GENOMIC REGIONS ASSOCIATED WITH

DIAPORTHE HELIANTHI AND *DIAPORTHE GULYAE* RESISTANCE

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College of Agriculture, Food and Environmental Sciences

National **Sunflower** ASSOCIATION

PHOMOPSIS STEM CANKER

- Yield losses ≥ 40% (Mathew et al. 2015)

- Caused by several fungi of *Diaporthe* genus

- *D. helianthi* and *D. gulyae* are predominant in the U.S. (Elverson et al. 2020)

RESEARCH JUSTIFICATION

- Phomopsis stem canker resistance is quantitative (Vear et al. 1997; Degener et al. 1999; Viguie et al. 1999)

- Identify genes that breeders can use to develop varieties with resistance to *D. gulyae* and *D. helianthi*

- Association mapping has not been performed for *D. gulyae* and *D. helianthi* resistance

RESEARCH OBJECTIVES

1. Assess the disease response associated with *D. helianthi* and *D. gulyae* in the USDA sunflower collection

2. Conduct genome-wide association mapping to identify genomic regions associated with *D. helianthi* and *D. gulyae*

3. Compare the genomic regions conferring resistance to *D. helianthi* and *D. gulyae* with the study by Pogoda & Hulke (2020)

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PREVIOUS RESEARCH

- Talukder et al. (2020) identified 15 QTLs that were associated with Phomopsis stem canker resistance

  - 11 chromosomes representing 5.24 to 17.39% of phenotypic variation

  - Recombinant Inbred line population derived from a cross between HA 89 (susceptible) and HA-R3 (resistant)

WORKFLOW

Manhattan-Plot
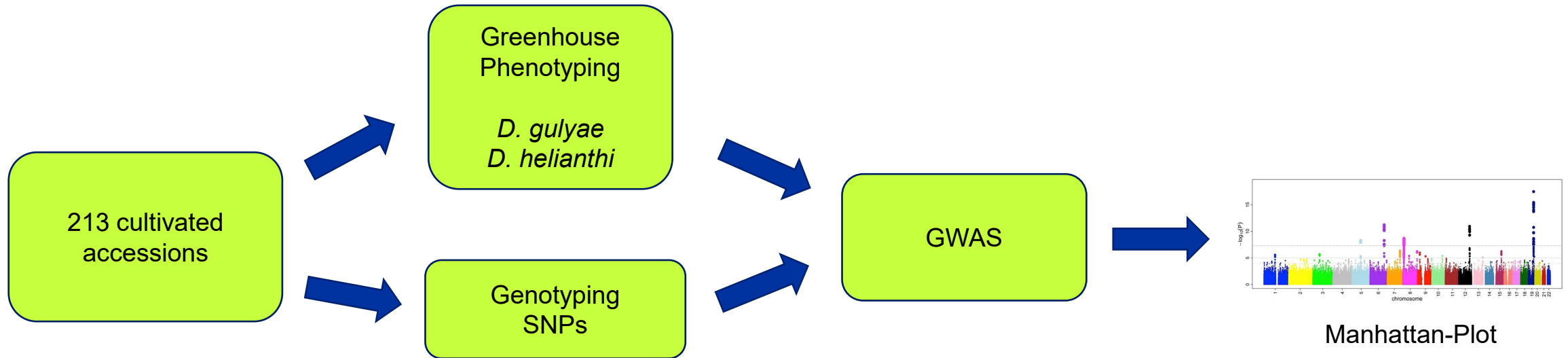
Sunflower pan-genome analysis shows that hybridization altered gene content and disease resistance

Sariel Hübner[1,2,3]*, Natalia Bercovich[1], Marco Todesco[1], Jennifer R. Mandel[4], Jens Odenheimer[5], Emanuel Ziegler[5], Joon S. Lee[1], Gregory J. Baute[1], Gregory L. Owens[1,6], Christopher J. Grassa[1,7], Daniel P. Ebert[1,8], Katherine L. Ostevik[1,9], Brook T. Moyers[1,10], Sarah Yakimowski[1], Rishi R. Masalia[11], Lexuan Gao[1], Irina Ćalić[11], John E. Bowers[11], Nolan C. Kane[1,12], Dirk Z. H. Swanevelder[13], Timo Kubach[5], Stephane Muños[14], Nicolas B. Langlade[14], John M. Burke[11] and Loren H. Rieseberg[1]

PHENOTYPING

- 213 cultivated accessions from USDA collection

- Consists of open-pollinated varieties, landraces and pre-bred lines (Mandel et al. 2013, 2011)

- Confection inbred 'HA 288' (PI552934) used as the susceptible check (Mathew et al. 2018)

PHENOTYPING

- Screening was performed in the greenhouse at 22-25°C and under a 16 h light/ 8 h dark cycle

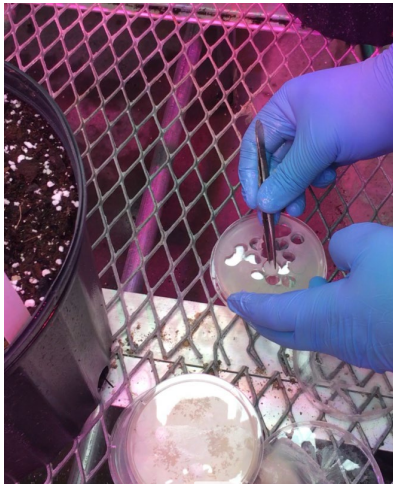- A single isolate of *D. gulyae* and *D. helianthi* used

PHENOTYPING

**Completely Randomized Design
6 plants per accession
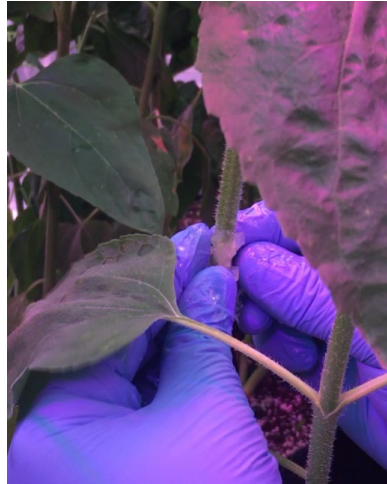Experiment repeated once**

PHENOTYPING

- Inoculation at V4 – V6 (four to six true leaves)

- Mycelial–contact method (Thompson et al. 2011)

- After inoculation, plants misted for 2 min every 2 h for 3 days

6-mm PDA plug

Placed at the third node

Covered with petroleum jelly

PHENOTYPING

- Disease severity evaluated for *D. gulyae* at 14 days after inoculation, and for *D. helianthi* at 30 days

- 0 to 5 disease rating scale (Mathew et al. 2015; Thompson et al. 2011)

0: No discoloration

1: low level discoloration

3: necrotic lesions 2–5 mm, leaf wilting and twisting

5: very severe necrosis and lesions, or plant death

PHENOTYPING DATA ANALYSES

- Disease severity data was analyzed separately for *D. helianthi* and *D. gulyae* using non-parametric statistics

	D. helianthi	*D. gulyae*
Shapiro-wilk test	$p < 0.0001$	$p < 0.0001$
Levene's test	0.24	0.60

PHENOTYPING DATA ANALYSES

	ANOVA type statistic	Degrees of freedom	p value
D. helianthi	2.16	4.12	p = NS
D. gulyae	10.85	1.54	$p < 0.0001$

- For *D. helianthi,* since *p*-value was not significant, disease severity associated with the accessions were not compared

- For *D. gulyae,* disease severity was expressed as relative treatment effects and compared using 95% confidence intervals

RESULTS - *D. gulyae*
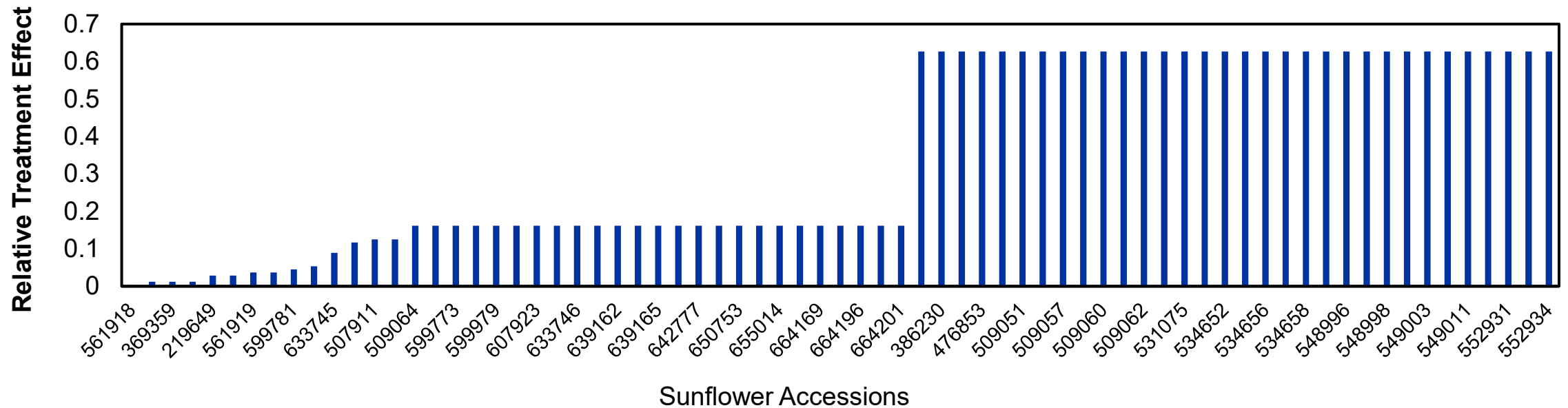
- 39 accessions had significantly lower RTE compared to HA 288 based on 95% confidence intervals

RESULTS

- 213 accessions were validated for their resistance to Phomopsis stem canker in a field with disease history (natural inoculum)

- PI 509060, PI 561918, PI 599782, and PI 633745 had significantly lower RTE compared to HA 288 in the field and greenhouse

GENOTYPING

- Genomic DNA extracted from each accession using CTAB protocol (Todesco et al. 2020)

- Whole-genome shotgun Illumina libraries prepared using TruSeq protocol (Rowan et al. 2015; Rohland and Reich 2012)

- Sequencing conducted on the HiSeq platform with 150-bp paired-end reads

GENOTYPING

- Sequences were trimmed (Trimmomatic v.036) and aligned to the *Helianthus annuus* XRQv1genome (3.6 Gbp) (NextGenMap (5.3) (Bolger et al. 2014)

- Variants calling followed the Genome Analysis Toolkit (Poplin et al. 2017)

- 3,647,583 biallelic SNPs with minor allele frequency > 0.03
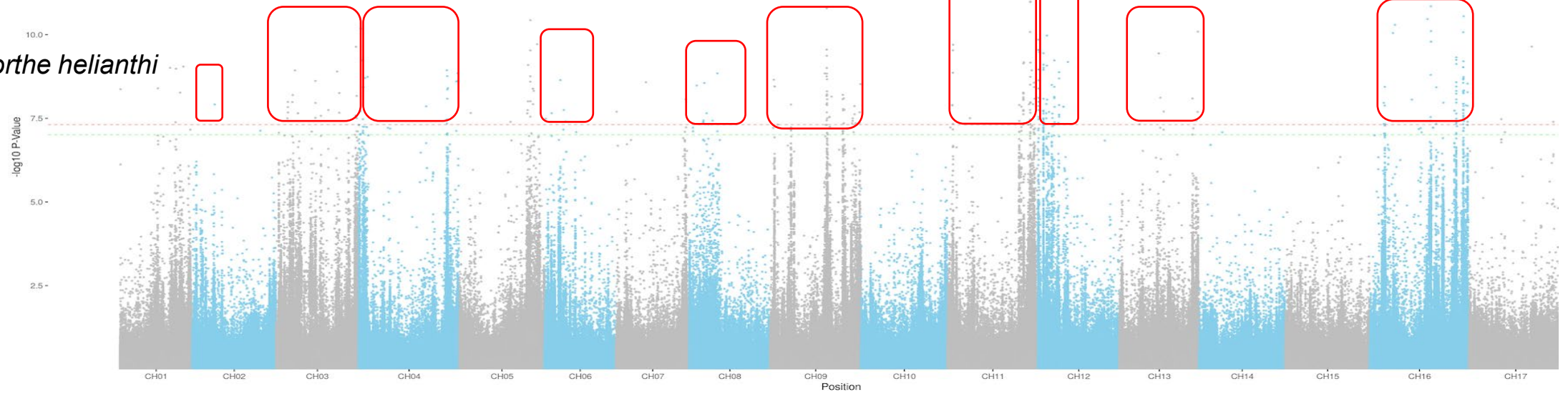
GENOME-WIDE ASSOCIATION

- EMMAX software with genotype as a fixed effect (Kang et al. 2010)

- To control false positives, a mixed model was employed with K- and P-matrices as covariates (Kang et al. 2010)

- To identify significant associations, a correction method for multiple testing was implemented by $- \log_{10} (0.05 \times Meff^{-1})$ (Gao 2011)
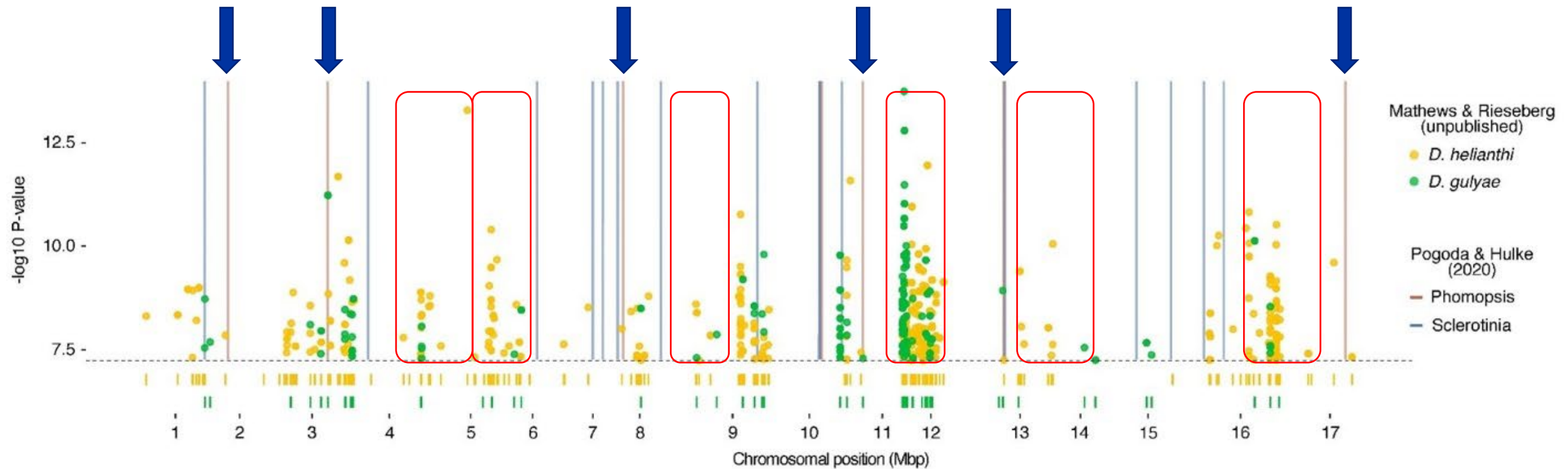
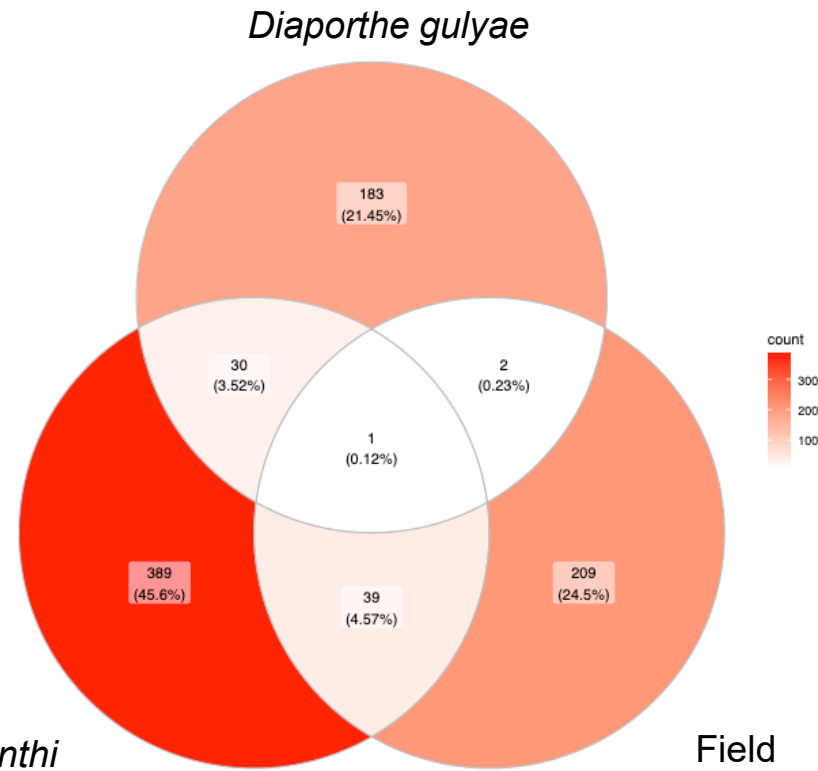*Diaporthe gulyae*
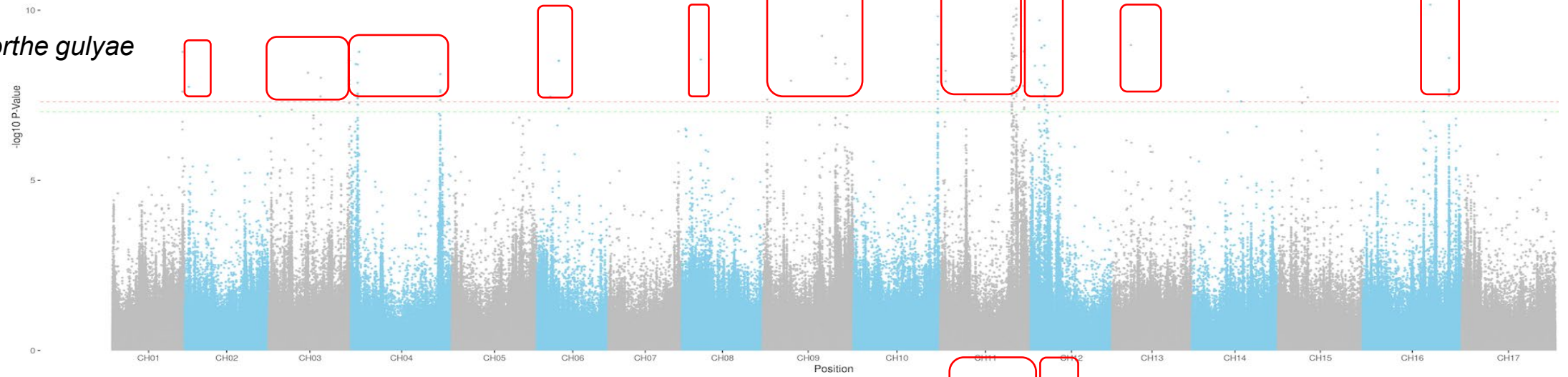
Diaporthe helianthi

Genetic loci underlying quantitative resistance to necrotrophic pathogens *Sclerotinia* and *Diaporthe* (*Phomopsis*), and correlated resistance to both pathogens

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SNPs overlap

Diaporthe gulyae

Diaporthe helianthi

Field

- 30 SNPs associated with resistance to *D. gulyae* and *D. helianthi*

Genes overlap

Diaporthe gulyae

Diaporthe helianthi

Field
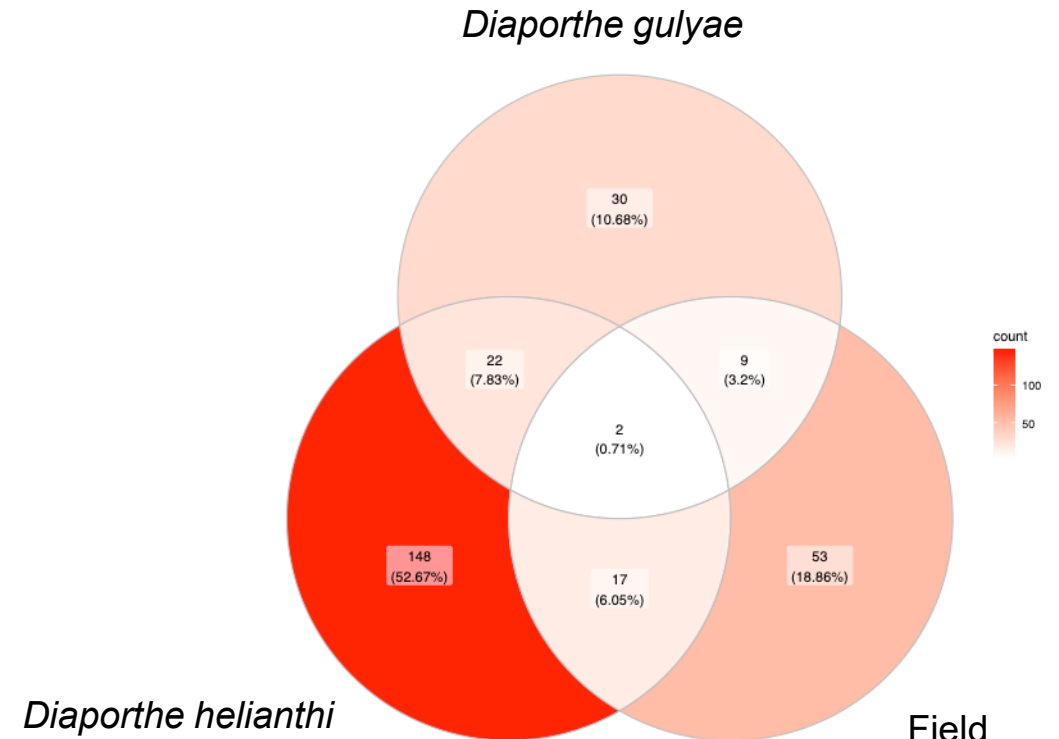
- 22 genes associated with resistance to *D. gulyae* and *D. helianthi*

SUMMARY

- This study provides information for sunflower improvement through identification of parental materials which can be exploited in breeding programs.

- 39 accessions had significantly lower RTE, among which PI 509060, PI 561918, PI 599782, and PI 633745 had significantly lower RTE compared to HA 288 in the field

SUMMARY

- 10 chromosomes (2, 3, 4, 6, 8, 9, 11,12, 13, and 16) were associated with resistance for *D. gulyae* and *D. helianthi*

- 6 chromosomes (2, 3, 8, 11, 13, and 17) were common between the study by Pogoda and Hulke (2020) and this study

SUMMARY

- 30 SNPs and 22 genes were associated with resistance to *D. gulyae* and *D. helianthi*

- SNPs with the highest effect size were identified on chromosomes 4 and 11

IMPLICATION

- Markers flanking the *D. gulyae* and *D. helianthi* resistance will facilitate marker-assisted selection in breeding

- Following breeding and development, disease resistant varieties can be incorporated into an IPM program for Phomopsis stem canker

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