

**CHARACTERIZATION OF SOYBEAN ACCESSIONS FOR
SCLEROTINIA STEM ROT RESISTANCE AND ASSOCIATION
MAPPING OF QTLS USING A GENOTYPING BY SEQUENCING
(GBS) APPROACH**

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Sclerotinia stem rot is the most important soybean disease in Eastern Canada. The development and deployment of resistant cultivars represents the most cost-effective means of limiting the impact of this disease. In view of ensuring durable resistance, it would be preferable to deploy different genetic resistances. For this, it is imperative to identify germplasm harbouring different resistance loci and to provide breeders with closely linked molecular markers to facilitate breeding. With this in view, we assessed resistance using a highly reproducible method we have developed in a diverse collection of 92 soybean lines previously reported to be resistant to *Sclerotinia* based on both natural and artificial inoculation. Of these, half (50%) exhibited a high level of resistance (equal to or better than our resistant checks), 40% showed intermediate resistance (between our resistant and susceptible checks) and 10% were very susceptible (more susceptible than our susceptible checks). This collection of lines was genetically characterized using a genotyping by sequencing protocol that we have optimized for soybean. After filtering for loci with a minor allele frequency > 0.05 , 8,396 single nucleotide polymorphisms (SNPs) were used to perform an association analysis. The mixed linear model as implemented in the TASSEL software was used to perform association mapping for SSR resistance. Population structure was described using a principal component analysis and genetic relatedness between lines was estimated using a kinship matrix. The model performed well in accounting for population structure and relatedness, as only 6.8% of the p-values were < 0.05 using 3 PCs. On chromosome Gm03, a cluster of seven markers had p-values $< 10^{-3}$, with the strongest association showing a p-value $= 2.03 \times 10^{-6}$. Other putative significant associations were found on chromosomes Gm08, Gm15 and Gm20 having p-values of $< 10^{-4}$. This work will facilitate the identification of lines to cross in order to combine different resistance genes and also to increase genetic diversity in Canadian soybean.

Characterization of Soybean Accessions for Sclerotinia Stem Rot Resistance and Association Mapping of QTLs using a Genotyping by Sequencing (GBS) Approach



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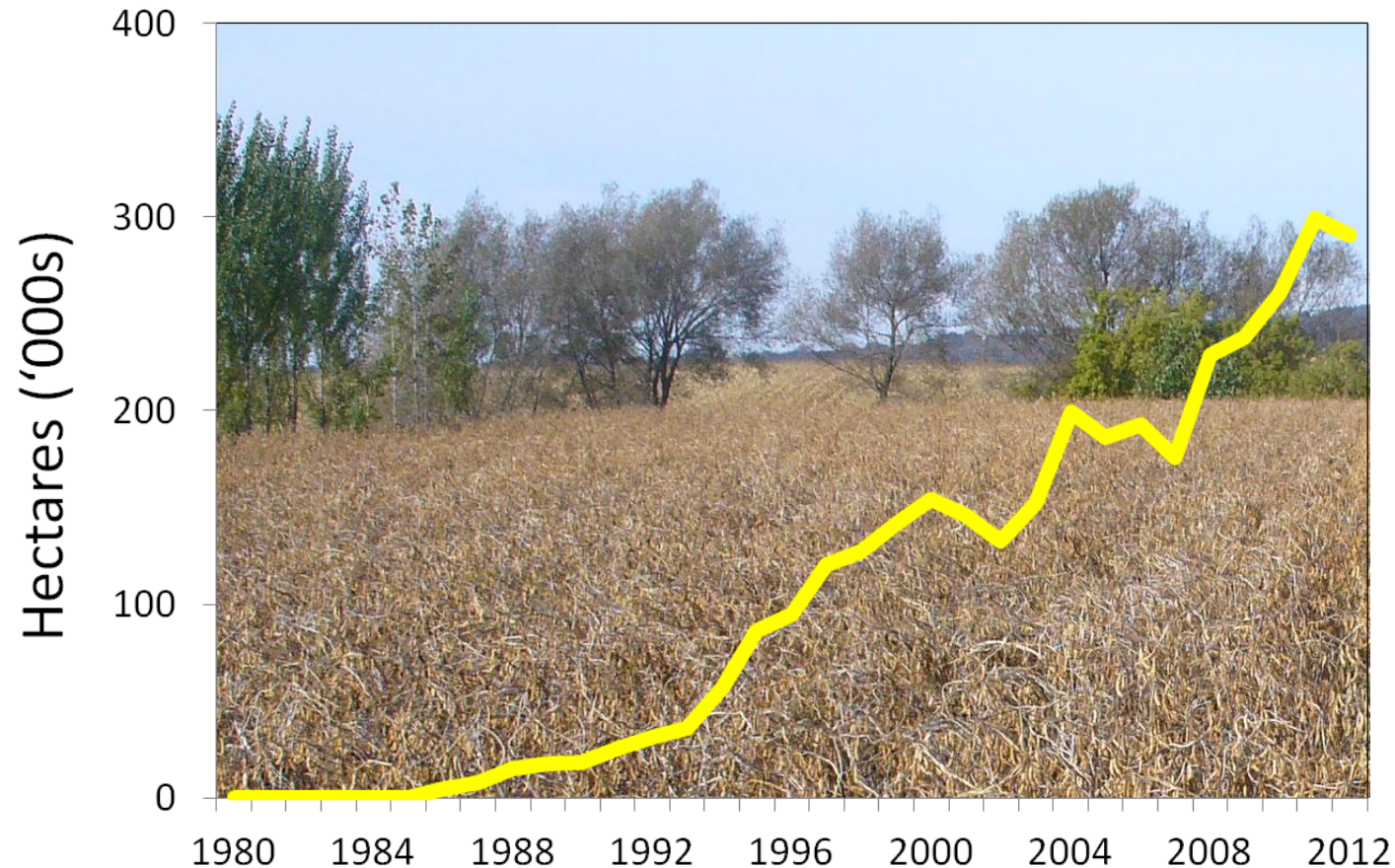
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The northern frontier for soybean



Soybean in the province of Quebec

A meteoric rise



Diseases tend to follow

❖ White mold (*Sclerotinia sclerotiorum*)

- “Cosmopolitan” pathogen found on >400 plants
- Prefers cool and humid conditions

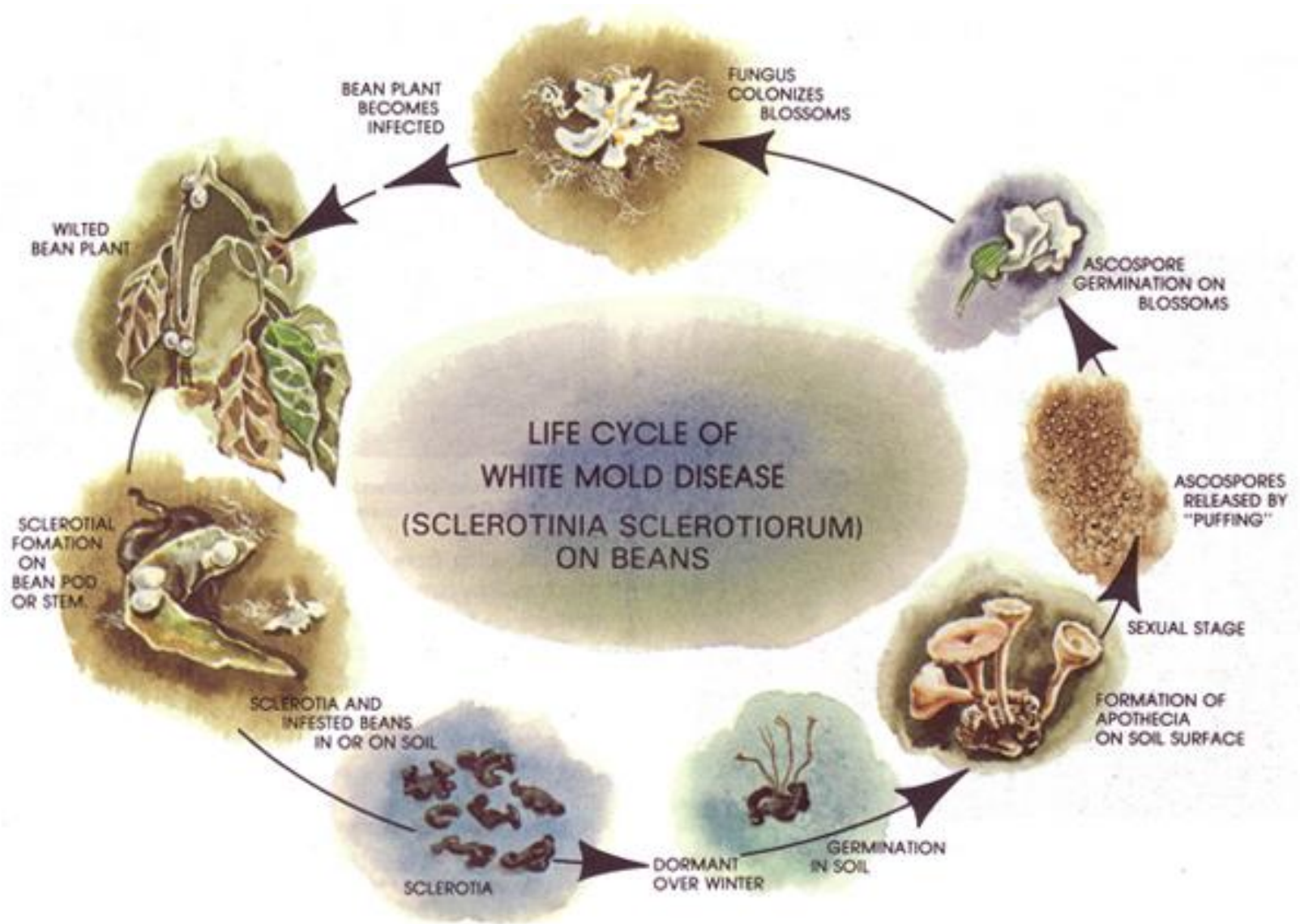
❖ Root rot (*Phytophthora sojae*)

- Favored by cool and wet soils in the Spring

❖ Rising concerns

- Soybean cyst nematode heading north
- Asian soybean rust?

Disease cycle of *S. sclerotiorum*



Challenges for breeders

- ❖ Natural incidence in the field is too sporadic and uneven to allow efficient selection
- ❖ Artificial inoculation methods have not always been well correlated with results seen in the field

Difficult to accurately measure white mold resistance
in germplasm and in breeding programs

How about using marker-assisted selection?

Challenges for breeders

- ❖ Resistance to white mold is conferred by multiple genes conferring partial resistance
- ❖ Numerous QTLs have been reported, mostly in adapted germplasm
 - Few common QTLs in different studies

Kim and Diers, (2000); Arahana et al. (2001); Guo et al. (2008)
Vuong et al., (2008); Huynh et al. (2010)
 - Not all resistance donors are attractive to the breeder (MG, poor adaptation, etc.)

How do we identify useful sources of partial resistance?

Objectives of the work

- ❖ Characterize white mold resistance in two sets of germplasm using the cotton pad method
 - Collection of PIs (MG 000-1), mostly from Asia
 - Some reported to be resistant (Hoffman et al. 2002)
 - Collection of lines from a private breeding program in Canada
- ❖ Genotype the lines with '000s of SNPs
- ❖ Perform a genome-wide association analysis to identify SNPs associated with resistance

Cotton pad method

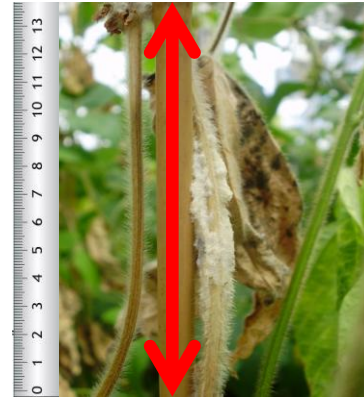
Bastien et al. 2012 *Canadian Journal of Plant Science* 92:279-288



Lesion length 7 days after inoculation



Resistant



Susceptible

White mold resistance in PIs

Elmer Iquira-Ph.D. student

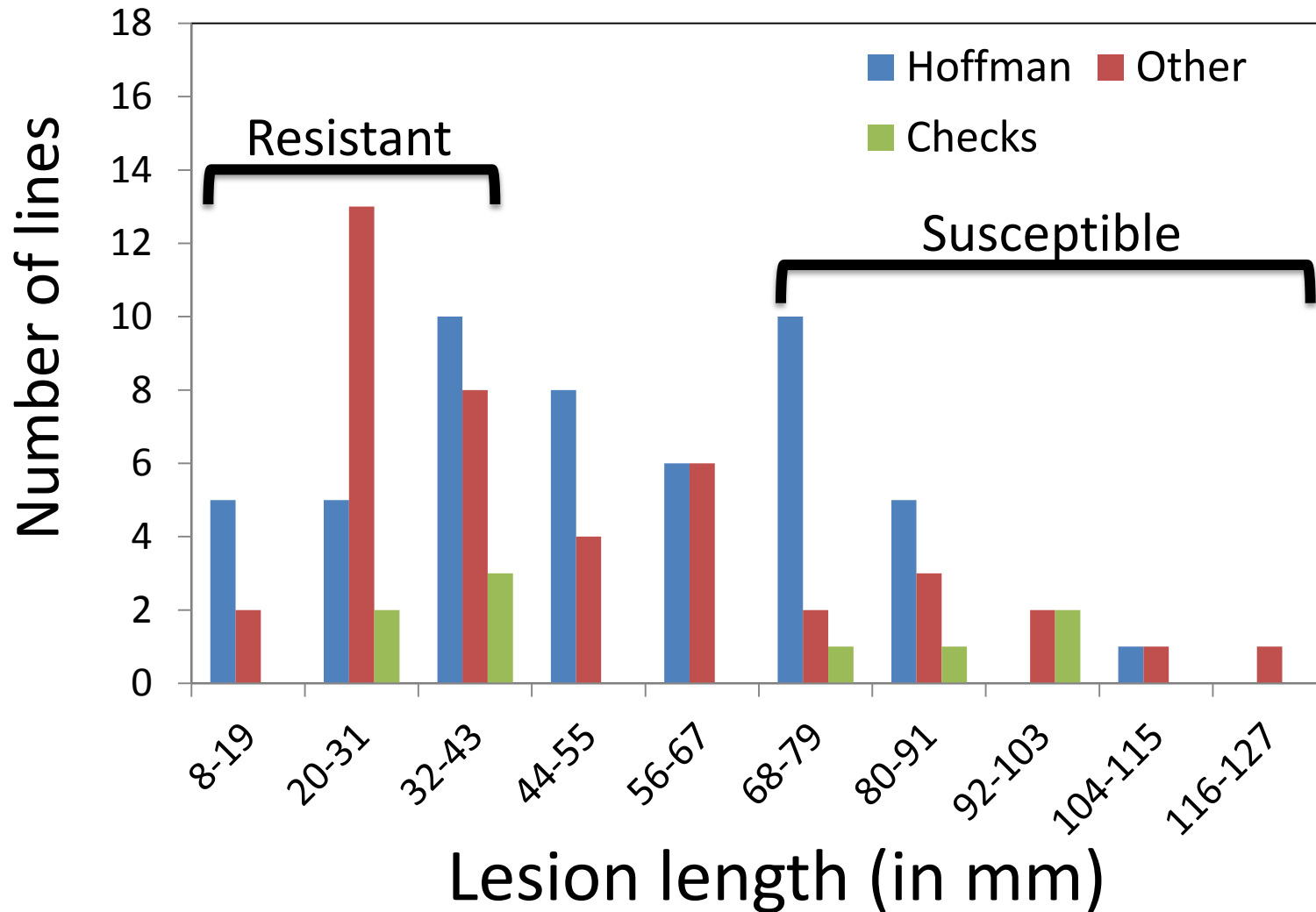
❖ 101 soybean accessions

- 50 PIs reported as resistant by Hoffman et al. (2002)
- 42 PIs of unknown resistance to white mold
- 9 checks
 - 4 resistant
 - 4 susceptible
 - 1 moderately susceptible

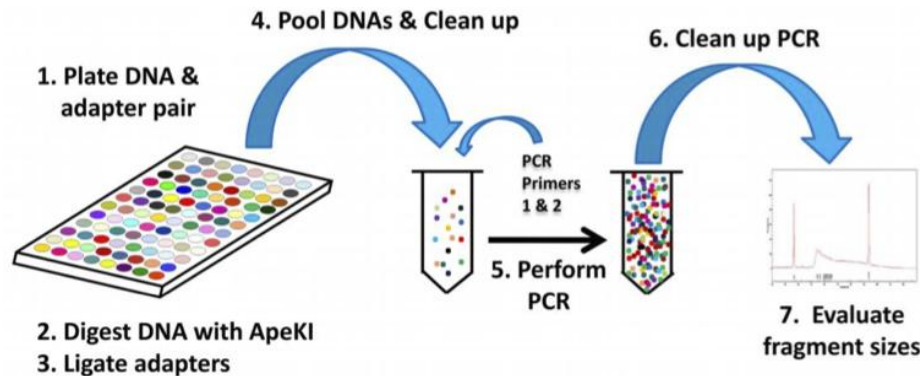


❖ Inoculated with cotton pad method in greenhouse

White mold resistance in PIs

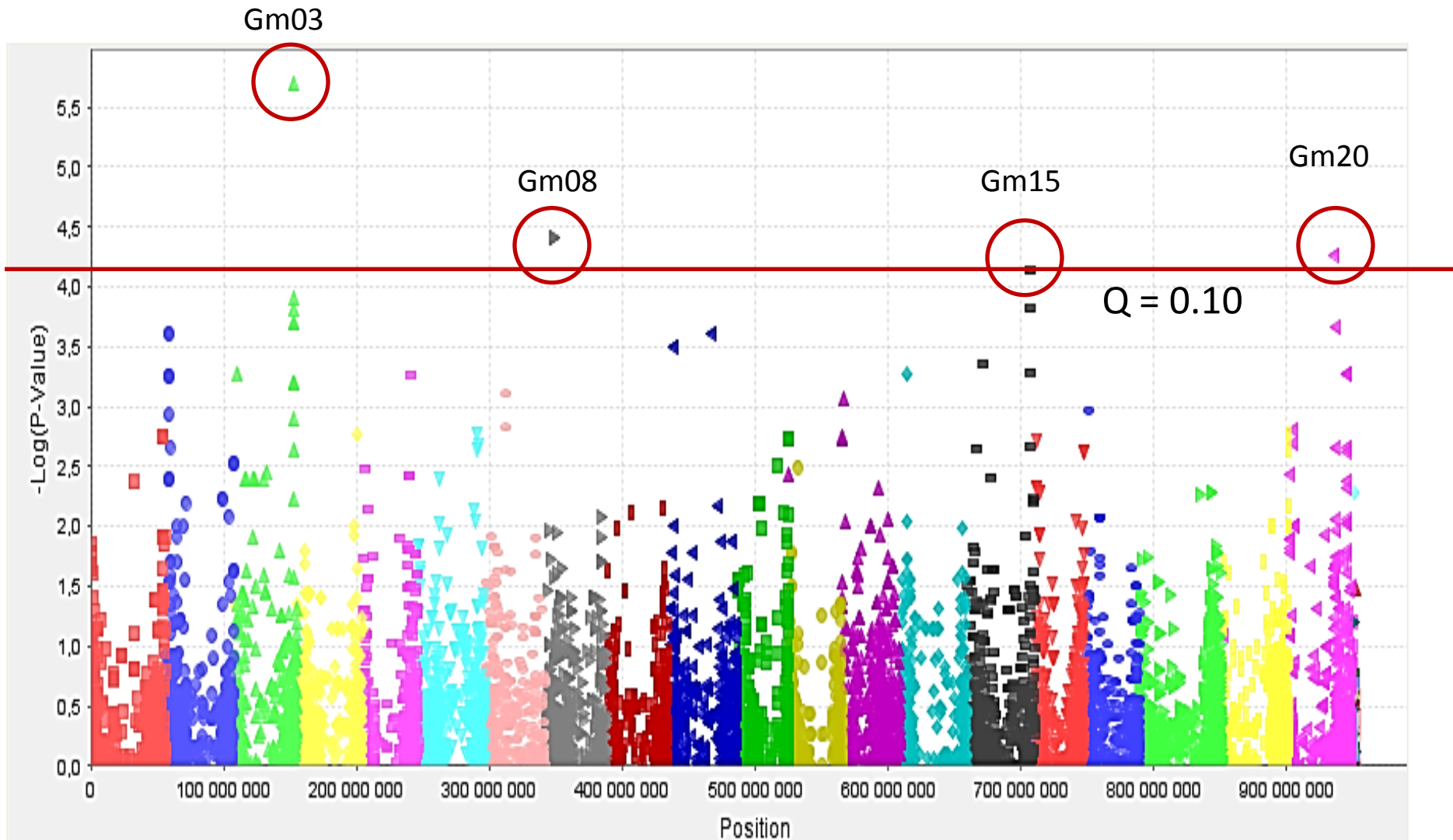


SNP genotyping by sequencing (GBS)



- ❖ GBS approach described in Sonah et al. (2013)
- ❖ After filtration for quality and minor allele frequency ($\text{maf} > 0.05$)
 - 8,397 SNP markers
 - Any missing data were imputed

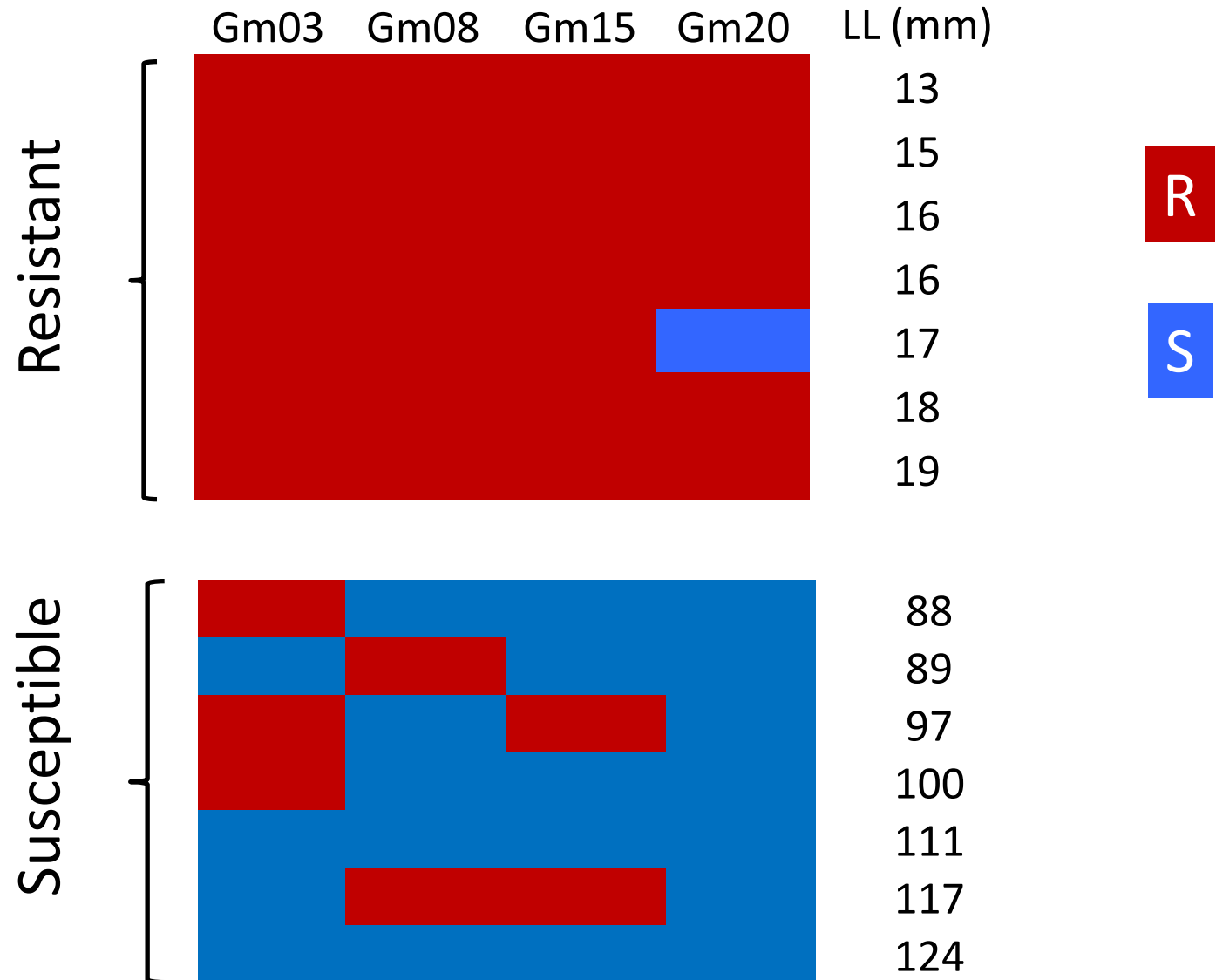
Genome-wide association analysis (GWAS)



Summary of significant SNPs

Chromosome	Q-value	R ² (%)	Δ lesion length (mm)
Gm03	0.01	21	32.7
Gm08	0.10	16	52.9
Gm15	0.10	15	21.7
Gm20	0.10	15	33.6

Allelic state at phenotypic extremes



Conclusions for PIs

- ❖ 7 highly resistant PIs
 - Lesions are shorter than 20 mm
 - Many PIs from Hoffman et al. (2002) are resistant to fungal spread in the plant, but some are highly susceptible (based on cotton pad inoculation)
- ❖ GBS identified more than 8,000 SNPs
- ❖ GWAS identified 4 regions with significant SNPs
- ❖ The most resistant PIs are practically all fixed for the favorable alleles

White mold resistance in a private breeding program

Maxime Bastien-Ph.D. student

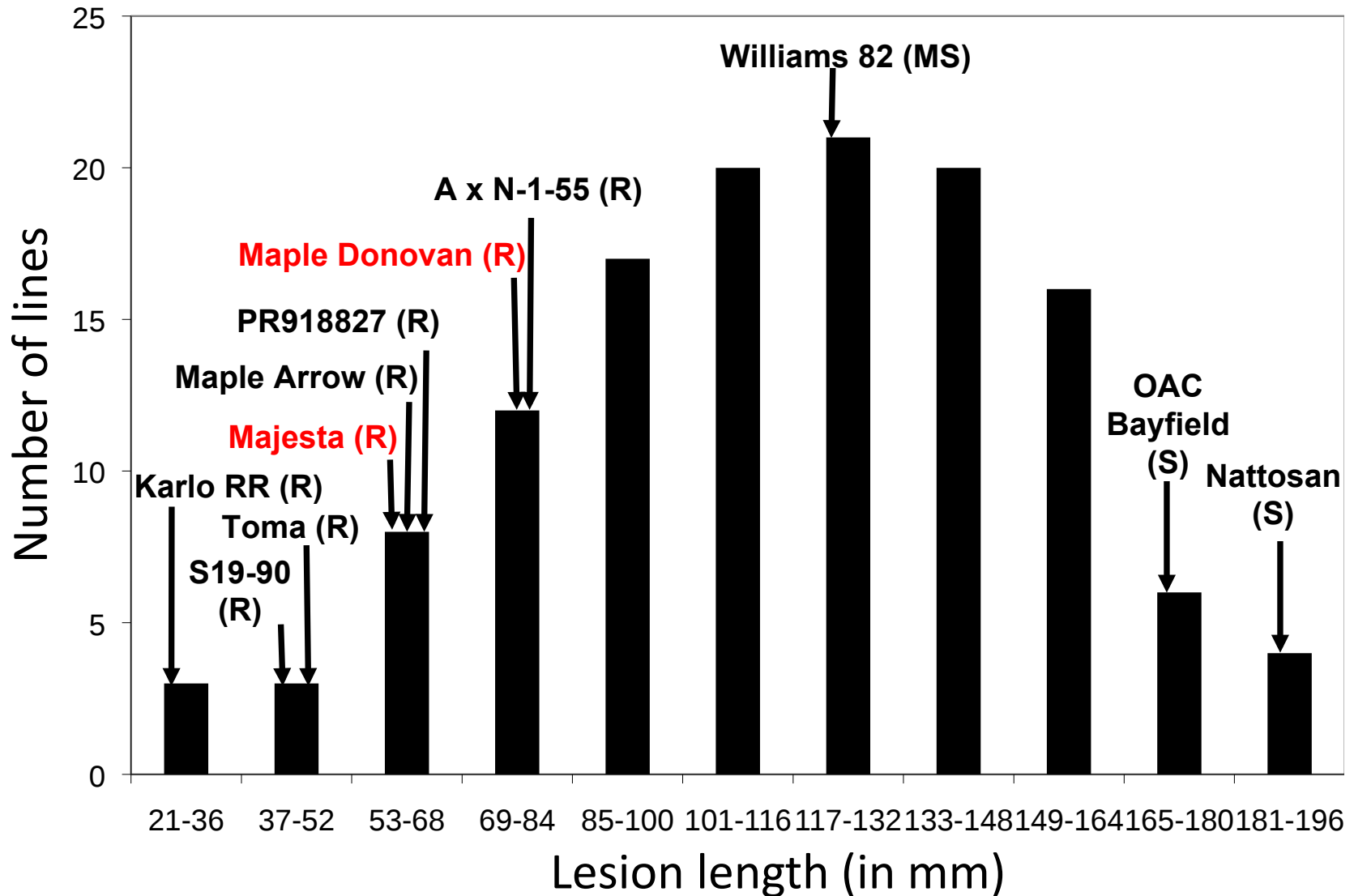
❖ 141 soybean accessions

- 130 soybean cultivars and lines from Semences Prograin
- 11 checks
 - 8 resistant
 - 3 susceptible

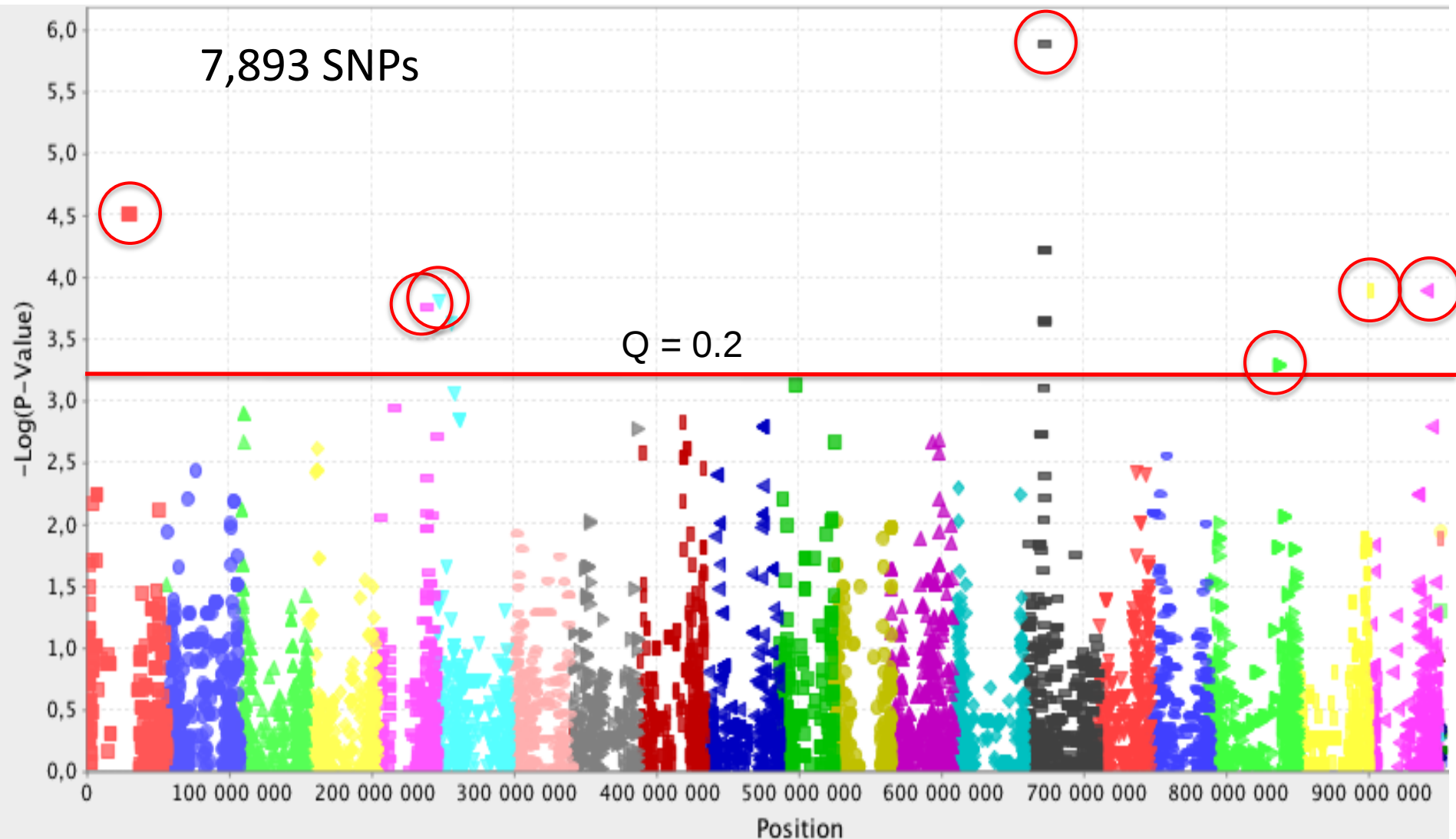


❖ Inoculated with cotton pad method in greenhouse

White mold resistance



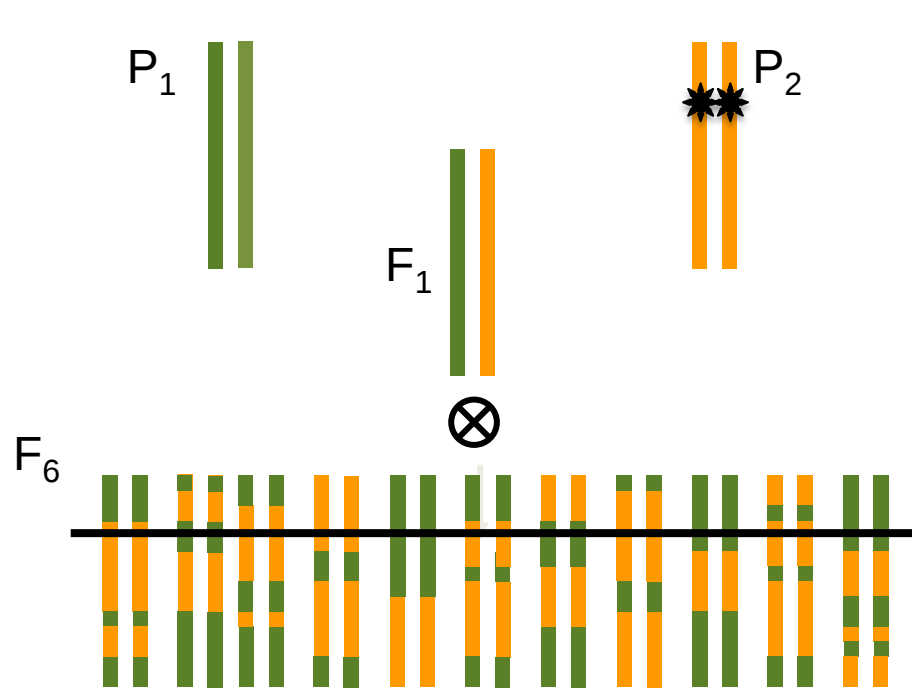
Genome-wide association analysis (GWAS)



Summary of significant SNPs

Chromosome	Q-value	Minor allele	Δ lesion length (mm)
Gm15	0,01	42	15.1
Gm01	0,04	51	5.4
Gm19	0,10	7	9.9
Gm20	0,10	42	7.9
Gm06	0,11	8	30.2
Gm05	0,12	15	29.8
Gm18	0,18	10	10.1

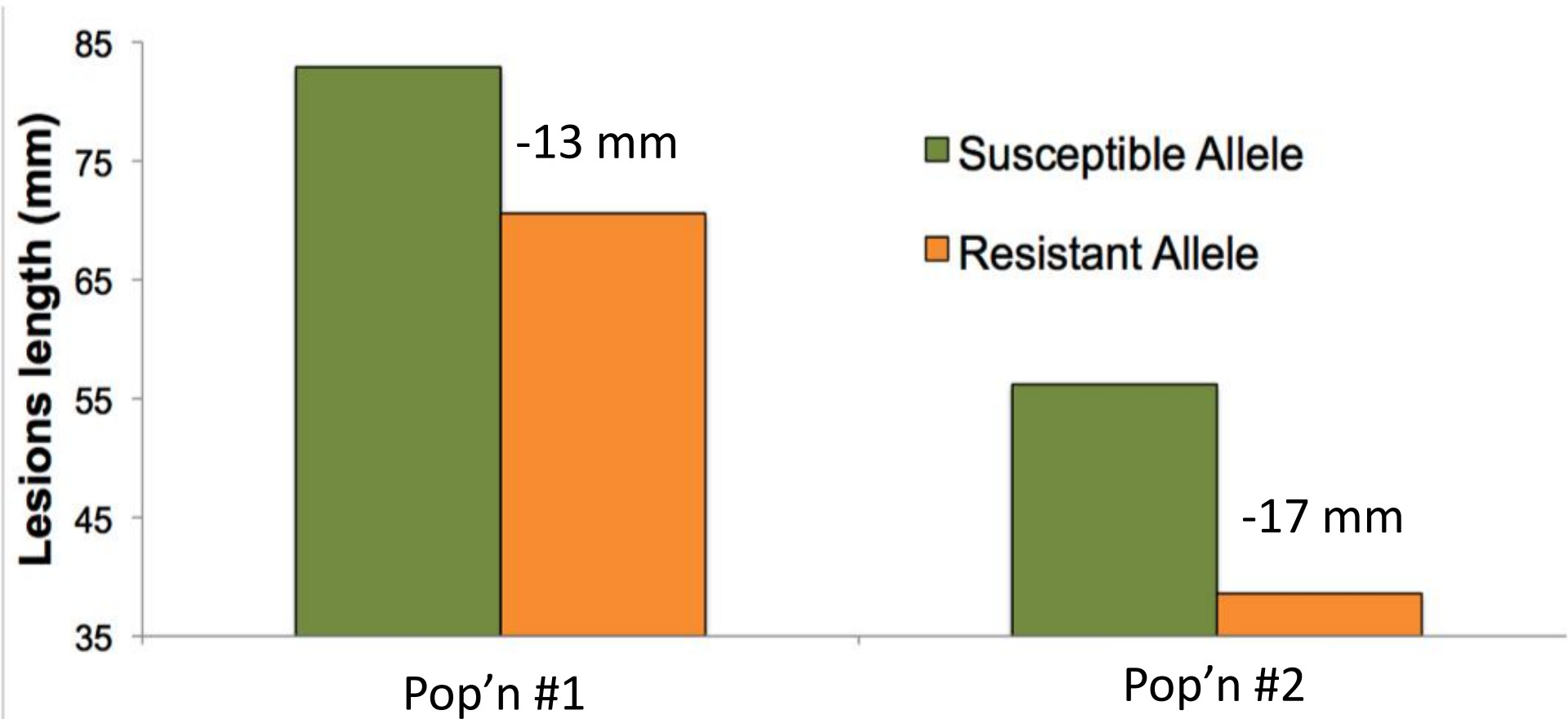
Validation of QTL on Gm15



Are lines with the R allele at the marker more resistant?

- ❖ Used two sets of RILs (F₆) segregating for marker on Gm15
- ❖ Developed a CAPS assay for marker on Gm15
- ❖ Genotyped 2 populations
 - 24 lines with R allele
 - 24 lines with S allele
- ❖ Tested for white mold resistance

Validation of QTL on Gm15



Conclusions for breeding lines

- ❖ The most resistant lines have lesions >20mm
- ❖ GBS identified close to 8,000 SNPs in this set
- ❖ GWAS identified 7 genomic regions that are associated with white mold resistance
- ❖ Marker on Gm15 was validated in 2 sets of RILs
 - The allelic effect was ~15mm in all populations

PIs vs Breeding lines

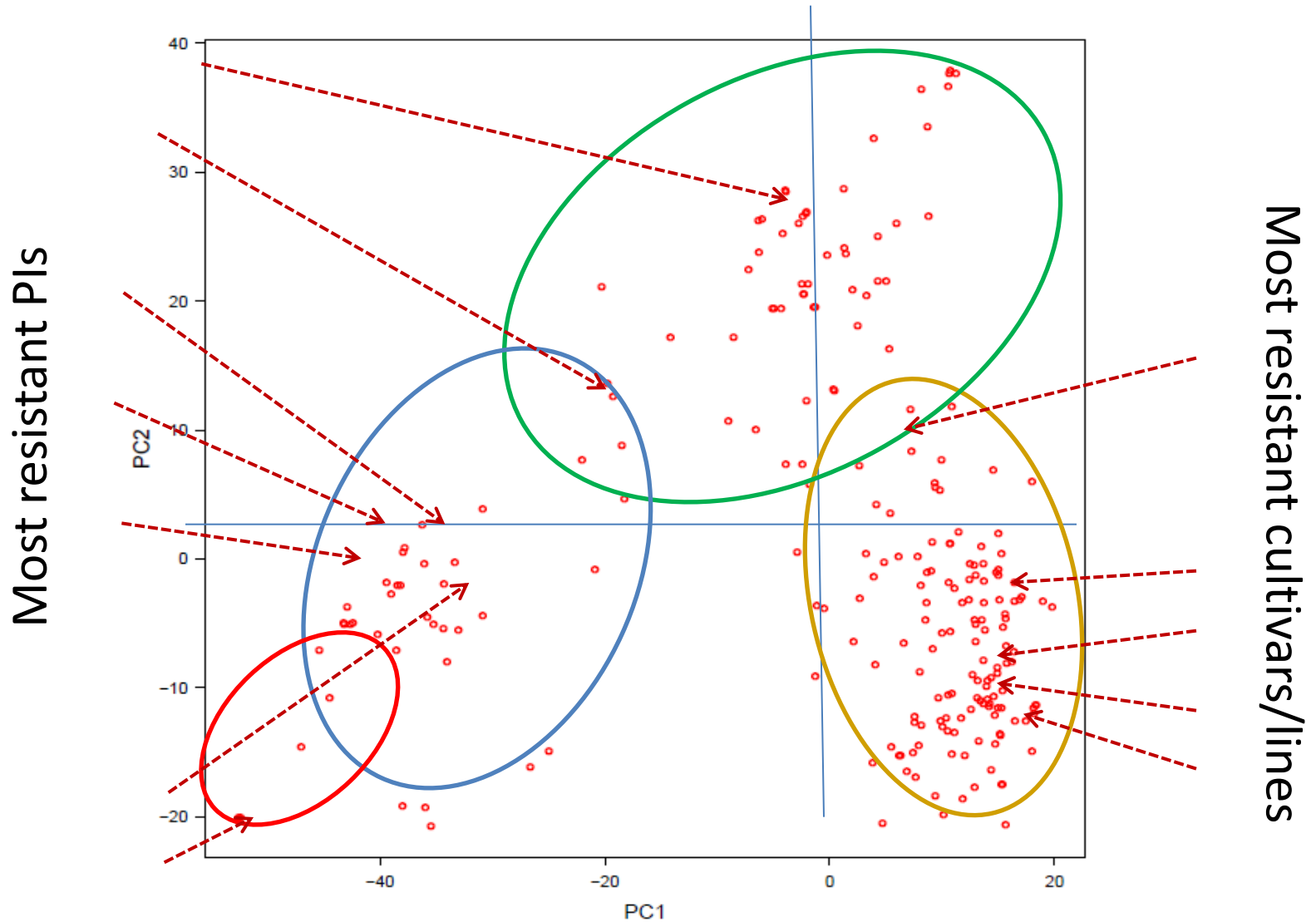
- ❖ QTLs identified in these two GWAS experiments are not the same

- Why?

- ❖ Individual QTLs result in modest reductions in lesion length

- How to best use these in breeding?

Two very different sets of germplasm



Implications for breeding

- ❖ The most resistant PIs carry the same alleles at all 4 loci
 - Can choose based on other traits (adaptation)
- ❖ The most resistant PIs are more resistant than the best cultivars/lines
 - There is scope for further improvement
- ❖ Each individual QTL has a modest effect
 - May need to follow many loci

Acknowledgements



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