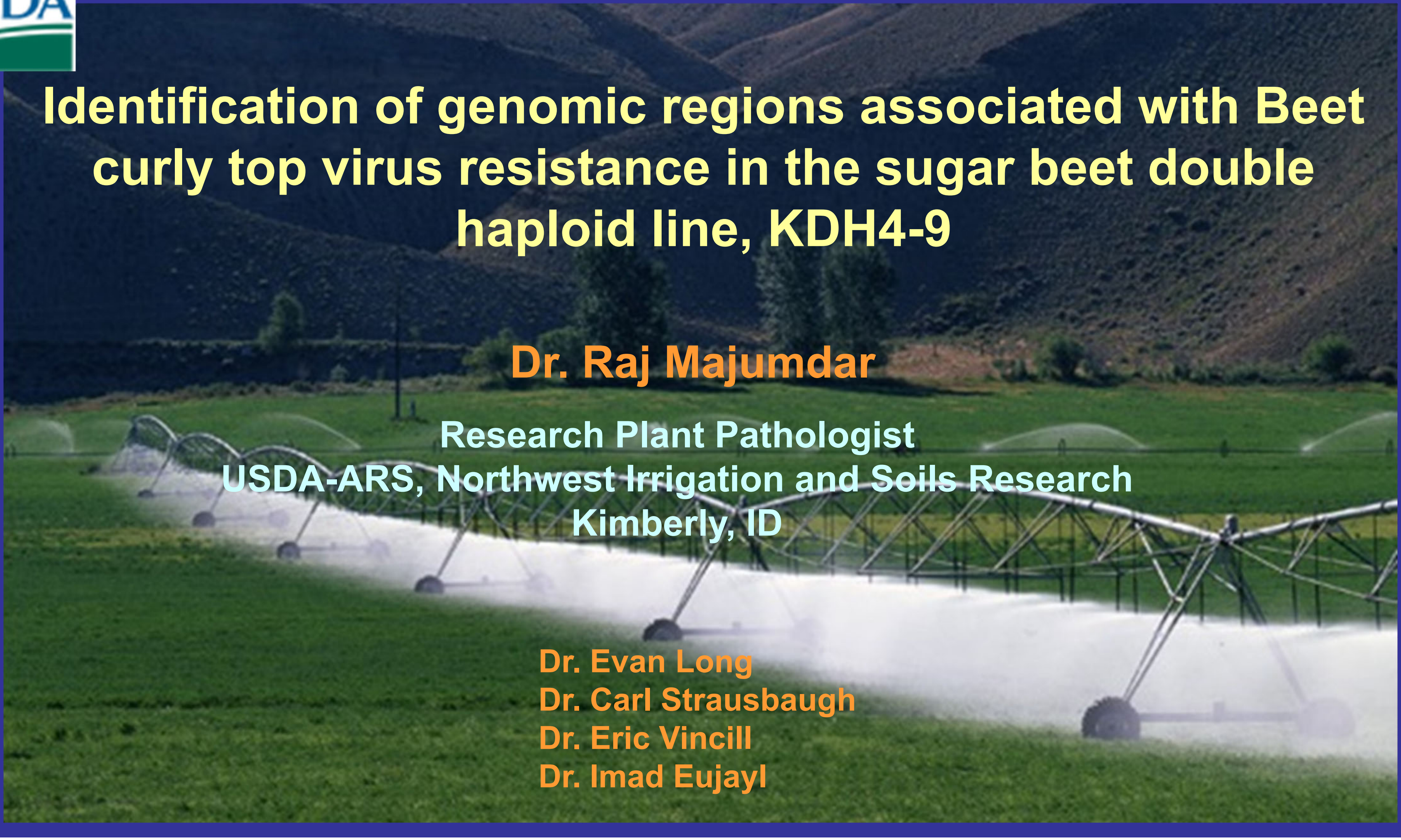

Identification of genomic regions associated with Beet curly top virus resistance in the sugar beet double haploid line, KDH4-9

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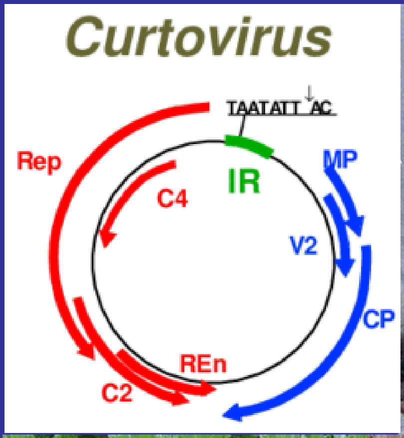
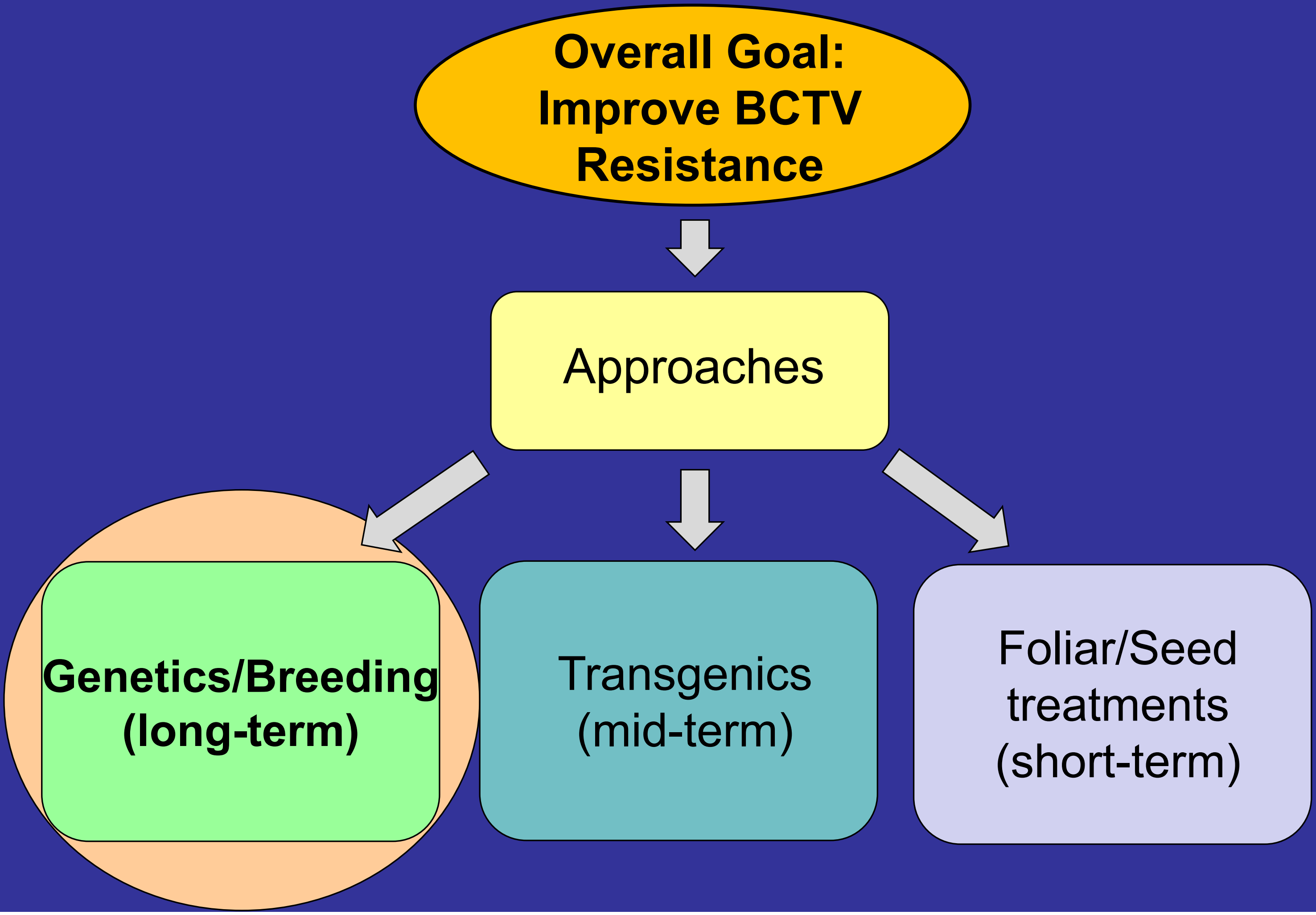
BCTV Infection in Sugar beet

- ➢BCTV is a single stranded DNA virus which significantly reduces yield/sugar content in semi-arid growing regions.
- ➢The virus is vectored by beet leafhopper (BLH).
- ➢Disease severity depends upon specific BCTV strains; Svr and CA/Logan.
- ➢Sources of genetic resistance are limited. Primarily dependent upon neonicotinoid seed treatment. Requires identification of novel resistance sources, and other effective and safe mitigation strategies.



BCTV Resistance in Sugar beet



Overall Goal: Improve BCTV Resistance

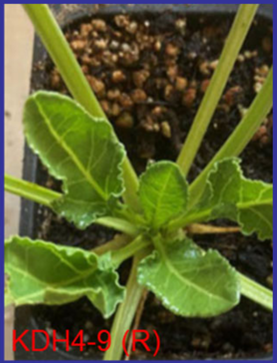
Approaches

Genetics/Breeding (long-term)

Transgenics (mid-term)

Foliar/Seed treatments (short-term)

Using Double Haploids and Graded-pool Sequencing to Dissect BCTV Resistance



Resistant
(parent)

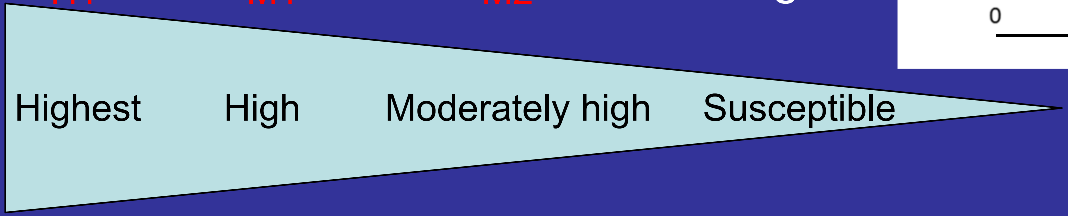
X

Susceptible
(parent)

F1

F2

H1 M1 M2 S



Highest High Moderately high Susceptible

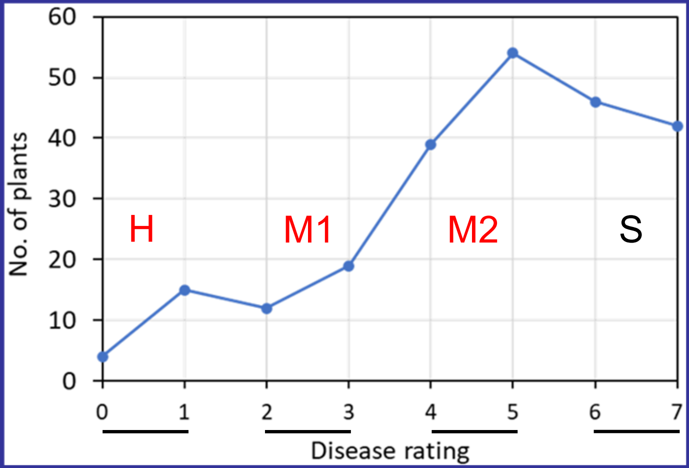
Phenotyping, sorting, pooling

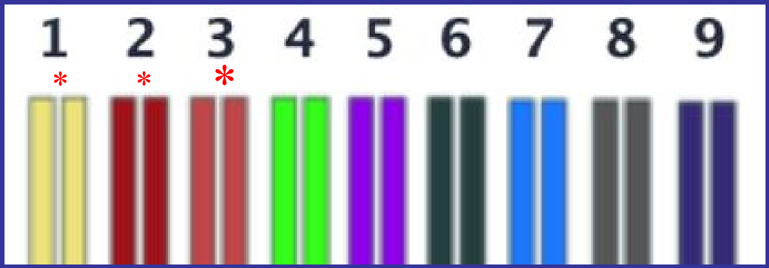
Sequencing (30X) bulked DNA from each group

Data alignment to the EL10.2 genome

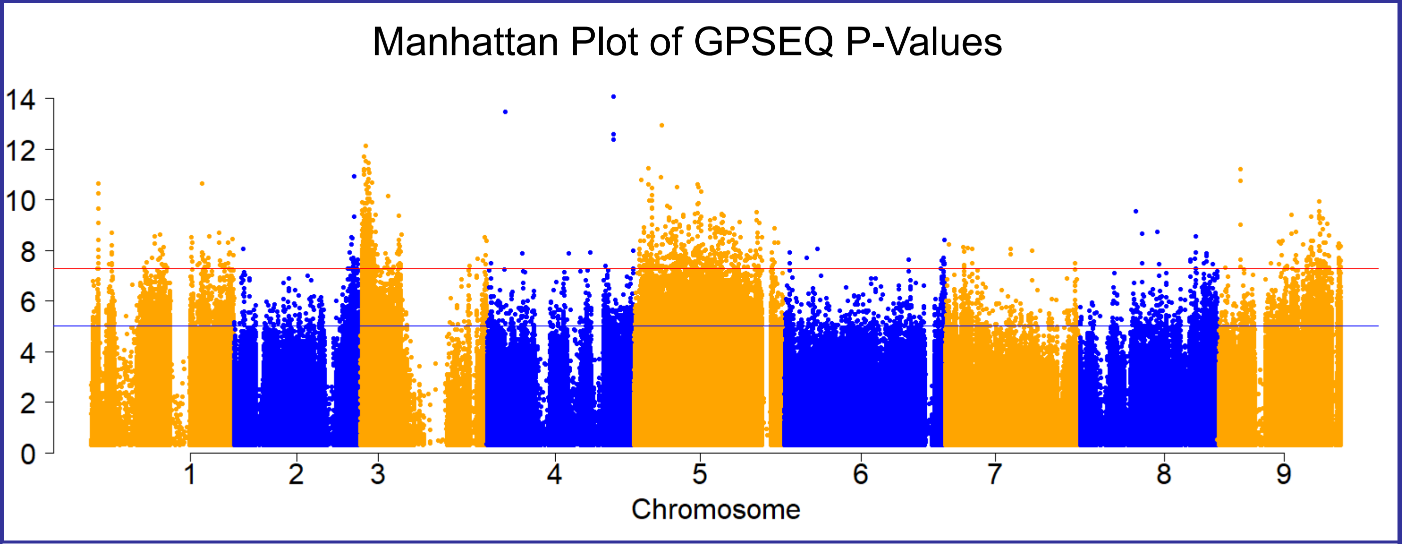
Background noise reduction

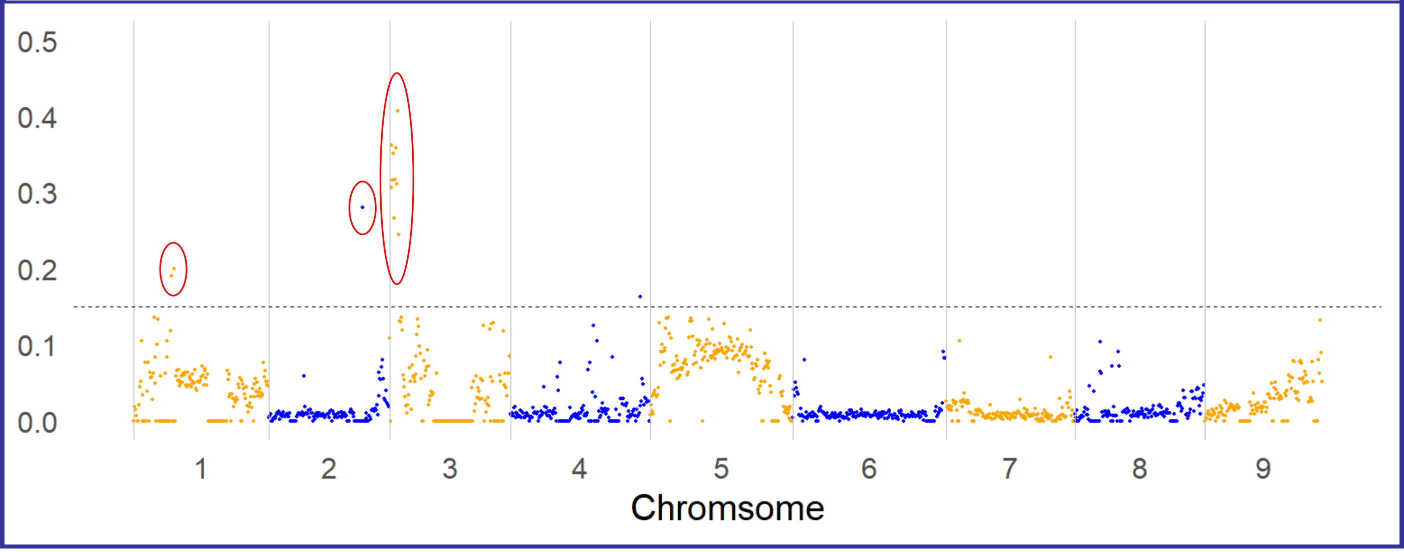
QTL identification





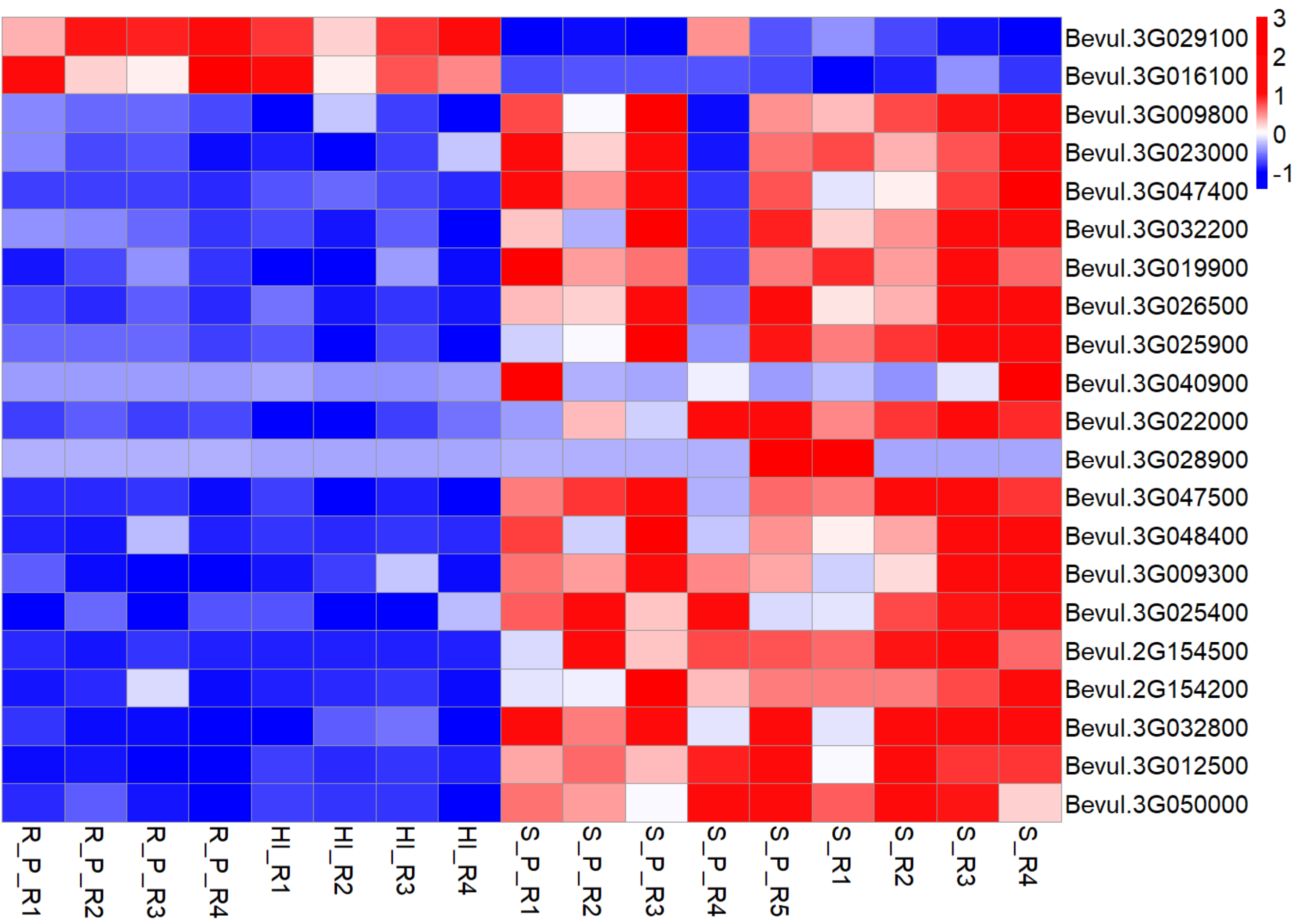
Quantitative Trait Loci (QTL) for BCTV Resistance in KDH4-9


Manhattan Plot of GPSEQ P-Values



SNP: single nucleotide polymorphism

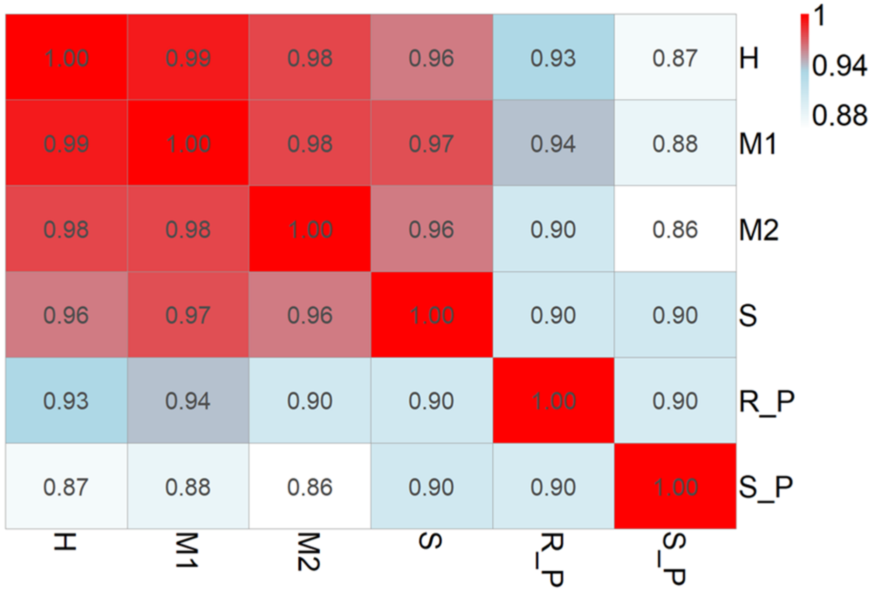
Putative Candidate Genes Combining GP-seq and RNA-seq



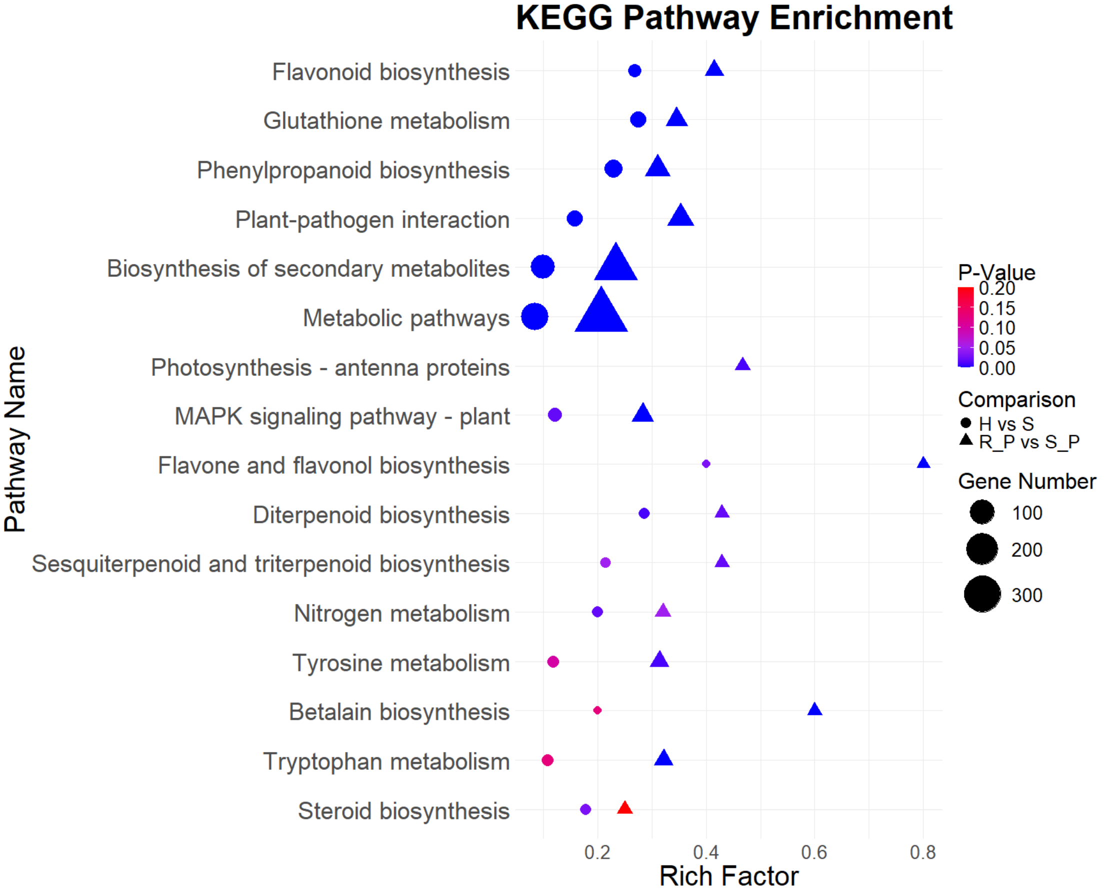
Growth-regulating factor 7
Phenylalanine--tRNA ligase
Inorganic phosphate transporter
Stomatin-like protein 2
Serine/threonine-protein kinase FLS2
yippee family
Nucleoside triphosphate hydrolases
Acidic endochitinase
DOWNY MILDEW RESISTANCE 6
Mta sah nucleosidase
Pathogenesis-related protein 5
Heat shock protein
Serine/threonine-protein kinase
Alpha-glucosidase
Myb4
NA
Amino acid permease 8
Disease resistance protein RGA1
Iron ascorbate-dependent oxidoreductase
Fk506-binding protein
Serine/threonine-protein kinase

R_P: resistant; S_P: susceptible (parents)
H1: highest resistance; S: susceptible (segregating)

Pathway Enrichment of DE Genes Among R and S Groups

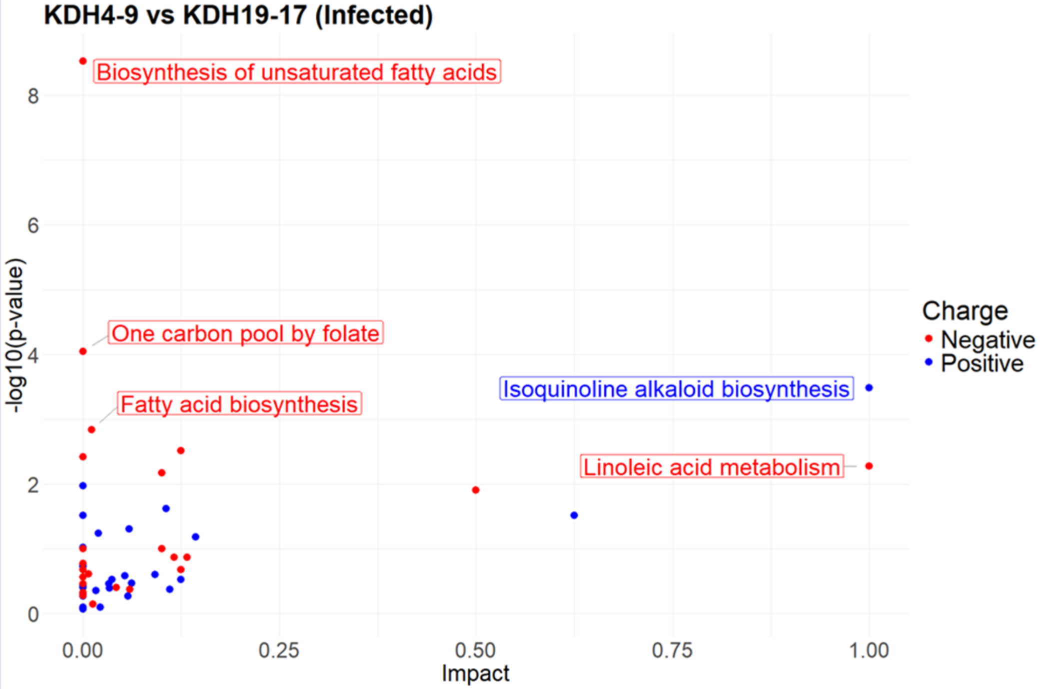


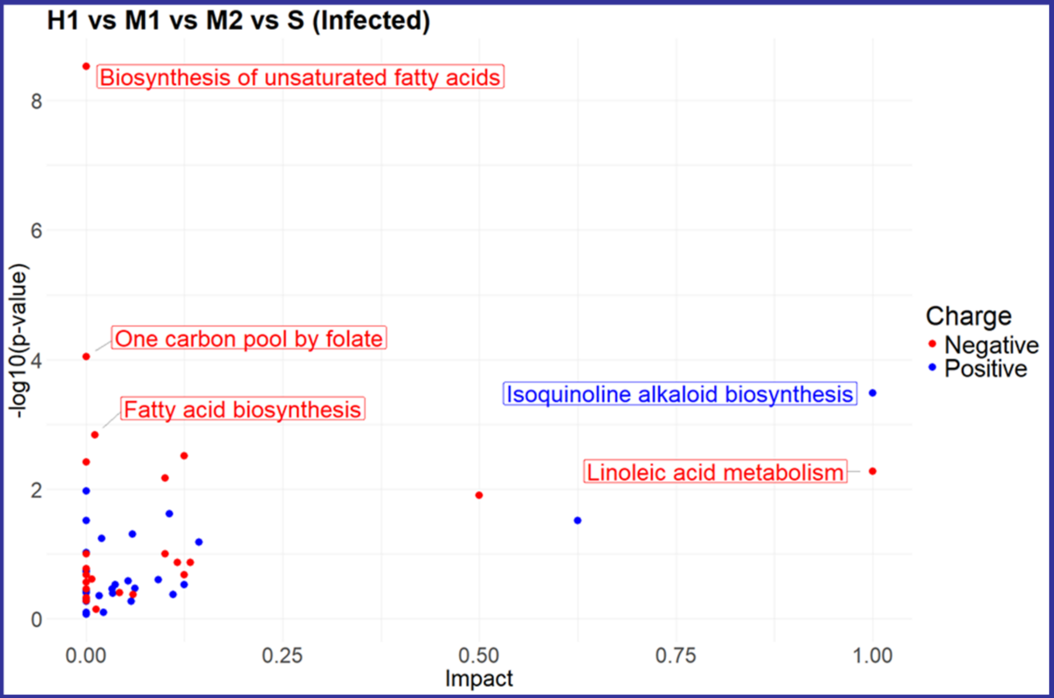
	H	M1	M2	S	R_P	S_P
H	1.00	0.99	0.98	0.96	0.93	0.87
M1	0.99	1.00	0.98	0.97	0.94	0.88
M2	0.98	0.98	1.00	0.96	0.90	0.86
S	0.96	0.97	0.96	1.00	0.90	0.90
R_P	0.93	0.94	0.90	0.90	1.00	0.90
S_P	0.87	0.88	0.86	0.90	0.90	1.00



R_P: resistant parent; S_P: susceptible parent
H: highest resistance (segregating)
M1: high resistance (segregating)
M2: moderately high (segregating)
S: susceptible (segregating)

Pathway Enrichment of Metabolites Among R and S Groups



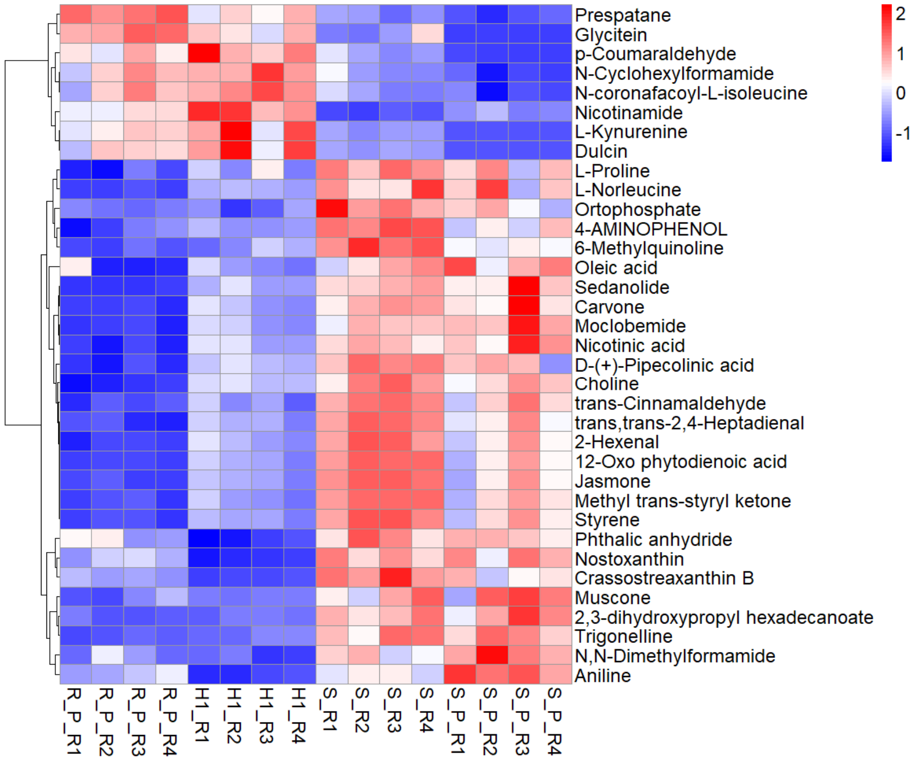


R_P: resistant (KDH4-9), S_P: susceptible (KDH19-17) [parents]

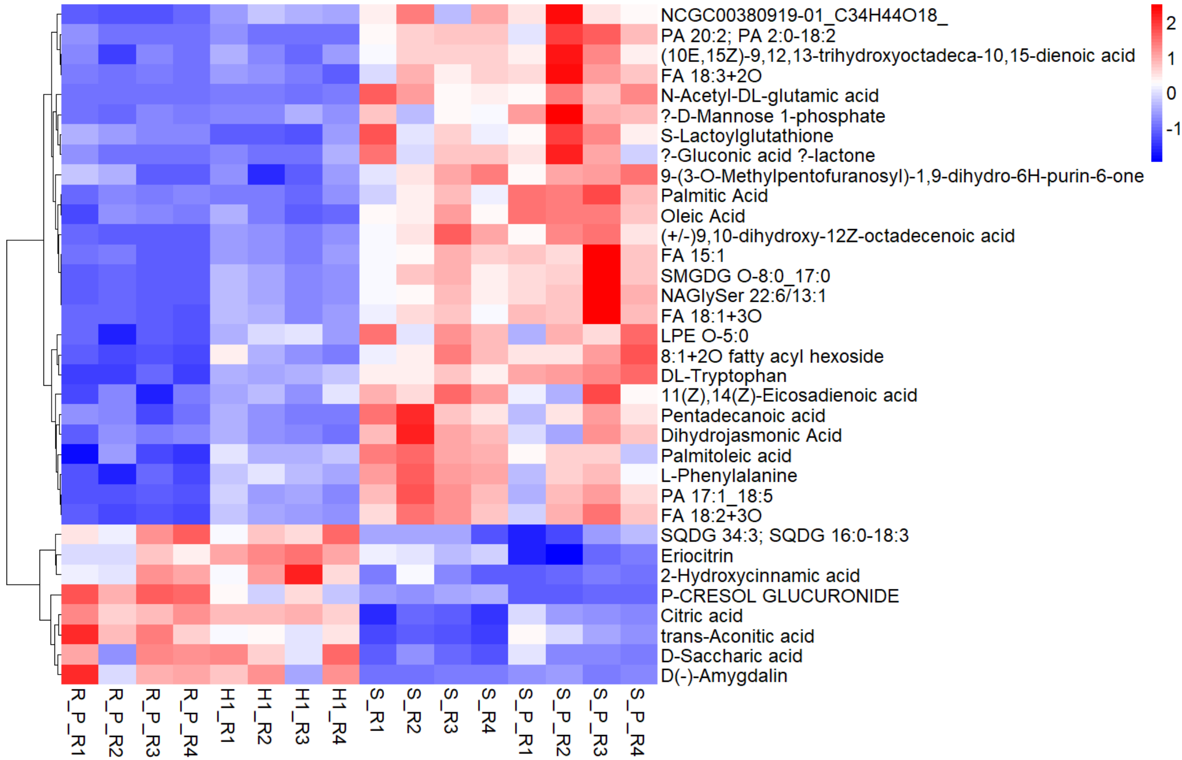
H1: highest resistance, M1: high resistance, M2: moderately high, S: susceptible (segregating)

Differentially Altered Metabolites Among R and S Groups

+ve ion mode



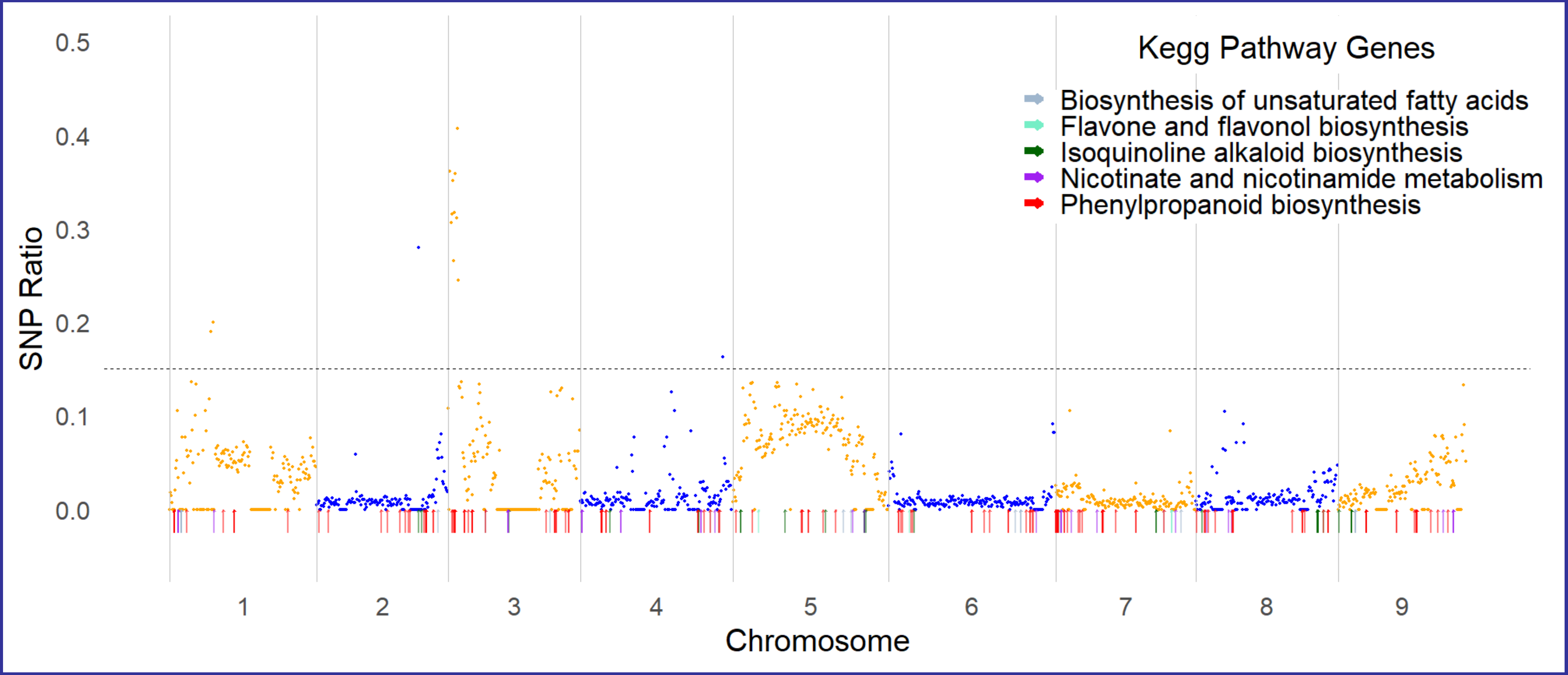
-ve ion mode



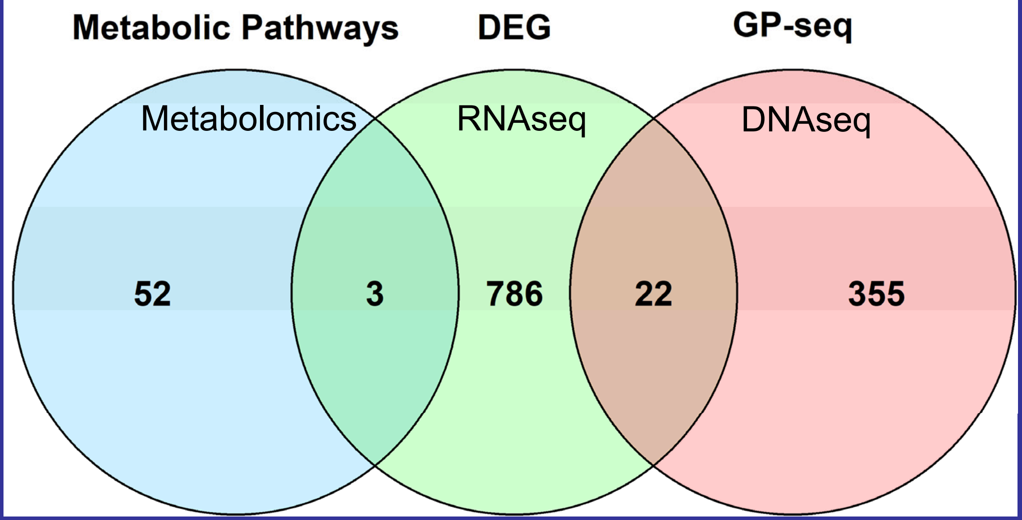
R_P: resistant; S_P: susceptible (parents)

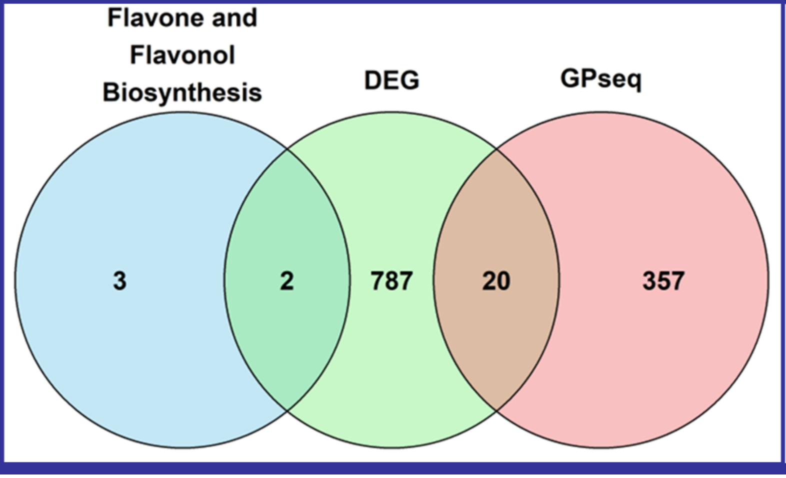
H1: highest resistance; S: susceptible (segregating)

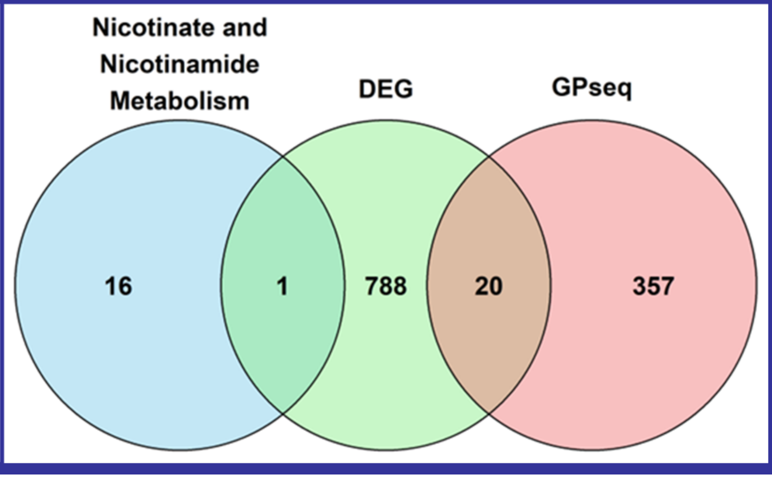
Overlaying Metabolite-related Genes in the Resistance Associated QTLs

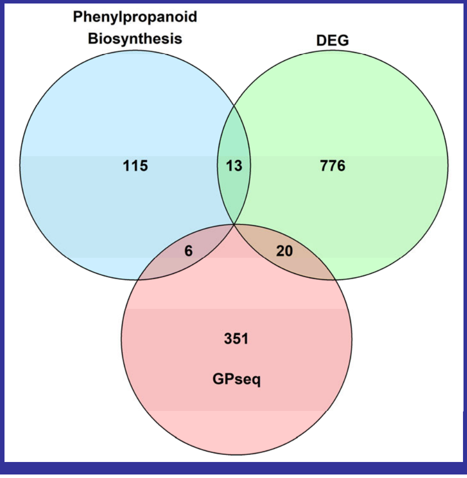


Overlaps Between Multi-omics Data Associated With Resistance





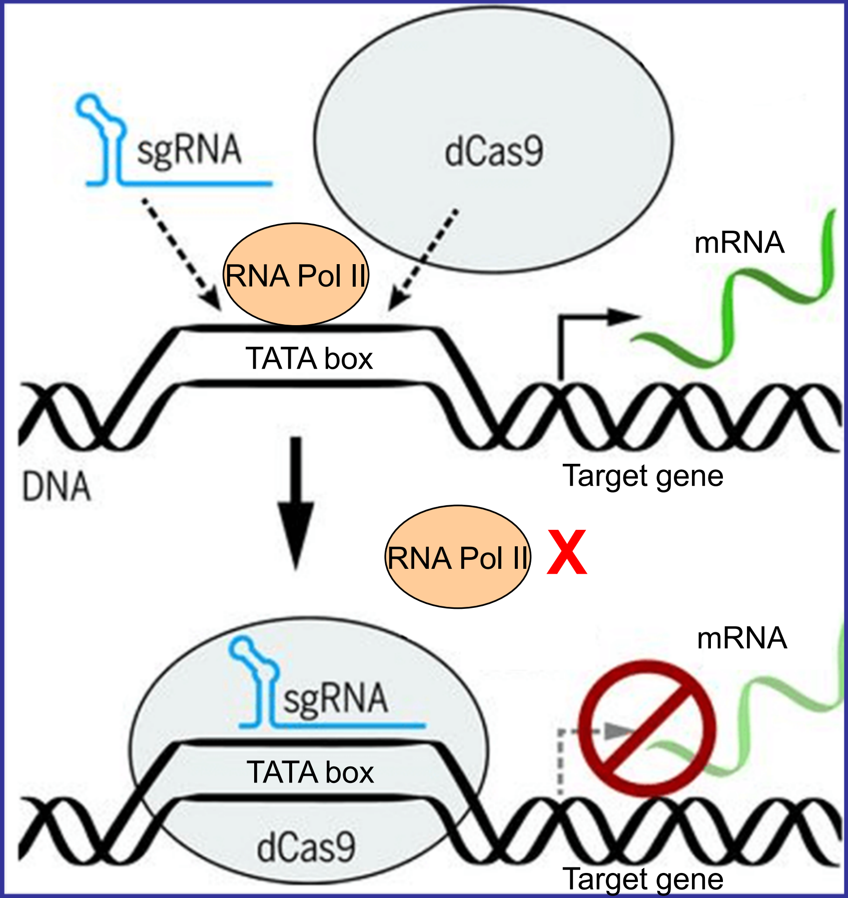




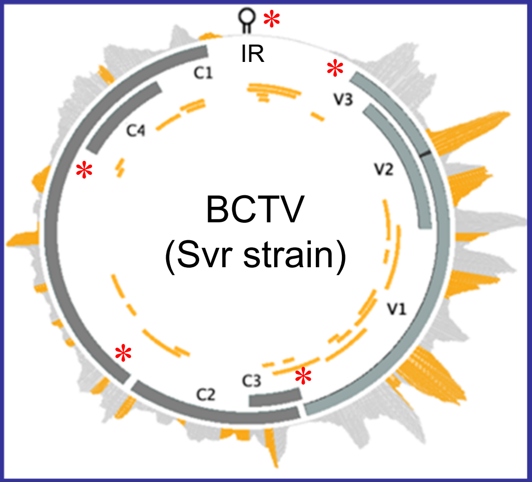
Functional Genomics: CRISPR interference (CRISPRi)

- Modified CRISPR-cas9 system where a mutated Cas9 (dCas9; does not alter DNA sequence) is used.
- CRISPR-dCas9 is designed to bind to the target gene (TATA site) and inhibits transcription. It can be used to block replication of viruses.

RNAi	CRISPRi
Post-transcription	Pre-transcription
Some off target effects	Highly specific
Moderate to high silencing of target gene	Greater silencing than RNAi



CRISPRi Implementation for BCTV Resistance



Design CRISPRi constructs containing sgRNAs to target BCTV/sugar beet genome ✓ ~6 weeks

↓

Agrobacterium mediated transformation of BCTV susceptible sugar beet genotype ✓

↓

Confirm sugar beet stable transformation events ✓

~7 months

↓

Grow transgenic plants (F1), vernalize, bolting, seed production ✓ ~8 months

↓

Evaluate transgenic F2 plants for BCTV resistance ~6 weeks

Conclusions and Future Directions

- Major QTLs (BCTV resistance) identified in Chr. 3, and minor QTLs in Chr. 1 and 2.
- Pathway enrichment of DE genes and metabolomics analysis revealed putative roles of metabolites such as phenylpropanoids, flavonoids in BCTV resistance.
- Assembling (chromosome level) of the KDH4-9 genome to capture structural and other variations associated with resistance.
- Using functional genomics tool to validate and improve sugar beet BCTV resistance.

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BEET SUGAR
DEVELOPMENT
FOUNDATION

Snake River Sugar Alliance

Thank you!