

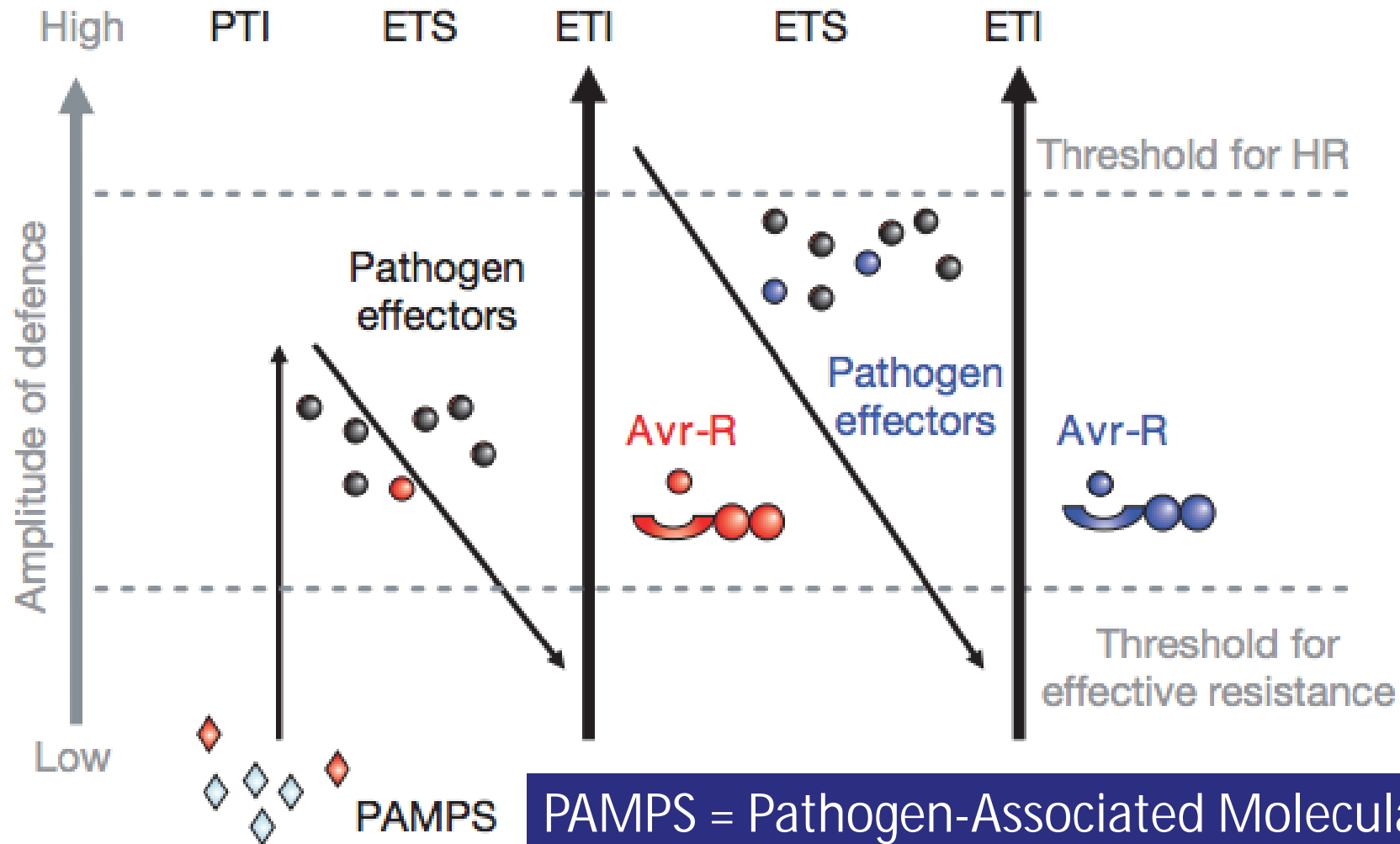
Plant-Pathogen Interactions for Leaf Rust and Spot Blotch of Wheat: Transcriptome/Epigenome Analysis

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Outline of Presentation

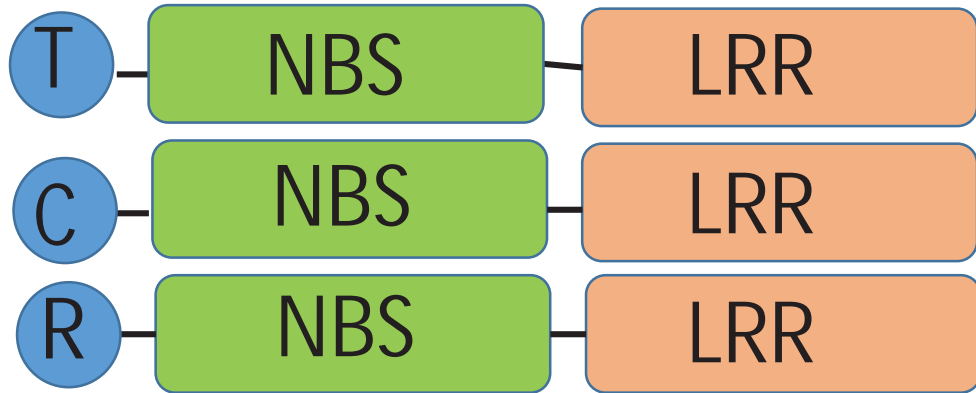
- Molecular Basis of Plant Immunity: Recent Work
- Seedling Leaf Rust Resistance (*Lr28*); APR (*Lr48*)
 - Transcriptomics: Upregulation/Downregulation
 - Epigenetic Regulation
 - DNA Methylation
 - Histone Modification
 - ncRNAs (miRNA and Target Genes)

Zig-Zag Model (Jones and Dangl 2006)



R Gene: Three Types of NLRs: TNL, CNL & RNL

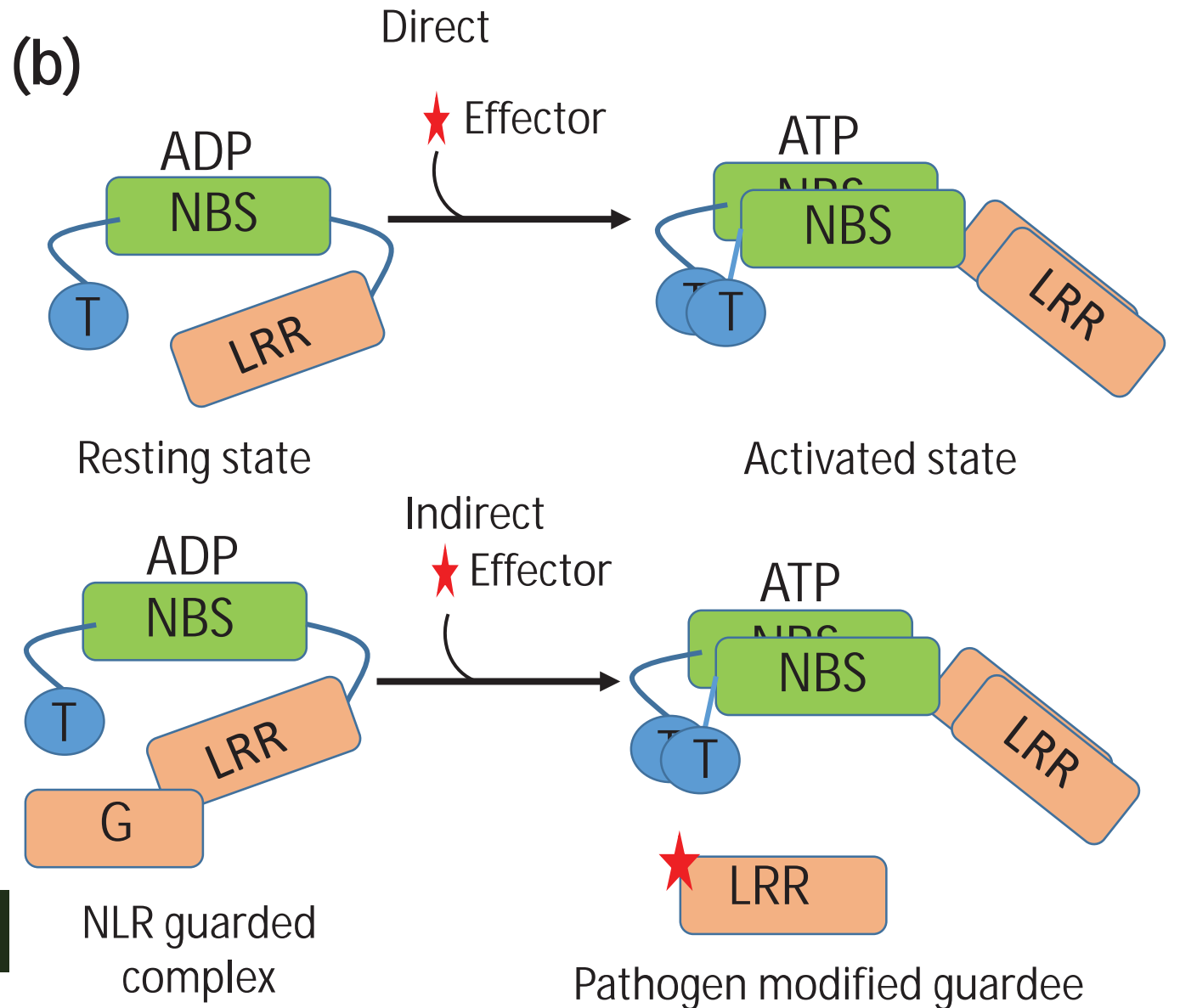
(a)



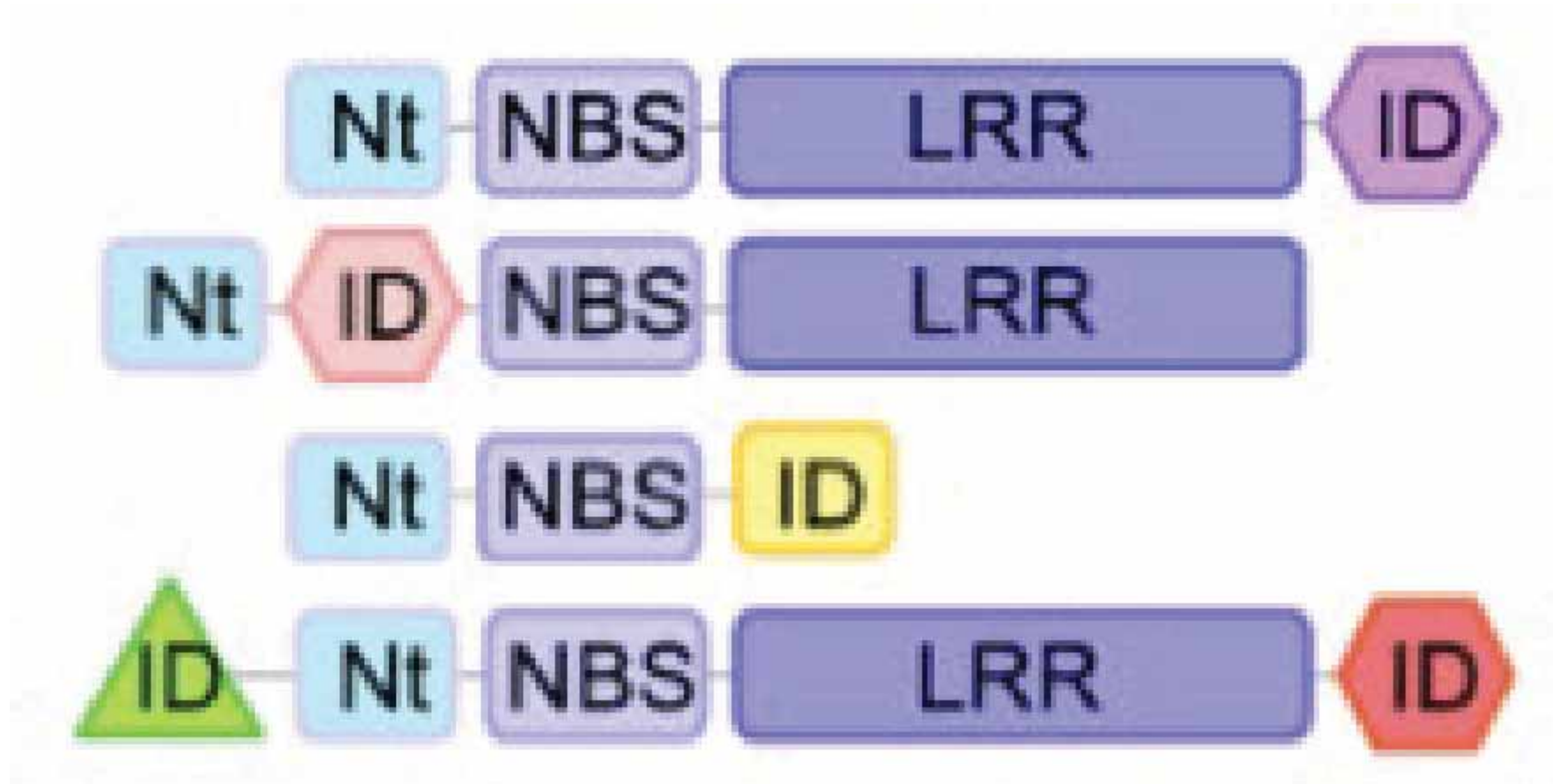
TNL Contains TIR at N-terminus
CNL contains CC at N-terminus
RNL contains RPW at N-terminus

NLR = Node Like Receptors or NBS-LRR

(b)



NLR-with Integrated Domains (IDs)



Leaf Rust in Wheat: Gene-for-Gene Model



		Host Genotype	
		<i>R</i>	<i>r</i>
Pathogen Genotypes	<i>Avr</i>	Resistance	Disease
	<i>avr</i>	Disease	Disease

Flor's Hypothesis

Leaf Rust = *P. triticina* = A Biotroph Fungal Pathogen

Inverse Gene-for-Gene Relationship: Spot Blotch in *Wheat-Bipolaris sorokiniana*



Pathogen
Genotype

Tox A

tox A

Host Genotype

Tsn 1

tsn 1

Disease

No Disease

No disease

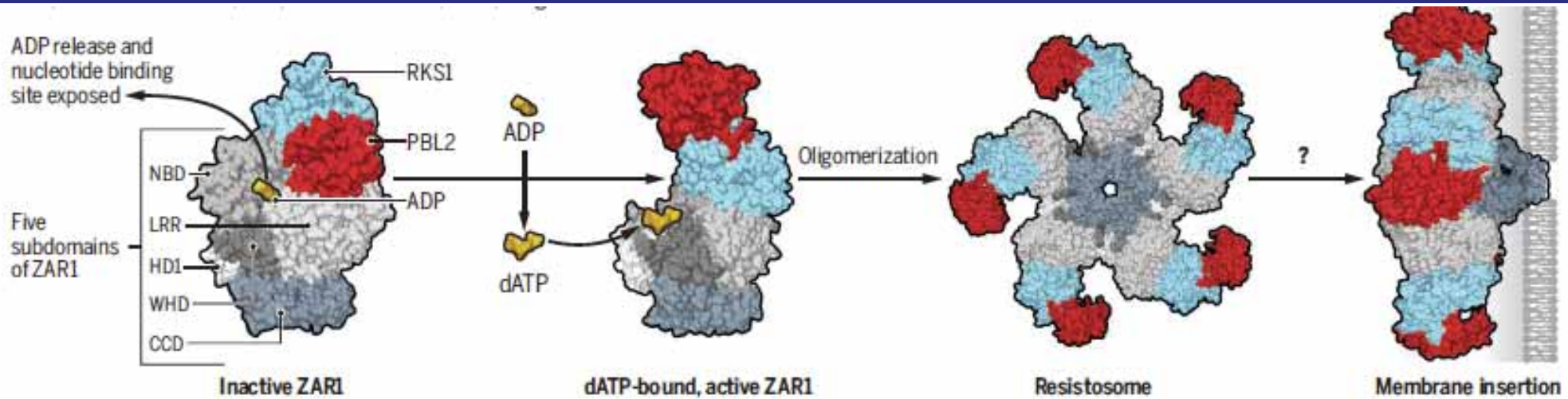
NO disease

Spot-Blotch = *B. sorokiniana*

Nawathe, S. et al, 2019 Plant Disease

1. NLR-Resistosome; Science Apr 5, 2019)

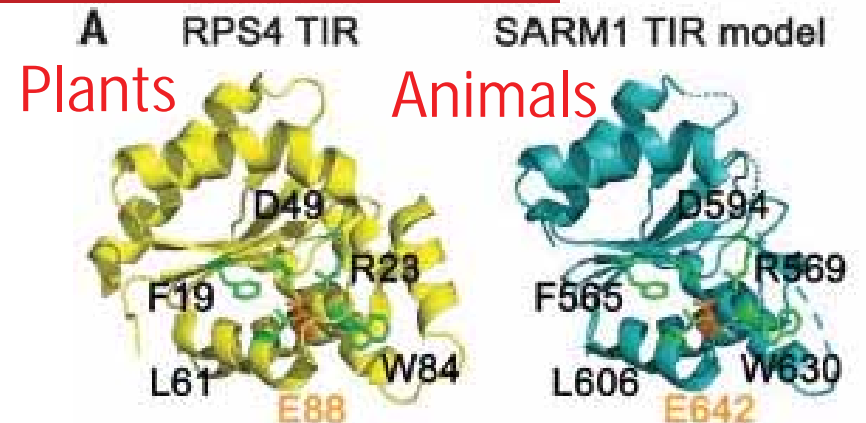
ZAR1 = HOPZ-ACTIVATED RESISTANCE 1 CNL in *A thaliana*: RKS1 = Resistance Related Kinase



ATP + ZAR1-RKS1-PBL2^{UMP} Penta-Oligomerization

ZAR1 = A CNL in Arabidopsis against AvrAc from *X. campestris* causing black rot disease in crucifers

2. NLR-TIR Domain as NADase (Science Aug 23, 2019)

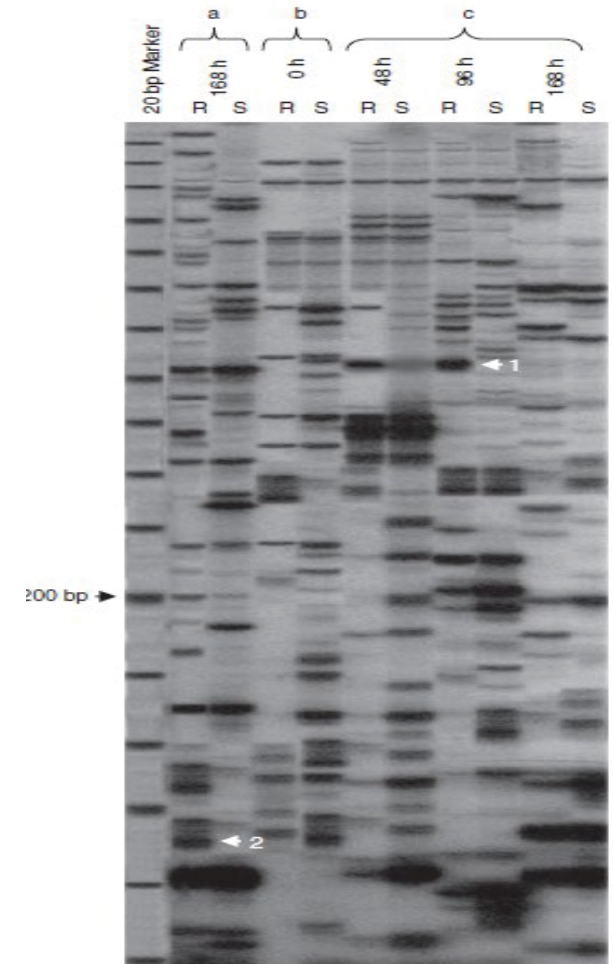
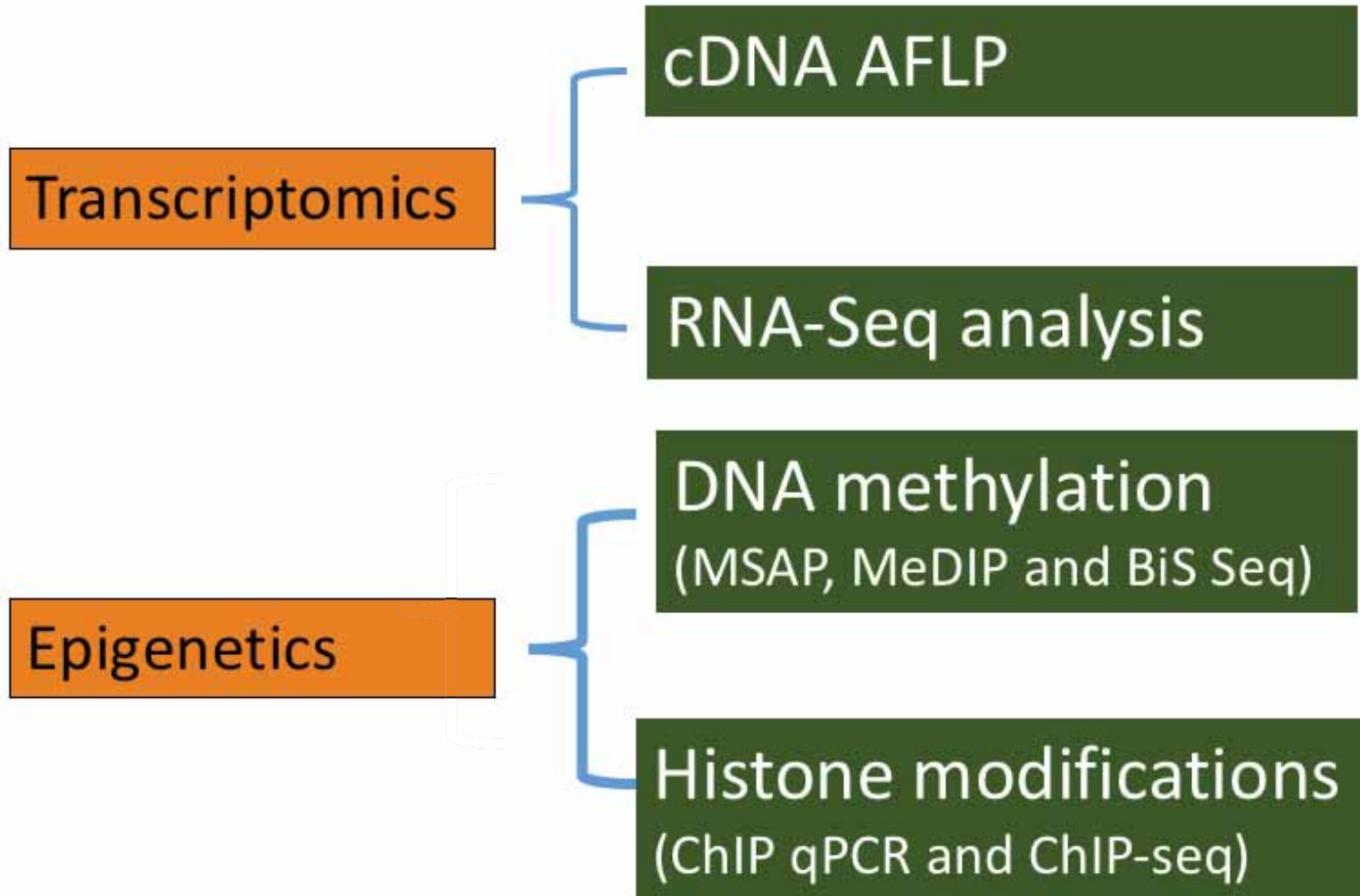


1. Seedling Resistance Gene *Lr28*/NILs
2. Adult Plant Resistance Gene *Lr48*

Transcriptome: cDNA-AFLP, RNA-seq,

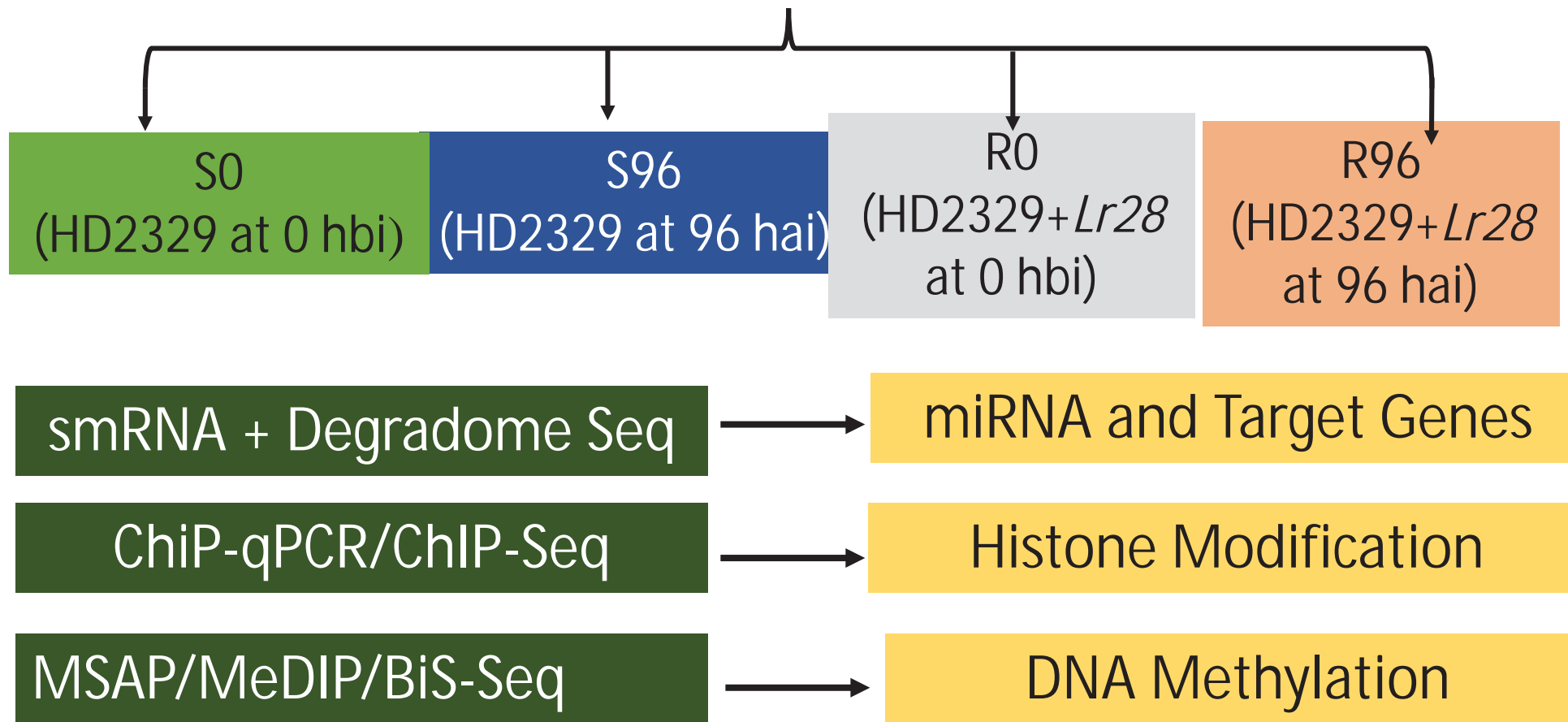
Epigenome: 1. DNA Methylation: MSAP/MeDIP, Bis-seq; 2. Histone Modifications: ChIP-qPCR/ChIP-Seq; 3. ncRNAs: (sRNA: miRNA + Degradome)

Gene Expression/Epigenetics for Leaf Rust Resistance (Due to *Lr28/Lr48*): Research at Meerut



Material Used for Epigenetic Studies: *Lr28*

Details of Samples: A Pair of NILs (HD2329)



Material Used for Epigenetic Studies: *Lr48* (Genotype CSP44 Selected from Austr cv Condor)

Pre-Adult
Stage
48/96 hai
 S_{48} S_{96}



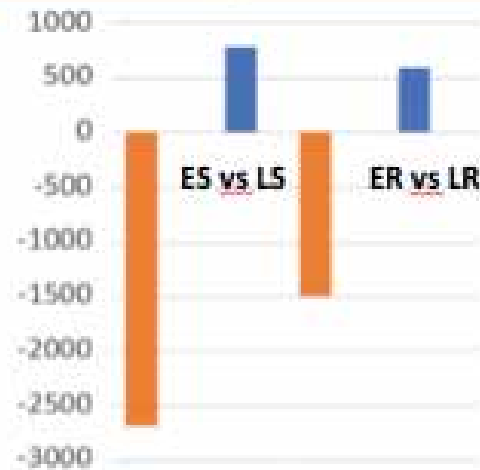
Post-Adult
Stage
48/96 hai
 R_{48} R_{96}

Early Susc
ROS Low

Adult Resist
ROS High

Transcriptomics Results : Lr28, Lr48

Lr28 Mediated Seedling Resistance



ES: Early susceptible
LS: Late susceptible
ER: Early resistant
LR: Late resistant

Sharma et al 2019 (FPB)

Lr48-Mediated Adult Plant Resistance



S = More Genes Active

R = More genes silent

Jain et al 2019 (FPB)

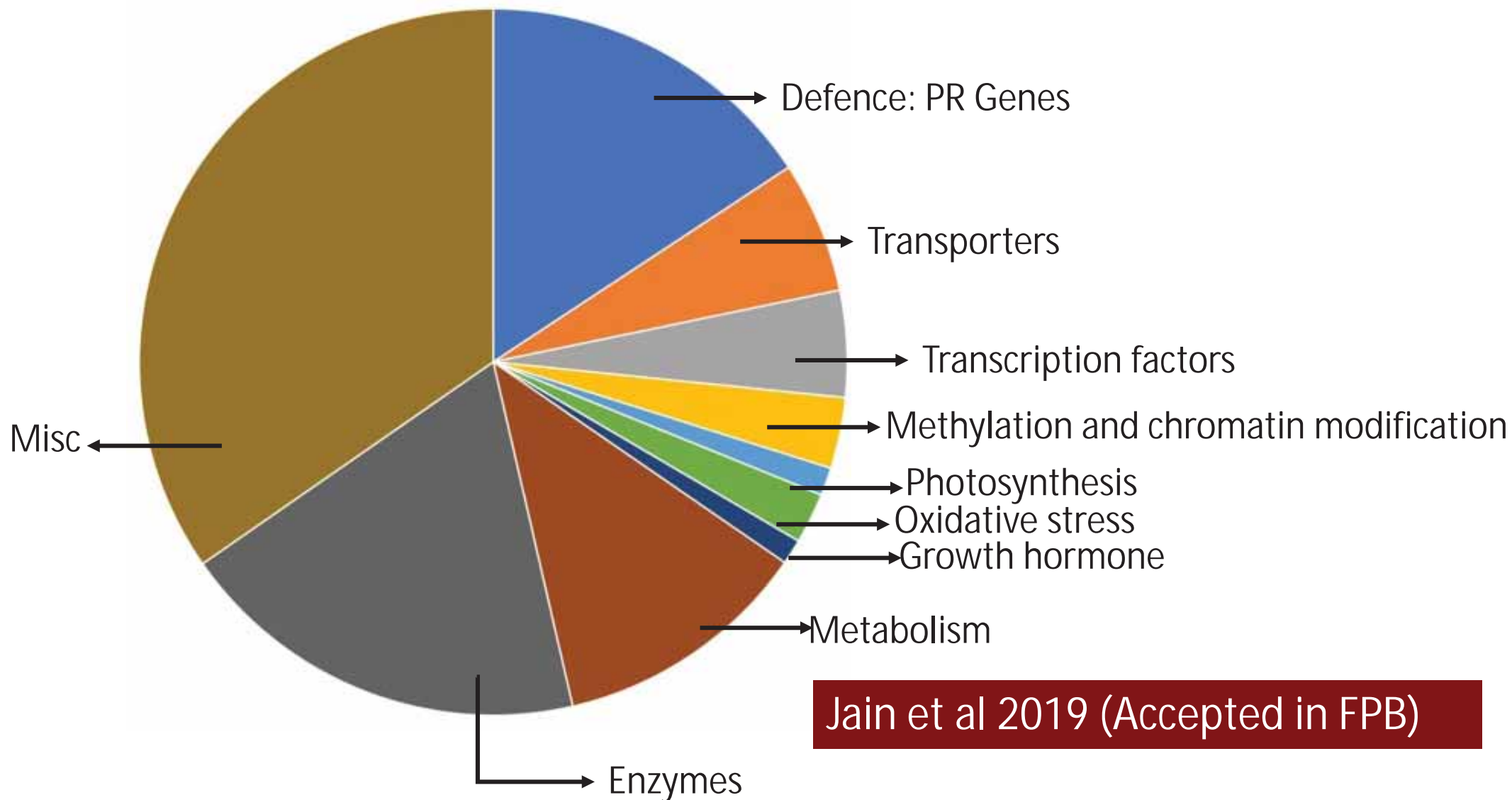


Early Susc
ROS Low

Adult Resist
ROS High

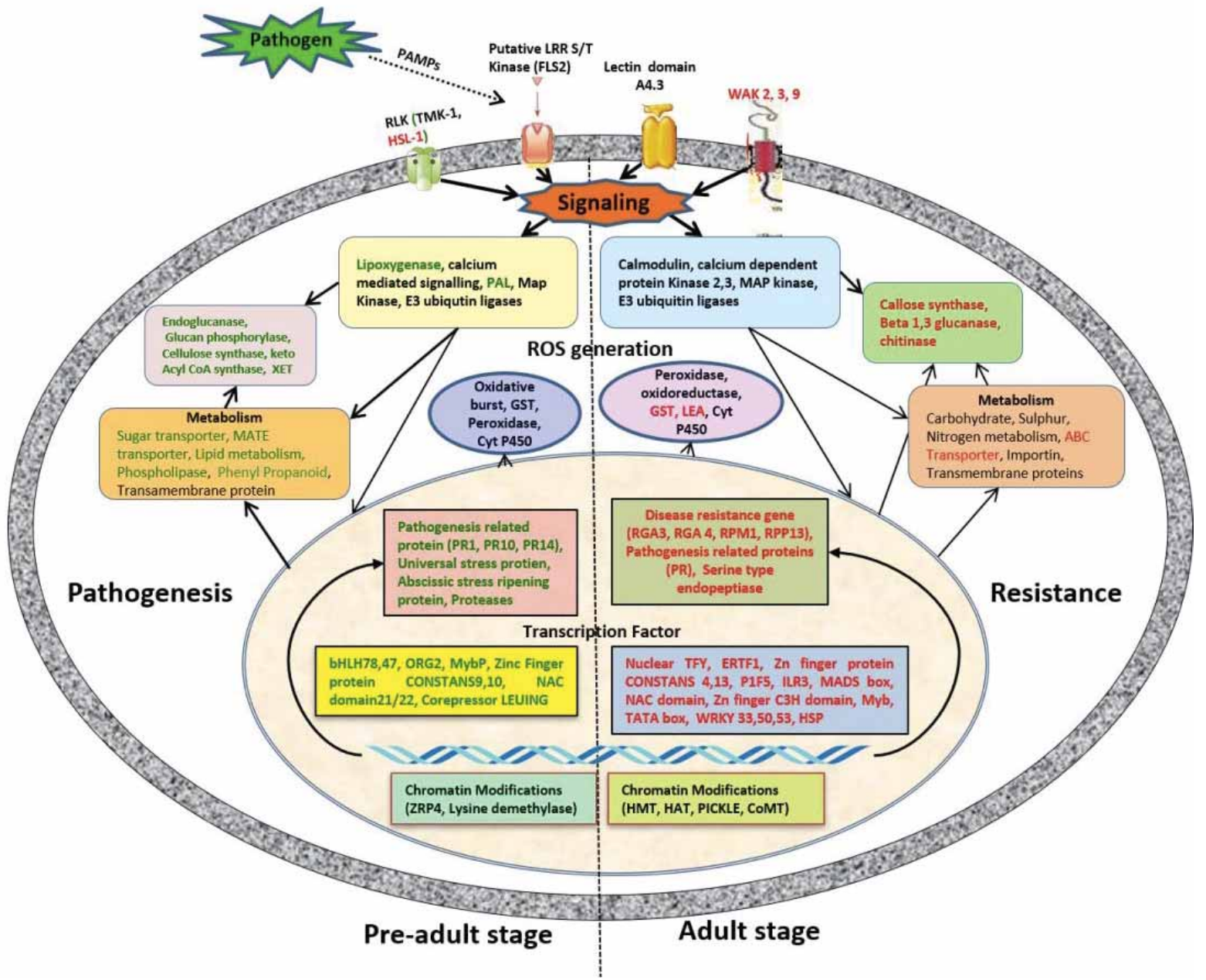
(Late S/R compared with Early)

Different Classes of DEGs Due to *Lr28/Lr48*



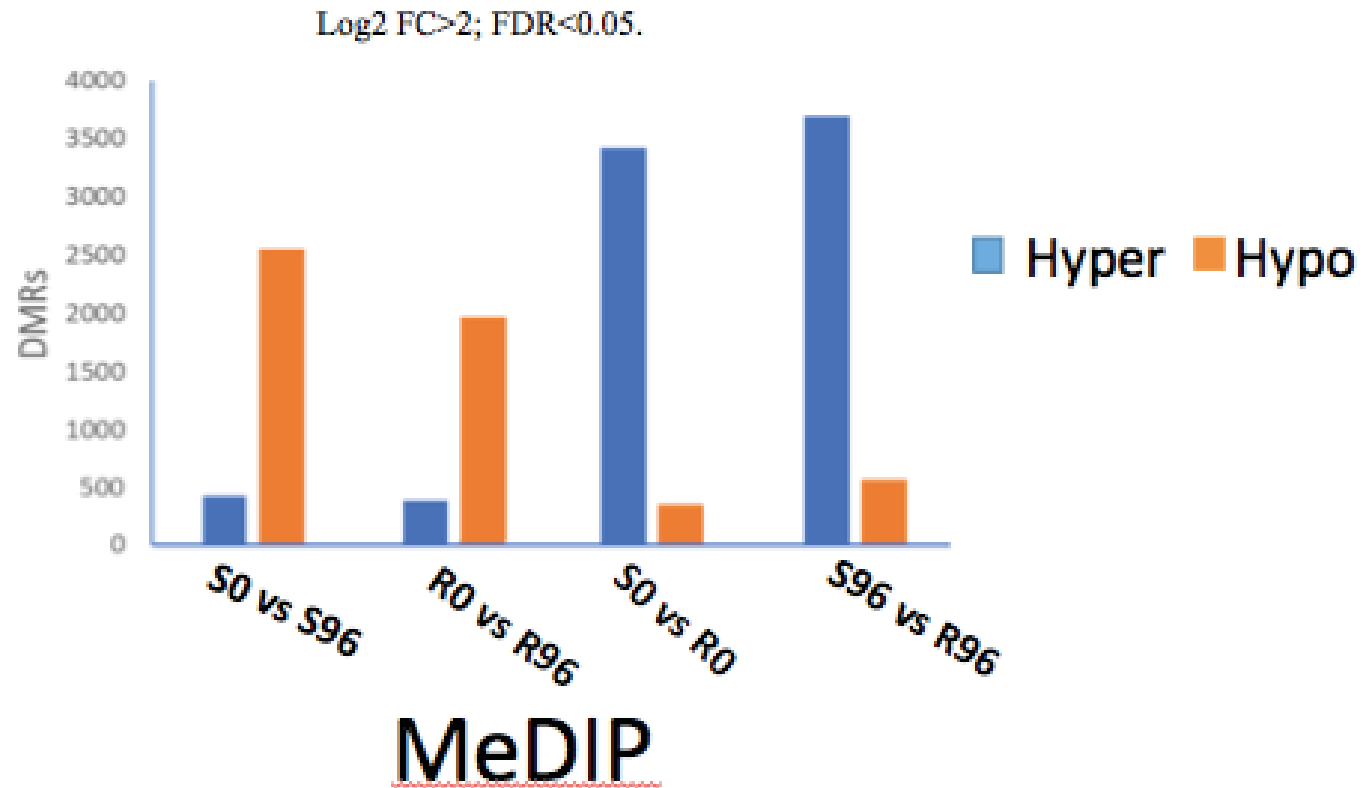
Jain et al 2019 (Accepted in FPB)

Network of DEGs During Infection: Lr48 at Pre-Adult and Post-Adult Stages



Hypomethylation of different sets of genes during progress of infection.

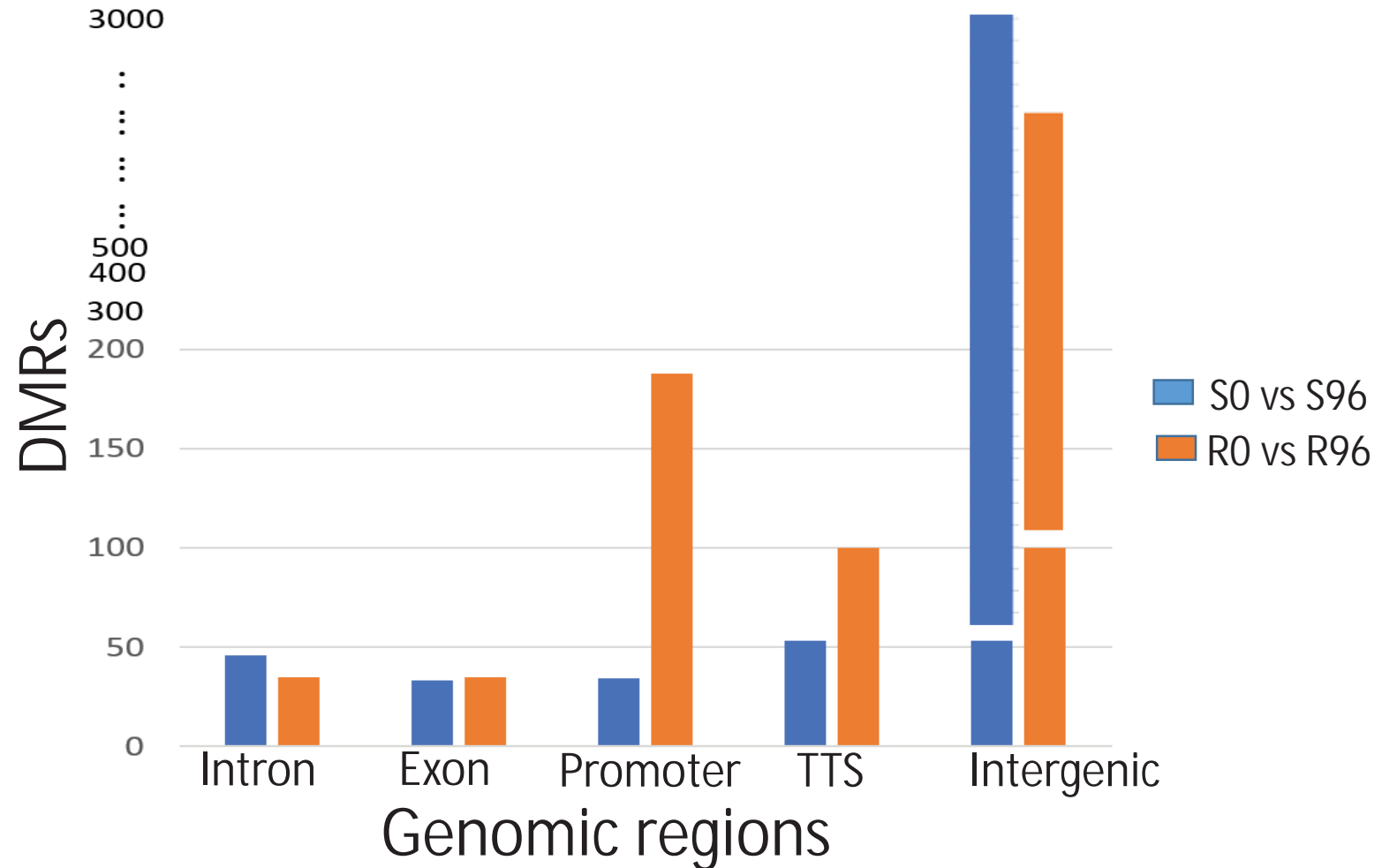
Hypermethylation of genes due to the presence of *Lr28*



Hyper/Hypo-methylated Differentially Methylated Regions (DMRs)

MeDIP: DMRs in Different Genomic Regions

A number of transposable elements like LTRs, Gypsy elements, etc. also identified in intergenic regions in each of the four treatment pairs



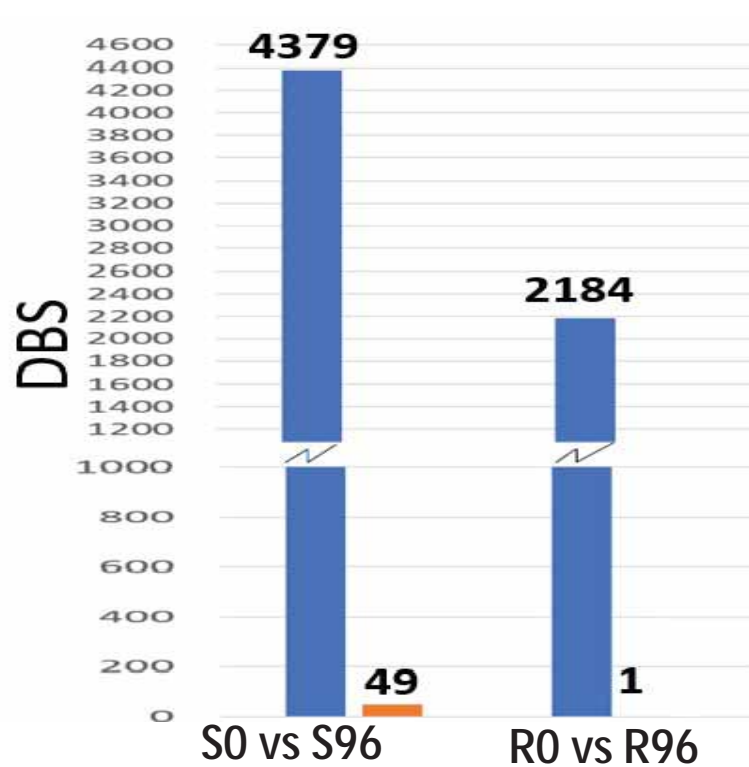
Chromatin Immunoprecipitation (ChIP):
qPCR/ Sequencing
H3K4ac/K9ac; H3K4me3/K27me3

ChIP-qPCR: Six Genes Selected from Transcriptome Study

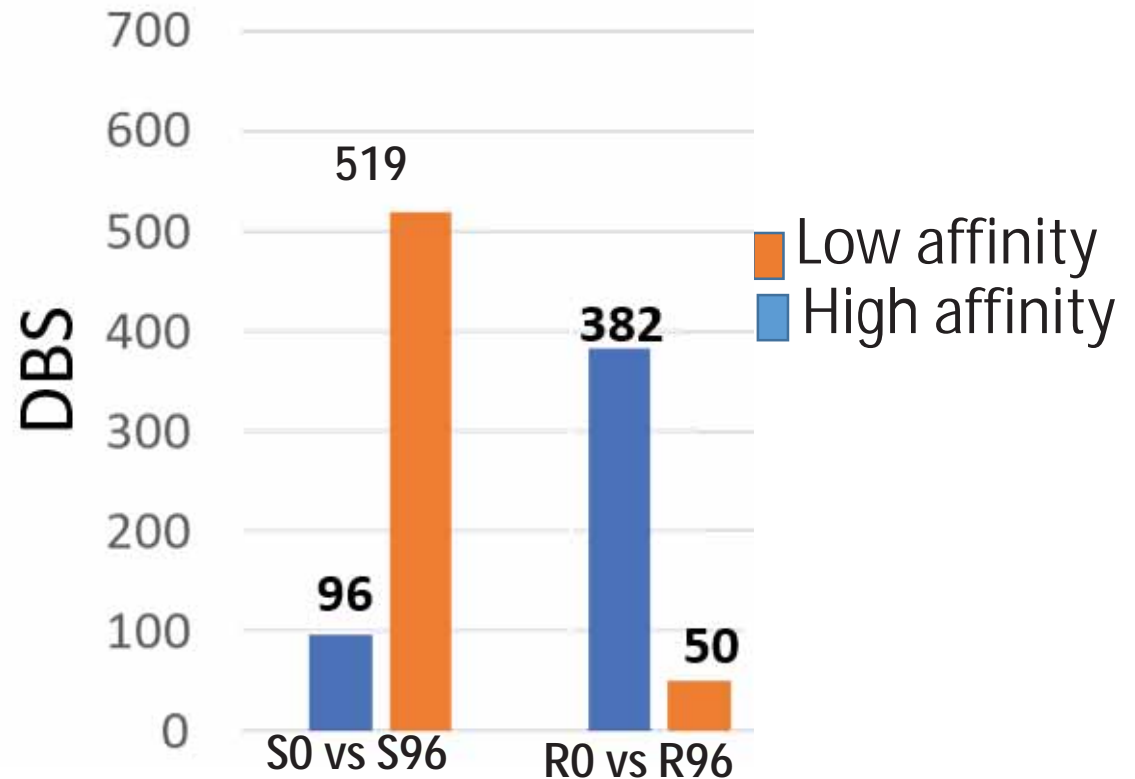
- ChIP Protocol Optimised in Wheat.
- Two Acetylation Marks: **H3K4ac and H3K9ac**
- ChIP qPCR for H3K4/K9 Acetylation of the Promoters (P1 & P2) in Following 6 Genes:

<i>1. N-acetyltransferase</i>	<i>4. Asr1 (ABA Stress Ripening)</i>
<i>2. WRKY40</i>	<i>5. Peroxidase12</i>
<i>3. WRKY70</i>	<i>6. Sarcosine oxidase</i>

ChIP-Seq: Differ Binding Sites (DBS): H3K4/27me3



H3K4me3; Activation



H3K27me3; Repressor

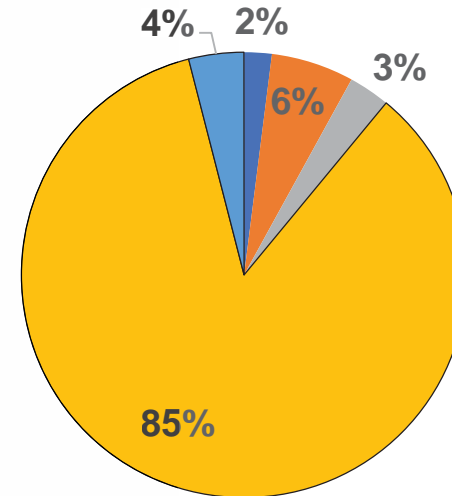
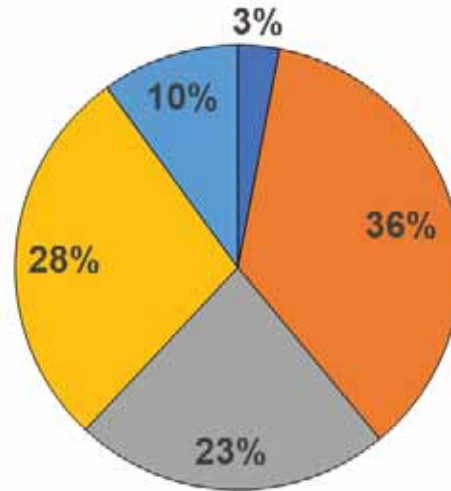
Susceptibility regulated by large scale activation of genes;
Resistance by suppression as well activation of genes

Distribution of DBS in Genomic Regions

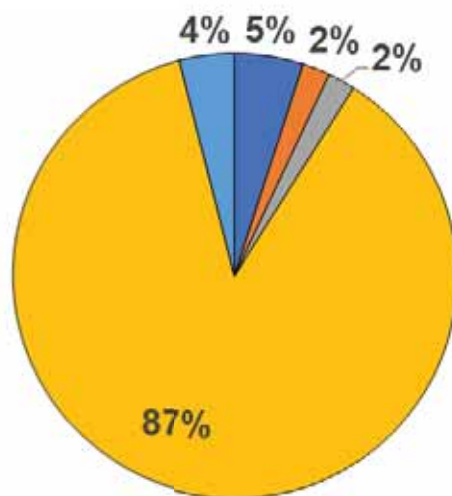
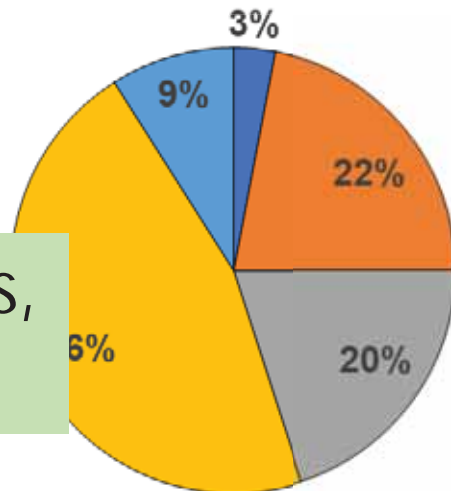
H3K4me3: Activation

H3K27me3: Repression

S0 vs S96



S96 vs R96



DBS distributed in exons, introns and intergenic

DBS mainly in intergenic regions

Bi-Sulphite-Seq (BiS-Seq)

- DNA samples from the Lr28 NILs are used for BiS-seq (C-Methyl-Seq).
- Differentially methylated regions/genes (DMRs/DMGs) to be identified and correlation of DMRs with DEGs is being studied.
- Raw data (80 to 90Gb per sample) is received and being analysed for C methylation in CG, CHG and CHH contexts

Bisulphite Sequencing +
ChIP sequencing



Epigenetic Modifications due to C
Methylation + Histone Modifications

Identification of Leaf Rust Responsive miRNAs

Leaf Rust Responsive miRNAs and Their Targets in Resistance and Susceptible NILs

miRNAs in Four Treatments

HOST

Pathogen

miRNA	S0	S96	R0	R96
Conserved miRNAs	862	733	892	800
Novel miRNAs	835	412	838	866
Novel miRNAs	15	20	21	20

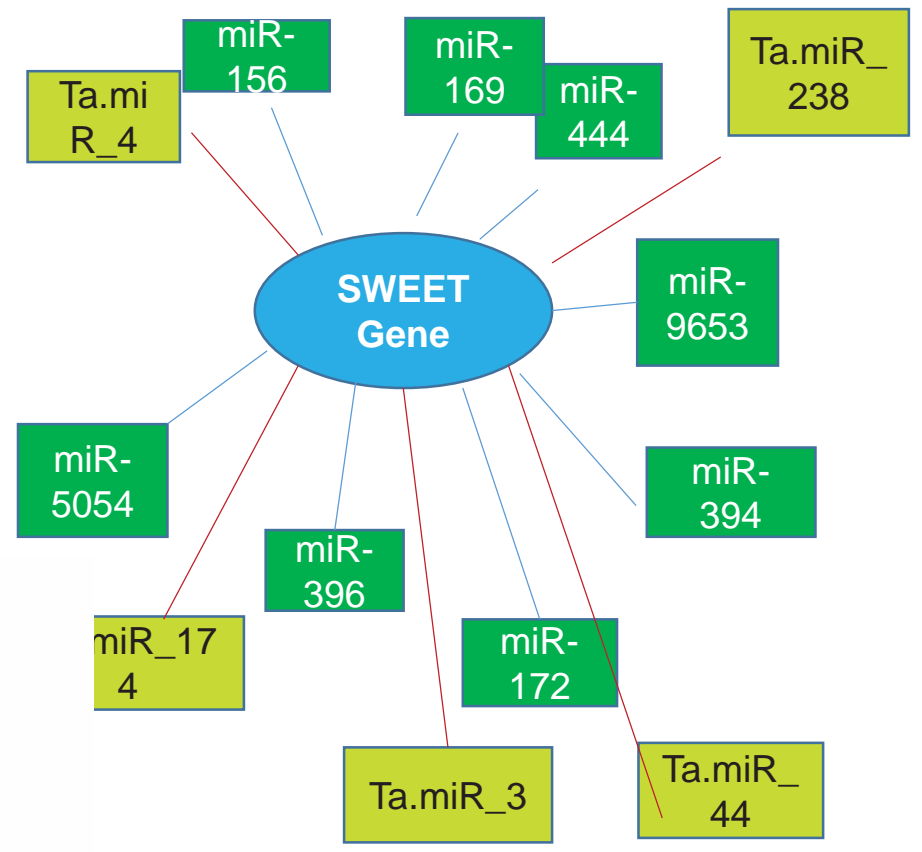
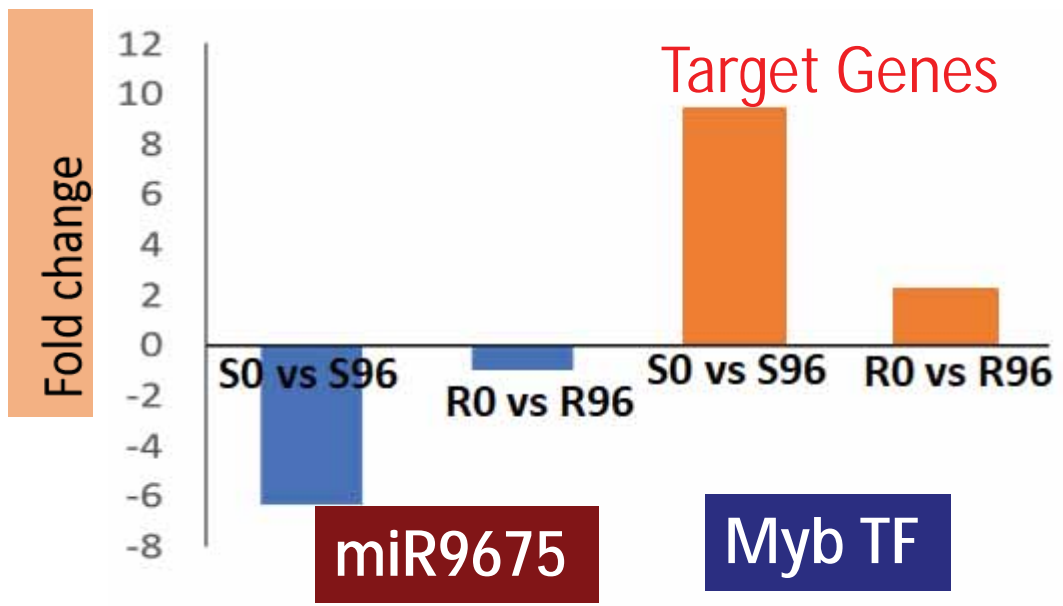
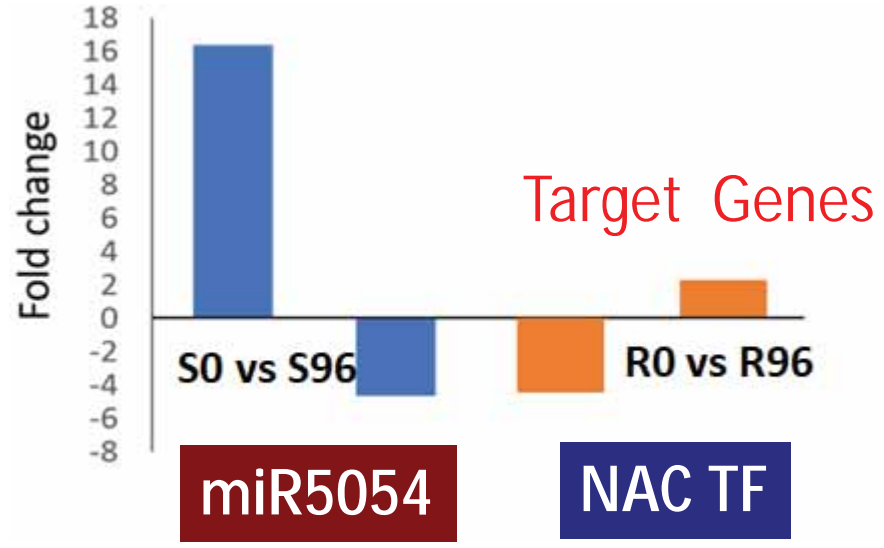
Identification of microRNAs

Treatments	DE miRs	Regulation
S0 vs S96	46	26↑ 20↓
R0 vs R96	66	30↑ 36↓

- 13 miRNAs were selected for validation using qRT-PCR;
- 12 miRNAs validated (except miR9669)
- 72 targets for HD96 and 110 for Lr96 were identified
- 11 targets validated using qRT-PCR

qRT-PCR for Two miRNA/Targets

Conserved miRNAs
Novel miRNAs



SWEET gene targeted by miRNAs in HD96 vs Lr96: A Review on SWEET Genes

Spot Blotch (Host): *Tsn1* in Indian Wheats

No.	Wheat species	Total genotypes	No. of <i>Tsn1</i> genotypes
1.	<i>Triticum aestivum</i>	182	76 (41.7%)
2.	<i>T. durum</i>	12	3 (25.0%)
3.	<i>T. dicoccum</i>	26	2 (7.7%)
	Total	220	81 (36.8%)

Spot Blotch (Pathogen): *ToxA* in Indian Isolates

- 110 Isolates of Spot Blotch from mega-environments of India
- 77 (70%) were *ToxA* positive

20 wheat cultivars (11 with *Tsn1* and 9 with *tsn1*) were inoculated with *ToxA*-positive isolates, seedlings of only those carrying *Tsn1* allele (not *tsn1*) developed necrotic spots surrounded by a chlorotic halo.

Summary & Conclusions

- R Gene Codes for NLR Protein (Recent Work 2019)
 - Gene-for-Gene & Inverse Gene-for-Gene
 - Oligomerizes into Resistosome
 - TIR Domain Carries NADase Activity Causing Cell Death
- Transcriptome: Lr28, Lr48 (APR); Many Genes Regulated
- Epigenome: Regulation of Genes Through Histone Modifications (H3Ac/Me), DNA Methylation and ncRNA (miRNA + Targets)

Project Group Members



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CCSU, Meerut



IIWBR, Shimla



DEPARTMENT OF BIOTECHNOLOGY

Ministry of Science & Technology

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