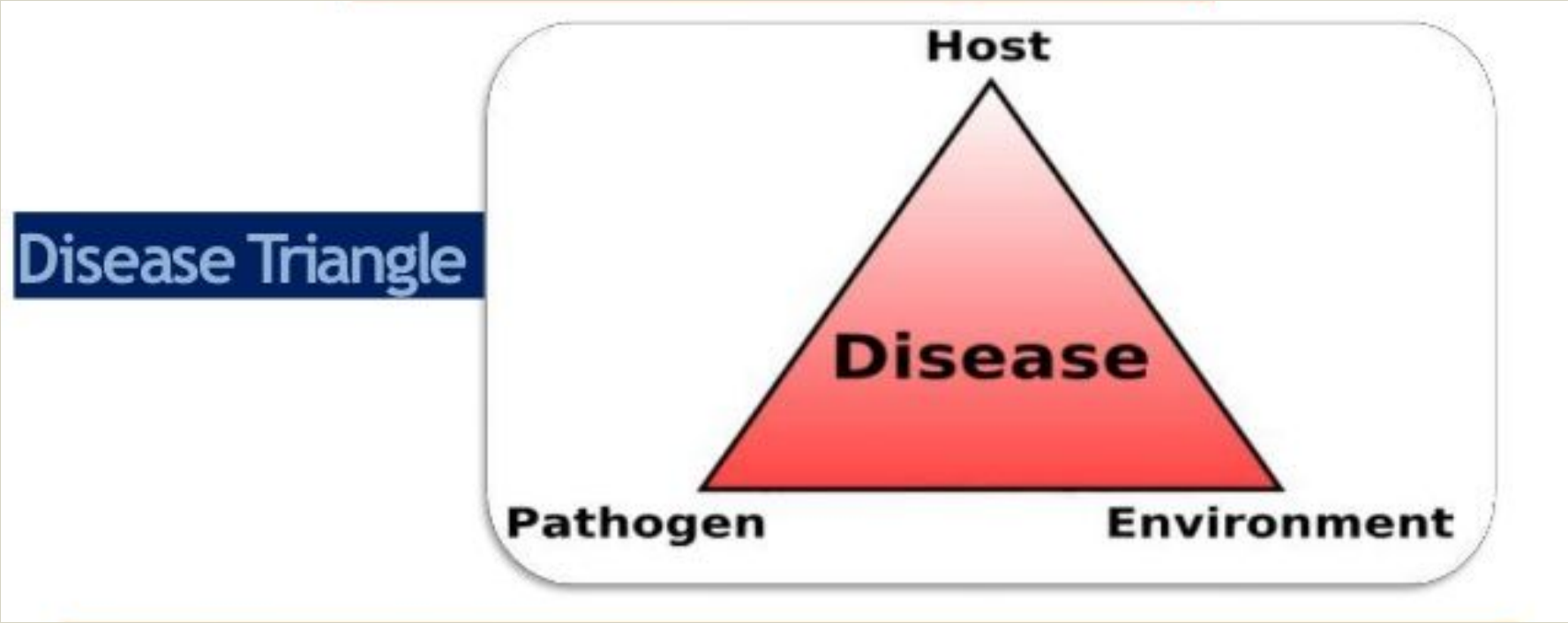
Dr. Nidhi S Chandra
Assistant Professor
Department of Microbiology
Ram Lal Anand College

OUTLINE

1. GENE FOR GENE CONCEPT



When Does Disease Develop?



1. Host should be susceptible
2. Pathogen should be virulent
3. Environment should be favorable

WHEN DOES THE IDENTIFICATION OF A PATHOGEN TYPICALLY OCCUR?

- Plant RESPONSES due to an **'incompatible'** and **'compatible'** reactions between the genes **R & Avr of host/ non-host and pathogen** respectively. (**gene-for-gene concept)**

Hypersensitive Response is the result is the result of "incompatible reaction"

Harold Henry Flor, 1900–1991

- Flor received a Ph.D. degree in 1929 from the University of Minnesota. He worked for the USDA for three years at Washington State University and then for the remainder of his career at North Dakota State University.
- In the 1940s, Flor developed the **gene-for-gene concept** to explain the genetic interactions between *Melampsora lini* and *flax*. His theories were put to use in Flor's own flax breeding program to successfully develop rust-resistant flax. This concept provided the underpinnings for research on the genetics of host-pathogen interactions for the next 70 years.

Incompatible reaction

- Found in biotrophic pathogens (obligate parasites) and is associated with hypersensitive response of the host.
- Only one of the four combinations would lead to the resistant response since the products of **R & Avr** would recognize & interact with each other.
- The product of alleles a & r rare unable to recognize each other, & there is no interaction between them, hence reaction of host become susceptible.

Plant Resistance /susceptibility genes	Pathogen Avirulence /virulence genes	
	A	a
R	Resistance	Susceptible
r	Susceptible	Susceptible

Agrios 2007

- Allele A of the virulence gene specifies avirulence.
- Allele a of the virulence gene governs virulence.

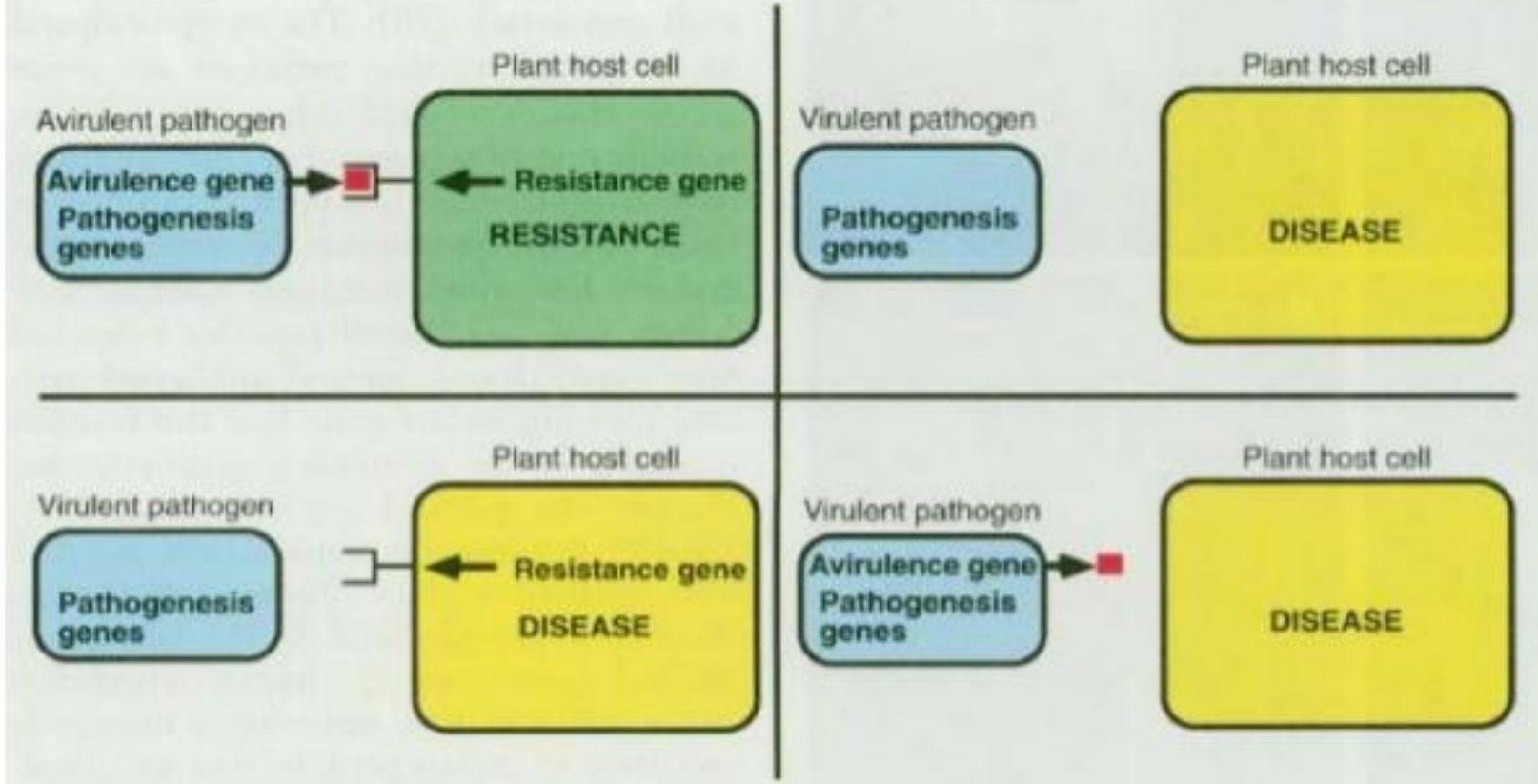


Fig. 1. Gene-for-gene interactions specify plant disease resistance. Resistance is only expressed when a plant that contains a specific *R* gene recognizes a pathogen that has the corresponding avirulence gene (upper left panel). All other combinations lead to lack of recognition by the host, and the result is disease. Green represents hypersensitive response; yellow represents susceptibility to disease.

(Staskawicz et al. 1995)

Compatible reaction

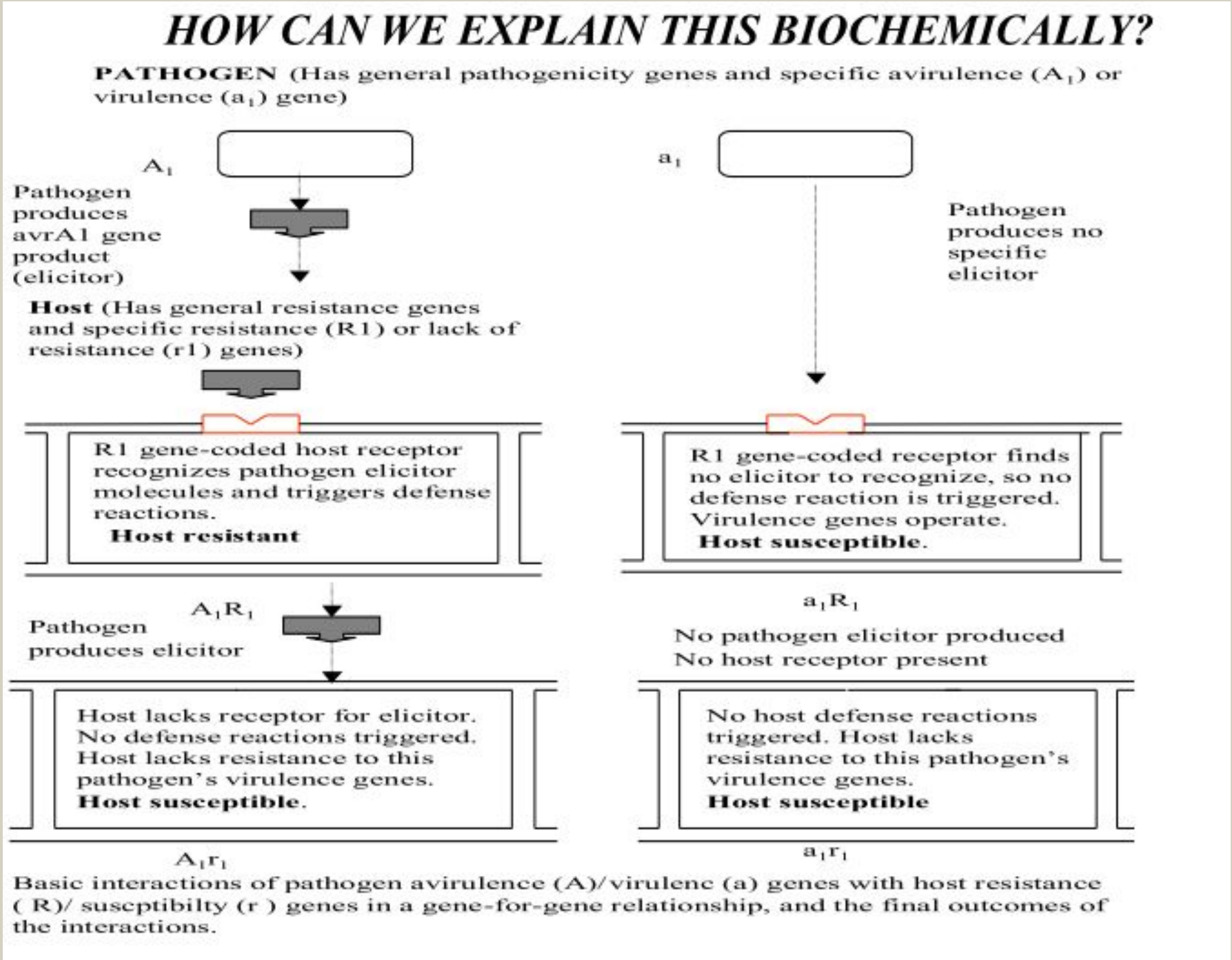
- Found in heterotrophic pathogens (facultative parasites).
- The allele for susceptibility of the host (r)and those for virulence in the pathogen produces specific compound, which interacts with each other to produce susceptible response.
- one of the four combinations would lead to susceptibility and rest leads to resistant.

Plant Resistance /susceptibility genes	Pathogen Avirulence /virulence genes	
	A	a
R	Resistance	Resistance
r	Resistance	Susceptible

- Allele A of the virulence gene specifies avirulence.
- Allele a of the virulence gene governs virulence.

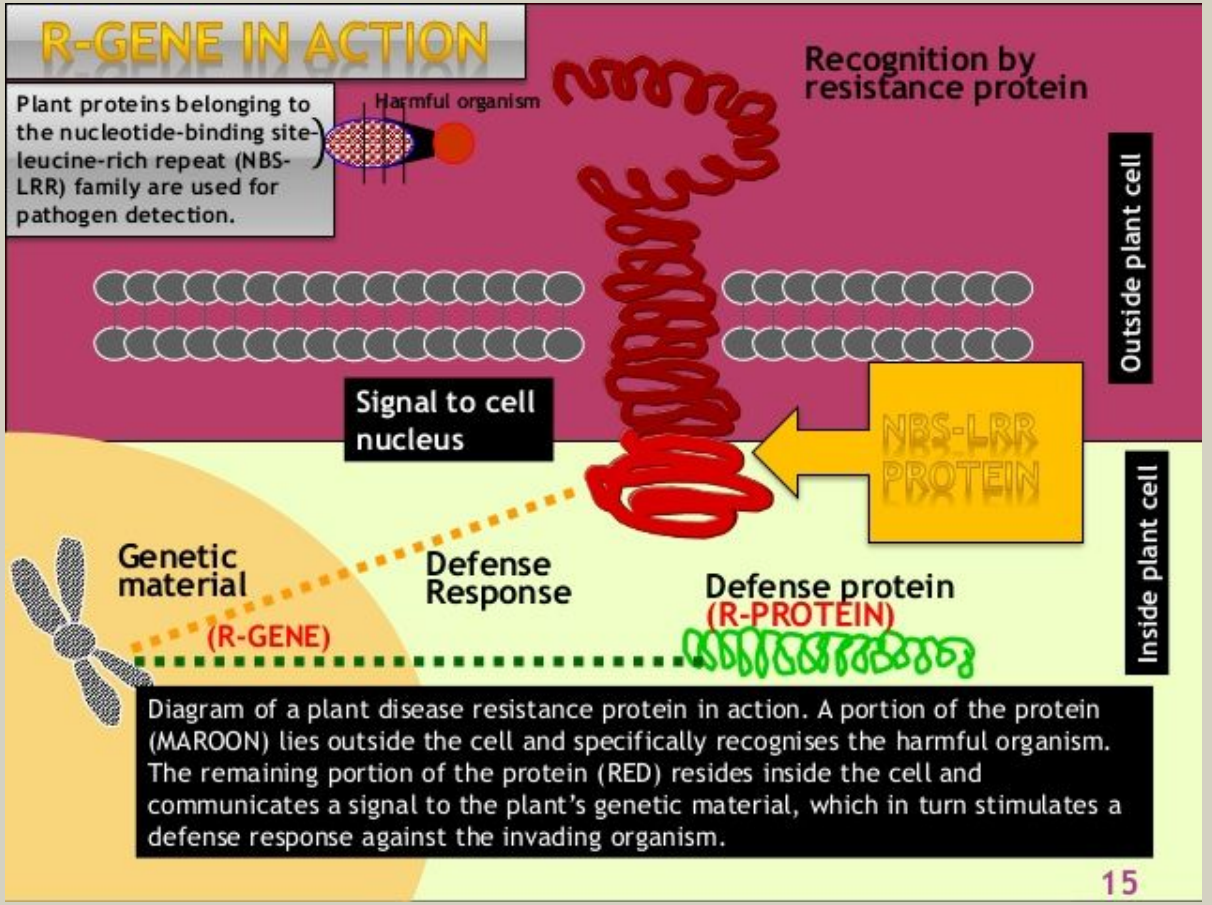
Receptor elicitor Model

- Albersheim and Anderson Prouty, 1975 proposed this model.
- The resistance allele of the plant encodes a **receptor** that recognizes an elicitor produced by the pathogen. Recognition of the pathogen elicitor by the plant receptor initiates plant defense responses that lead to plant resistance.
- If the pathogen produces the elicitor, it is avirulent.
- If the pathogen does not produce the elicitor, it is virulent



R genes

- Present in host plant.
- Polymorphic genes.
- Controls a major step in the recognition of the pathogen and plays a major role in expression of resistance.
- Control Gene-for-Gene interaction.
- R gene product inactivates the toxin.
- Most R proteins contain amino acid leucine rich domain (LRR- leucine rich repeats),
- Depending on R protein LRR reside : cytoplasmic LRRs or extracytoplasmic LRRs.
- Leucine-rich repeats (LRR) region of R-genes is involved in recognizing pathogens



Major Classes of R Gene

(Gururani *et al 2012*)

S. NO	MAJOR R-GENE CLASSES	EXAMPLE
1	NBS-LRR-TIR	N, L6, RPP5
2	NBS-LRR-CC	I2, RPS2, RPM1
3	LRR-TrD	Cf-9, Cf-4, Cf-2
4	LRR-TrD-Kinase	Xa21
5	TrD-CC	RPW8
6	TIR-NBS-LRR-NLS- WRKY	RRS1R
7	LRR-TrD-PEST-ECS	Ve1, Ve2
8	Enzymatic R-genes	Pto, Rpg1

LRR - Leucine rich repeats; **NBS** - Nucleotide-binding site; **TIR** -Toll/Interleukin-1- receptors; **CC** - Coiled coil; **TrD** -Transmembrane domain; **PEST** -Amino acid domain; **ECS** - Endocytosis cell signaling domain; **NLS** - Nuclear localization signal; **WRKY** -Amino acid domain; **HC toxin reductase** - Helminthosporium carbonum toxin reductase enzyme.

Avr Genes, Feature and function

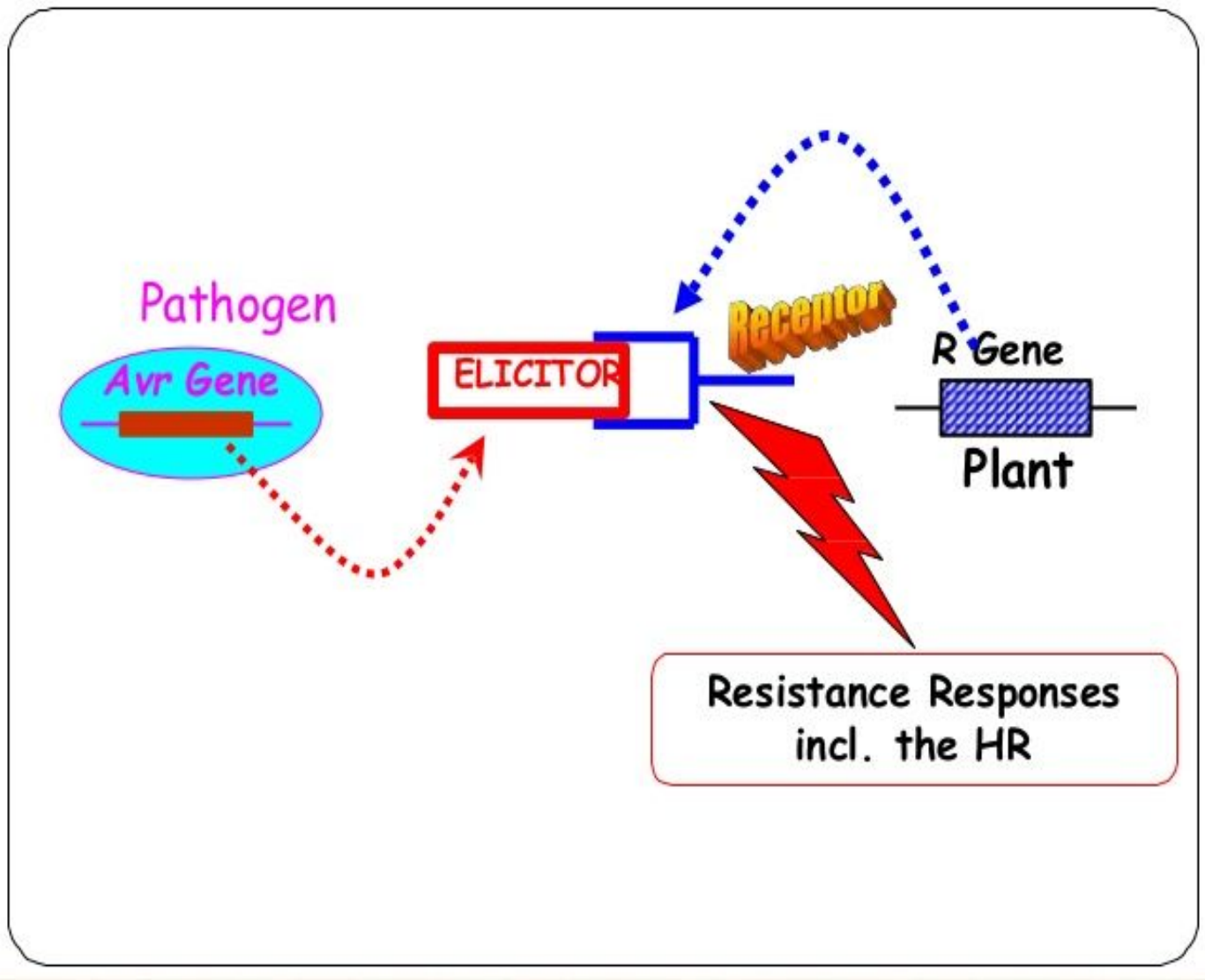
- Avr genes were first identified by H. H. Flor in 1950.
- Mild genes of pathogen.
- Responsible for activation of certain defense response in host.
- Leads to resistance including **hypersensitive response**.
- Hydrophilic- lacking stretches of hydrophobic amino acids
- enable them to be anchored in cell membranes May produced and localized in pathogen cytoplasm or secreted through membrane pores If secreted externally directly acts as elicitors If localized in cytoplasm of pathogen acts indirectly as enzyme to produce elicitor molecules Acting as avirulence factors in **elicitor receptor model** (plant defense)
- Contribution towards the virulence of pathogen. eg. AvrBs2 gene of *X. campestris* pv. *Vesicatoria* Avr proteins interact with specific plant proteins (virulence target)
- enhances availability of nutrients to pathogen .

Examples of Avr genes and corresponding R- genes

Plants	Avirulent gene	Pathogen	Matching R genes	References
Rice	AvrPITA	*Magnaporthe grisea*	Pi-ta	Valent (1998)
Tomato	AvrPto	*Pseudomonas syringae pv. tomato*	Prf	Salmeron et. al. (1996)
Tomato	AvrRpp8	*Meloidogyne incognita and Macrosiphum euphorbia*	Mi	Milligan et. al. (1998) Rossi et. al. (1998)
Potato	Coat protein	Potato virus X (PVX)	Rx	Bendahmane et. al. (1999)
Potato	Elicitin or AvrD	*Phytophthora infestans*	Pto	Cai et. al. (2001)
Tobacco	Replicase	Tobacco mosaic virus(TMV)	N	Whitham et. al. (1994)

Second Gene for Gene Hypothesis

- **Flor's gene –for- gene hypothesis** is purely a hypothesis of **identities**.
- The resistance gene in the host and the corresponding virulence gene can be identified by this hypothesis.
- But it does not tell us about the gene quality.
- A second gene for gene hypothesis proposed by **Vanderplank,** which is an extension of Flor's hypothesis, tells us about the **quality of genes**.
- The quality of resistance gene in the host determines the fitness of matching gene in the pathogen to survive, when this gene for virulence is unnecessary.
- Unnecessary gene means-a gene for virulence in the pathogen population against which matching resistance gene in the host is not present.
- Reciprocally, the fitness of the virulence gene in the parasite to survive when it is unnecessary determines the quality of matching resistance gene in the host.
- For example there are ten or more genes in the host for resistance to disease R_1.....R_{10}.



Some Concepts regarding plant immune systems

- Performed resistance
- Induced resistance
- Gene for gene interaction
- Hypersensitive Response
- Systemic acquired resistance

WHY STUDY PLANT RESISTANCE?

80% of total calories consumed by human population come from only six crops: wheat, rice, maize, potatoes, sweet potatoes, and manioc (Raven, P.H. et al, 1999).

We lose 12% of total crop yields to pathogen infection– equivalent to nine hundred million tons worldwide annually (Krimsky S. and Wrubel R., 1996).

HYPERSENSITIVE RESPONSE (HR)

- The plant 'hypersensitive response' (HR), a defense mechanism, involves interaction between products of an 'avr' gene of the pathogen and a matching 'R' gene of the host plant (Dodds et al., 2006).

- **It is a mechanism used by plants to prevent the spread of infection by microbial pathogens.**

- The HR is one kind of **programmed cell death (PCD)** associated with the death of a small number of cells at and around the site of infection

- HR serves to inhibit the growth of the **invading pathogen** by killing **infected and uninfected cells,** producing a physical barrier composed of dead cells.
- During HR, the dying plant cells strengthen their cell walls and accumulate certain toxic compounds like **phenols and phytoalexins**

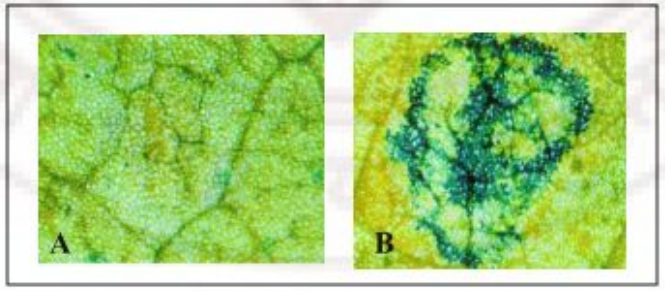


Fig 1.1 Hypersensitive response on a tobacco leaf during pathogen infection

Hypersensitive response (HR) manifested with the development of necrotic lesions (stained with Evans blue) followed by localized desiccation and browning of the affected cells. A) initial hours of infection and B) late hours of infection (adapted from Wright *et al*., 2000).

What are elicitors?

Specific signal molecule produced by the pathogen and recognized by plant receptor molecule

- A variety of **bacterial or fungal products elicit** defensive plant responses in both **host and non-host plants** and these non-specific elicitors are the prime inducers of defense responses in non-host plant-pathogen interactions.
- An example of **non-specific elicitors** from bacteria are the **'harpins' (heat stable proteins)** encoded by 'hrp' (hypersensitive response pathogenicity) gene cluster of some Gram-negative phytopathogenic bacteria (Collmer et al., 2000).
- These bacteria have limited host ranges and specialized in colonizing the apoplast and trigger diseases in plants causing rots, spots, vasucular wilts, cankers and blights.
- The majority of these pathogens are Gram-negative rod shaped bacteria from the genera *Erwinia, Pseudomonas, Xanthomonas* and *Ralstonia*

PROTEIN AND FACTORS INVOLVED

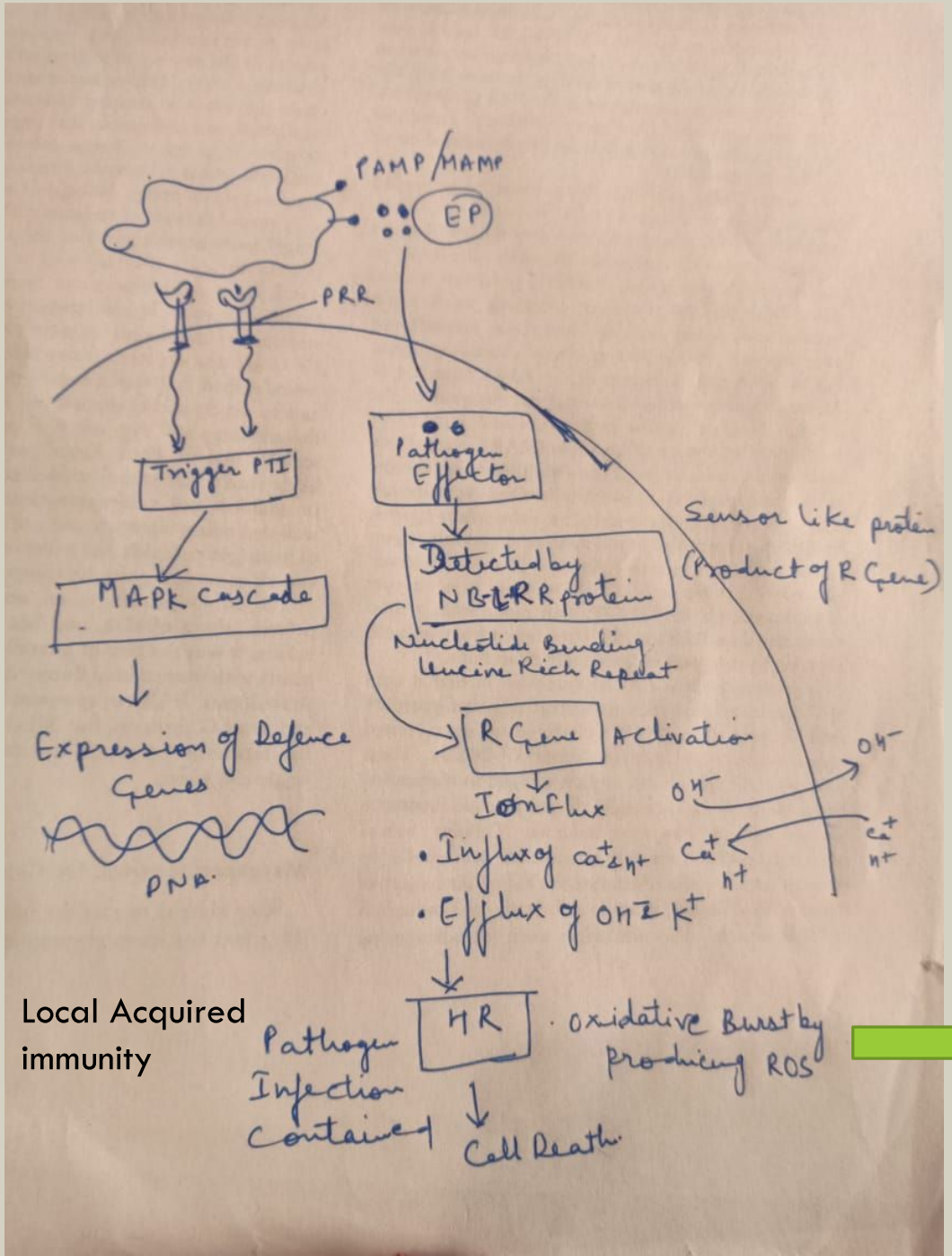
Plant Cell

- PRR (Pattern Recognition Receptor)
- R Gene → R protein (Resistance Protein)

Pathogen

- PAMP/MAMP (Pathogen Associated molecular protein/ Microbe Associated molecular protein)
- Pathogen Effector

What does HR in plants lead to?



Local Acquired immunity

Systemic Acquired Immunity

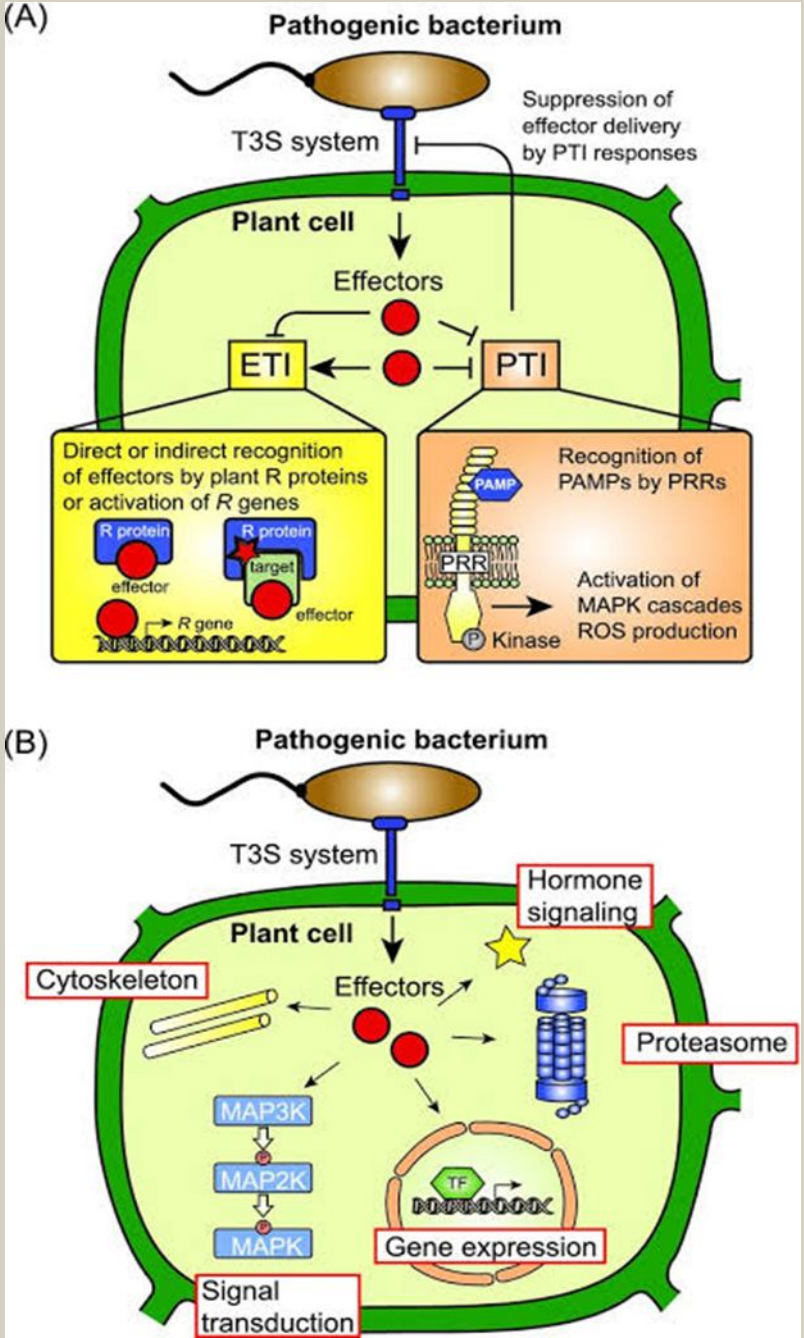
1. Activates the genes of phytolaxins and chitinases (Degarde the cell wall of fungal pathogen)
2. Activates Hormonal Signaling (JA pathway [Necrotrophic Pathogen], SA pathway [Biotrophic pathogen])
   release stress phytohormone activates PR genes (defense gene) form PR protein which combat pathogen

Plants usually defend themselves against microbial attacks by two levels of defense responses:

1. **PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI)**
2. **Effector-triggered immunity (ETI).**

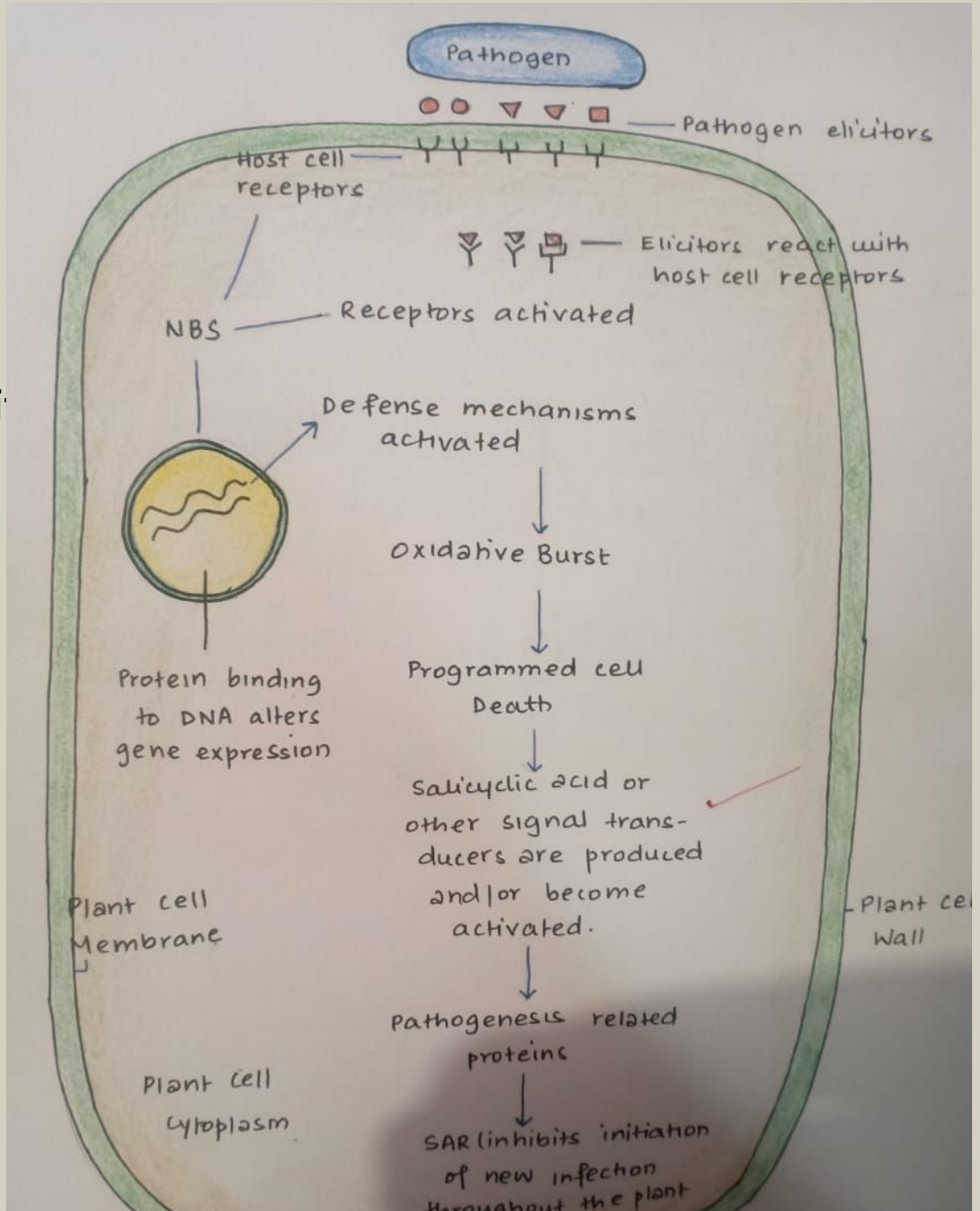
- **PTI** is one of the first defense responses and is activated upon recognition of PAMPs by plant pattern recognition receptors (PRRs).
- PAMPs are conserved microbial molecules such as flagellin, bacterial elongation factor (EF)-Tu, peptidoglycan, chitin or cell-wall-derived molecules, which are essential for pathogen survival or fitness.
- PTI responses can be overcome by the actions of translocated type III effector proteins, which interfere with PTI responses and thus promote bacterial virulence.

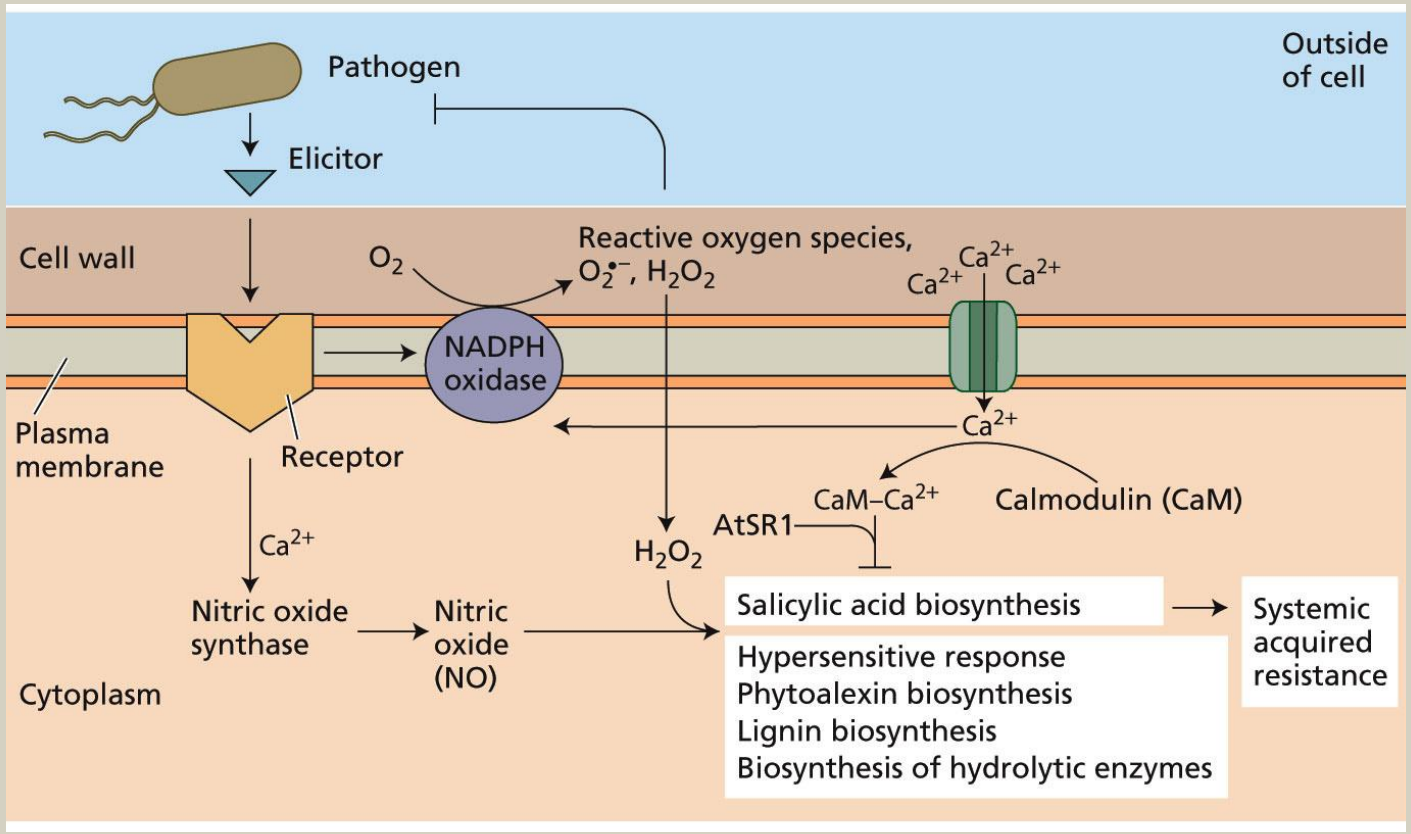
- The effector-triggered susceptibility is counteracted by a second defnse of plant defense responses, **ETI**, which is activated by the products of plant resistance (*R*) genes upon detection of individual effector proteins.
- Plant *R* genes often encode NB (nucleotide binding, also termed NB ARC [nucleotide-binding adaptor shared by Apaf1])-LRR (leucine-rich repeat) receptors



BASIC EVENTS IN INCOMPATIBLE HOST-PATHOGEN INTERACTION

- Burst of oxygen reactive species around infection si
- Synthesis of antimicrobial phytoalexins
- Accumulation of Salicylic Acid (SA)
- Directly kill and damage pathogens
- Strengthen cell walls, and triggers apoptosis
- Restrict pathogen from spreading
- Rapid and local





- Activation of R genes triggers an influx of Ca^{2+} and H^+ and an efflux of OH- and K^+ to the outside the cells.
- Generation of oxidative burst by producing reactive oxygen species (ROS), superoxide anions, H_2O_2, OH radical and NO molecules. These compounds exhibits cell-wall modification by lipid peroxidation, accumulation of PR-proteins (chitinase, gluconase), phytoalexins as well as phenolic compounds. This local response induces systemic alterations known as systemic acquired resistance (SAR). As a result the plant acquires resistance to a pathogen, which would previously spreading to the other cells.

Crosstalk between MAPK Signaling and the Radical Burst

ROS participate in the activation of MAPK.

MAPK cascades are major signal transduction modules in eukaryotes and consist of at least three protein kinases, :

a MAPK kinase kinase (MAPKKK)

phosphorylation

activates a MAPK kinase (MAPKK)

phosphorylation

activates a MAPK.

2 MAPKs, tobacco salicylic acid-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK), *and their orthologue in other plant species play pivotal roles in disease responses to several pathogens and development and diverse physiological processes.*

BIOLOGOLICAL FUNCTION OF ROS AND NO

- ROS and NO play important roles independently or coordinately in plant innate immunity. ROS generated on the plasma membrane can directly cause strengthening of the cell walls via cross-linking of glycoproteins against secondary infection and simultaneously activating the Ca^{2+} channel to increase the level of cytosolic Ca^{2+}(act as inducer and defense).
- However, NO signaling includes various messenger molecules, such as cGMP, cADP ribose and Ca^{2+} and responsible for posttranslational modification of target proteins, such as NO-dependent cysteine *S*-nitrosylation that can modulate the activity and function of pathogen proteins.
- NO induces activation of MAPK and the expression of defense genes, such as *Phenylalanine ammonia-lyase* and *pathogenesis-related proteins*.NO ASSOCIATED1 (NOA1) participates in NOS (nitrogen synthase) and NR (nitrate reductase) activity playing a key role in interaction between pathogen-plants and also triggered NO Burst .
- NO can also react with O_2^- to form the reactive molecule peroxynitrite ($ONOO^-$) which is responsible for tyrosine nitration, that is the major toxic reactive nitrogen species in cells. In plants, NO and O_2^- are produced simultaneously through a convergent signaling MAPK cascades.

SYSTEMIC ACQUIRED RESISTANCE