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Candidate resistance genes associated with *Phomopsis*

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Outline

- Introduction
- Rationale
- Research Objective
- Materials and Methods
- Results and Summary
- Future work

Phomopsis stem canker

- A yield-limiting disease, >40% yield loss

(Mathew et al. 2015)

- Disease incidence across fields ranged from 0 to 100% in the Dakotas

(NSA survey 2023)

- *Phomopsis helianthi* and *P. gulyae* are prevalent in the U.S.

(Elverson et al. 2020)



Disease Management

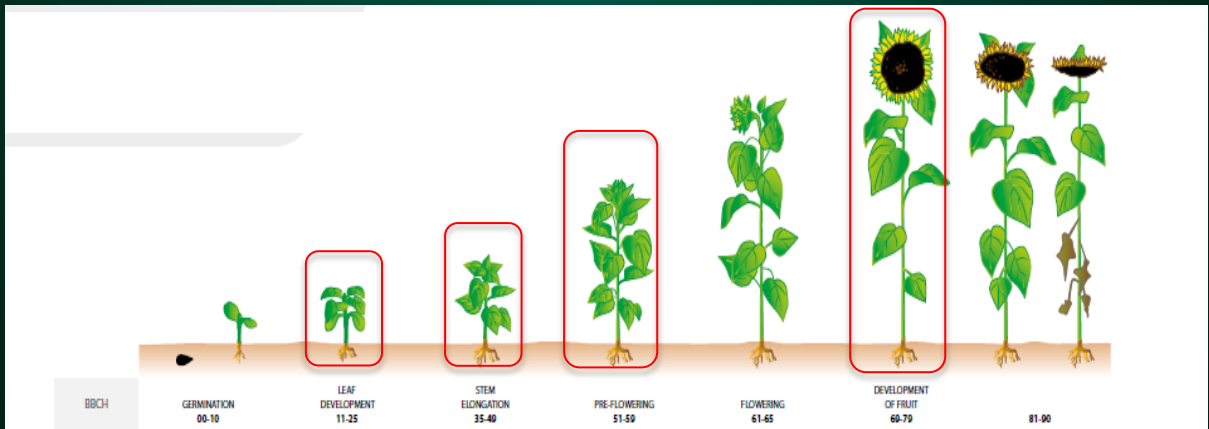
- Use of foliar fungicides containing pyraclostrobin (Kashyap et al. 2023)
- Weed management
- Crop rotation with non-hosts
- Tolerant hybrids (Mathew et al. 2018)

Host Resistance to *Phomopsis*

- Guidini et al. (2023)
 - ✓ Genome Wide Association Studies
 - ✓ USDA cultivated sunflower accessions (213) and one susceptible check (PI 552934)
 - ✓ 11 candidate genes associated with *P. helianthi* resistance
- Kashyap (2022)
 - ✓ RNA Sequencing analysis
 - ✓ Two resistant (PI 561918 and PI 509062) and one susceptible (PI 552934) genotypes inoculated with three *P. helianthi* isolates
 - ✓ Two candidate genes may be associated with resistance to the toxins produced by *P. helianthi*

Rationale

- ✓ Stem lesions are visible at or after flowering (R5) growth stage
- ✓ Endophytic species of *Phomopsis* recovered from the early vegetative stage (Dangal 2022)
- ✓ No information on the expression pattern of the identified genes
- ✓ RTqPCR can prioritize the identified candidate genes



Research Objective





To validate the expression level of candidate genes associated with resistance to *Phomopsis helianthi* in sunflower

Materials and Methods

- Completely randomized design in a growth chamber
 - ✓ N4HM354 (Susceptible) and PI 561918 (Resistant)

Materials and Methods

- Completely randomized design of in a growth chamber
 - ✓ N4HM354 (Susceptible) and PI 561918 (Resistant)
 - ✓ For each sampling, nine replication (plants) per genotype

Sampling time (dpi)	0	3	15	23	35
Growth stage	V4 -V6	V6	V8	R1	R5
Disease scores on susceptible genotype		 0: No discoloration	 1: low level discoloration	 3: necrotic lesions 2–5 cm	 5: severe necrosis or plant death

Materials and Methods

- Completely randomized design of in a growth chamber
 - ✓ N4HM354 (Susceptible) and PI 561918 (Resistant)
 - ✓ For each sampling, nine replications (plants) per genotype
 - ✓ Growth chamber temperature: 20 to 25°C
 - ✓ Experiment repeated once

Materials and Methods

Inoculation

- V4 to V6 growth stage (four to six true leaves)
- Isolate DIA-59 from Polk County, MN (Guidini et al. 2023)
- Mycelial contact inoculation method (Thompson et al. 2011)
 - Third internode
 - Secured with Parafilm.
- Non-inoculated plants as controls

RNA Extraction and cDNA synthesis



- ✓ QIAGEN RNeasy Plant Mini Kit (Qiagen Sciences, Maryland, USA)

- ✓ NanoDrop spectrophotometer
- ✓ Quality and quantity of RNA

- ✓ cDNA synthesis
- ✓ High-Capacity RNA-to-cDNA™ Kit (Thermo Fisher Scientific Baltics UAB)

Quantitative Reverse Transcription PCR

- Primers designed for candidate resistance genes

Candidate Gene	Biological function description	Reference
<i>Ha412HOChr09g0421111</i>	F-box/kelch-repeat protein At1g74510	Guidini et al. 2023
<i>Ha412HOChr12g0549821</i>	G-type lectin S-receptor-like serine/threonine-protein kinase	
<i>Ha412HOChr04g0150221</i>	Disease resistance protein RPV1 isoform X1	
<i>Ha412HOChr16g0758001</i>	Probable WRKY transcription factor 31	
<i>Ha412HOChr03g0104501</i>	Probable lipoxygenase gene	Kashyap 2023
<i>Ha412HOChr08g0365321</i>	Germacrene D synthase gene-like isoform	

- Melting curves generated at 65 to 95 °C to check primer specificity

Quantitative Reverse Transcription PCR

- QuantStudio 3 Real-Time PCR System and PowerTrack™ SYBR Green Master Mix kit (Thermo Fisher Scientific Baltics UAB)
- Cycle conditions of 3 min at 95°C and 40 cycles of 10 s at 95°C and 60 s at 56°C.
- Actin gene to normalize gene expression
- PCR efficiency: 96.8 to 98.8 %

Data Analysis

- The genes' relative expression levels were calculated using the $2^{-(\Delta\Delta CT)}$ method (Schmittgen and Livak 2008)

where, $\Delta CT = CT(\text{target gene}) - CT(\text{reference gene})$
 $\Delta\Delta CT = \Delta CT(\text{inoculated plant}) - \Delta CT(\text{uninoculated})$

- A fold change of '1' at $P < 0.05$ was regarded as cutoff for differential expression
- One-way ANOVA and Tukey HSD using 'Agricolae' package in R v4.3.0

Results

Homogeneity of Variance

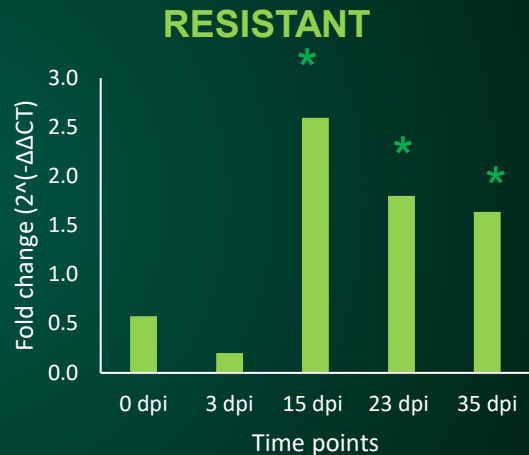
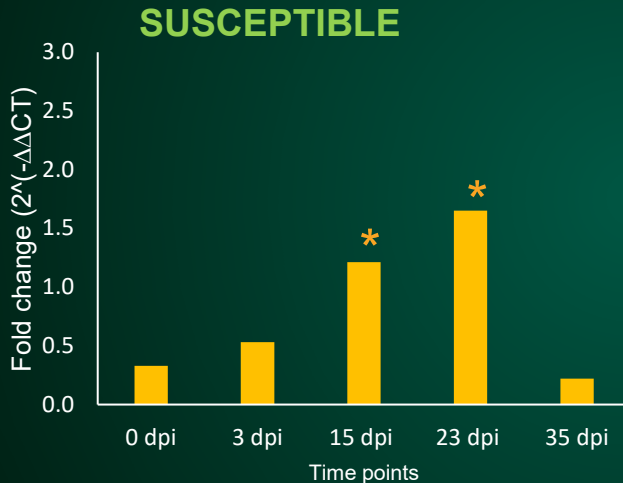
- Between experiments

Sl. No.	Gene		P value
1	<i>Ha412HOChr09g0421111</i> (F-box/kelch-repeat protein)	N4HM354 (S)	0.97
		PI561918 (R)	0.95
2	<i>Ha412HOChr12g0549821</i> (G-type lectin S-receptor-like serine/threonine-protein kinase)	N4HM354 (S)	0.09
		PI561918 (R)	0.10
3	<i>Ha412HOChr04g0150221</i> (Disease resistance protein RPV1)	N4HM354 (S)	0.13
		PI561918 (R)	0.99
4	<i>Ha412HOChr16g0758001</i> (Probable WRKY transcription factor)	N4HM354 (S)	0.09
		PI561918 (R)	0.11
5	<i>Ha412HOChr03g0104501</i> (Probable lipoxygenase gene)	N4HM354 (S)	0.80
		PI561918 (R)	0.12
6	<i>Ha412HOChr08g0365321</i> (Germacrene D synthase gene-like isoform)	N4HM354 (S)	0.10
		PI561918 (R)	0.25

Relative Expression Analysis

Ha412HOChr09g0421111 (F-box/kelch-repeat protein)

- A significant time effect was observed for susceptible ($P < 0.0001$) and resistant ($P < 0.0001$) genotype

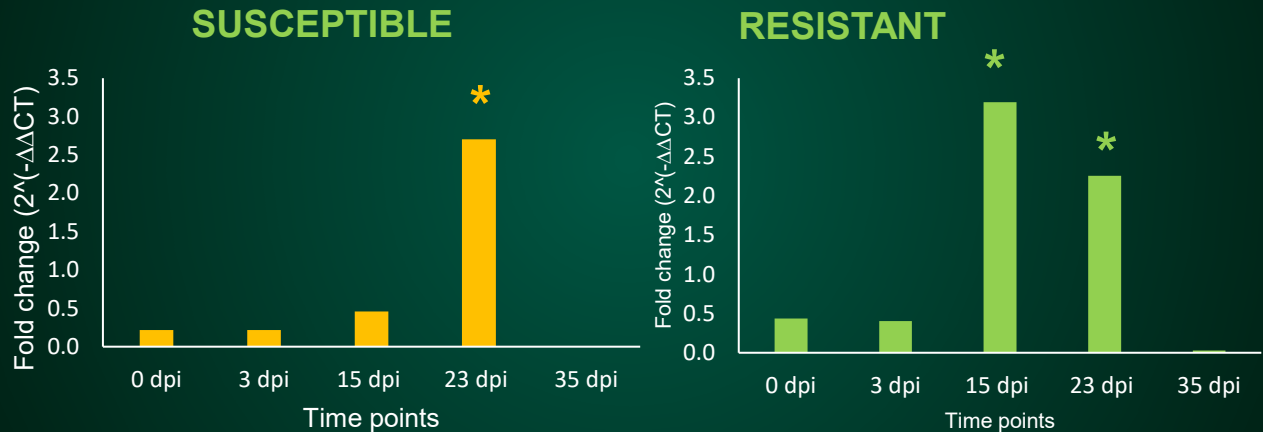


F-box/Kelch-repeat protein VpEIFP1, was strongly induced in grapevine species naturally resistant to *Erysiphe necator* (Wang et al. 2017)

Relative Expression Analysis

Ha412HOChr12g0549821 (G-type lectin S-receptor-like serine/threonine-protein kinase)

- A significant time effect was observed for susceptible ($P=0.01$) and resistant ($P=0.008$) genotype



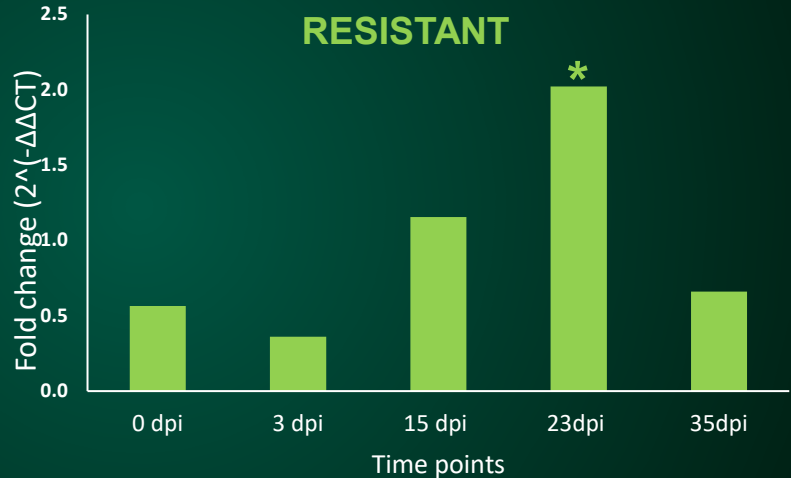
In rice (*Oryza sativa*), the G-type LecRLK, Pi-d2, was reported to provide resistance against the fungal pathogen *Magnaporthe grisea* (Chen et al. 2006)

Relative Expression Analysis

Ha412HOChr04g0150221 (Disease resistance protein RPV1)

- A significant time effect was observed for resistant ($P=0.0004$)

- 23 dpi
- Fold change = > 2.0



Homologous to a nod like receptor (NLR) resistance gene, *RPV1* conferring resistance to *Plasmopara viticola* in grapevine (*Vitis vinifera*).

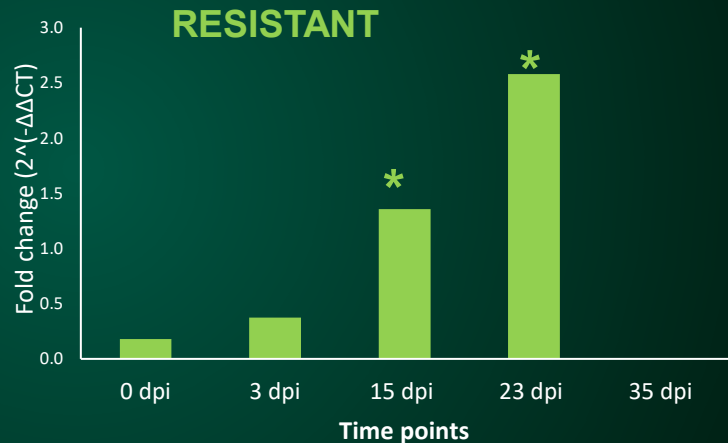
(Williams et al. 2016)

Relative Expression Analysis

Ha412HOChr16g0758001 (Probable WRKY transcription factor 31)

- A significant time effect was observed for resistant ($P < 0.0001$)

- 15 dpi, Fold change = > 1.0
- 23 dpi, Fold change = > 2.0



Genes belongs to *WRKY* transcription factor family play critical roles in response of sunflower to *Verticillium dahliae*

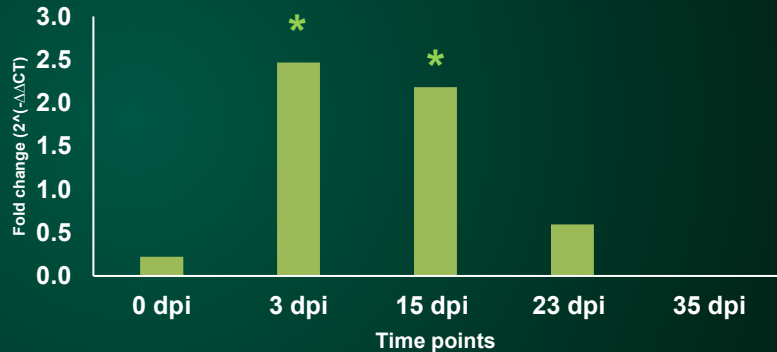
(Guo et al. 2017)

Relative Expression Analysis

Ha412HOChr03g0104501 (Probable lipoxygenase gene)

- A significant time effect was observed for resistant ($P < 0.0001$)

RESISTANT GENOTYPE



- 3 dpi and 15 dpi
- Fold change = > 2.0

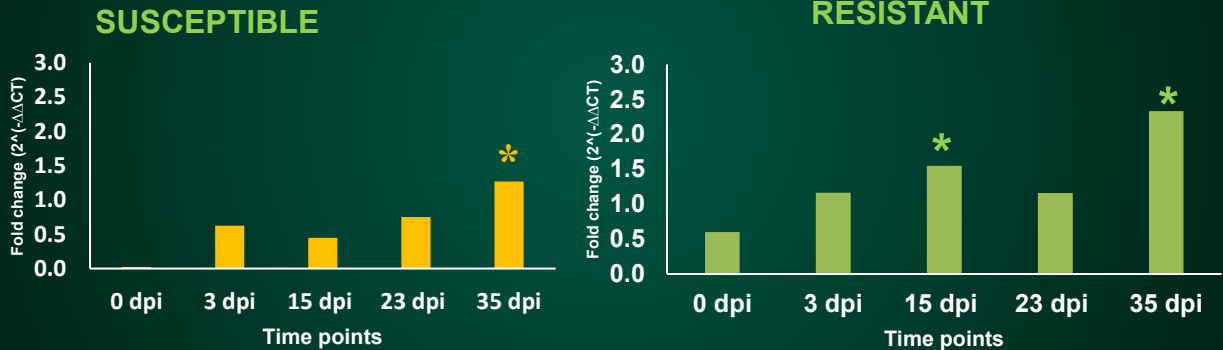
Genes belongs to lipoxygenase family expressed in corn against aflatoxin produced by *Aspergillus flavus*

(Hawkins et al. 2018)

Relative Expression Analysis

Ha412HOChr08g0365321 (Germacrene D synthase gene-like isoform)

- A significant time effect was observed for both susceptible and resistant ($P < 0.001$) genotype



Homologue to a *Germacrene D synthase gene* expressed against aflatoxin produced by *Aspergillus flavus* in wheat and corn

(Hawkins et al. 2018)

Summary

- ✓ Our study demonstrated the expression pattern of the candidate genes associated with resistance to *P. helianthi* at different growth stages of sunflower

Genotype		Ha412HOCh r09g0421111	Ha412HOChr 12g0549821	Ha412HOChr 04g0150221	Ha412HOChr 16g0758001
Significant upregulation	N4HM354 (S)	✓	✓	x	x
	PI561918 (R)	✓	✓	✓	✓
Growth stage of crop	N4HM354 (S)	V8 & R1	R1	-----	-----
	PI561918 (R)	V8, R1 & R5	V8 & R1	R1	V8 & R1

✓ Significant upregulation
X No upregulation

Summary

- ✓ Our study demonstrated the expression pattern of the candidate genes probably associated with *P. helianthi* toxin at different growth stages of sunflower

	Genotype	<i>Ha412HOChr03g0104501</i>	<i>Ha412HOChr08g0365321</i>
Significant upregulation	N4HM354 (S)	x	✓
	PI561918 (R)	✓	✓
Growth stage of crop	N4HM354 (S)	-----	R5
	PI561918 (R)	V6 & V8	R1 & R5

✓ Significant upregulation
 X No upregulation

Future work

- Validate the role and function of *Ha412HOChr04g0150221*, *Ha412HOChr16g0758001* and *Ha412HOChr03g0104501* in the response of sunflower to *D. helianthi*.

Acknowledgement

Dr. Laura Marek (ISU)
Dr. Alison Pokrzywinski (Nuseed)
Julie Hochhalter (NDSU)

My Lab:

Bijula Sureshbabu
Brian Kontz
Dilorom Rasuleva
Nitha Rafi
Tasneem Fathima
Taofeek Mukaila

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Thank you.....