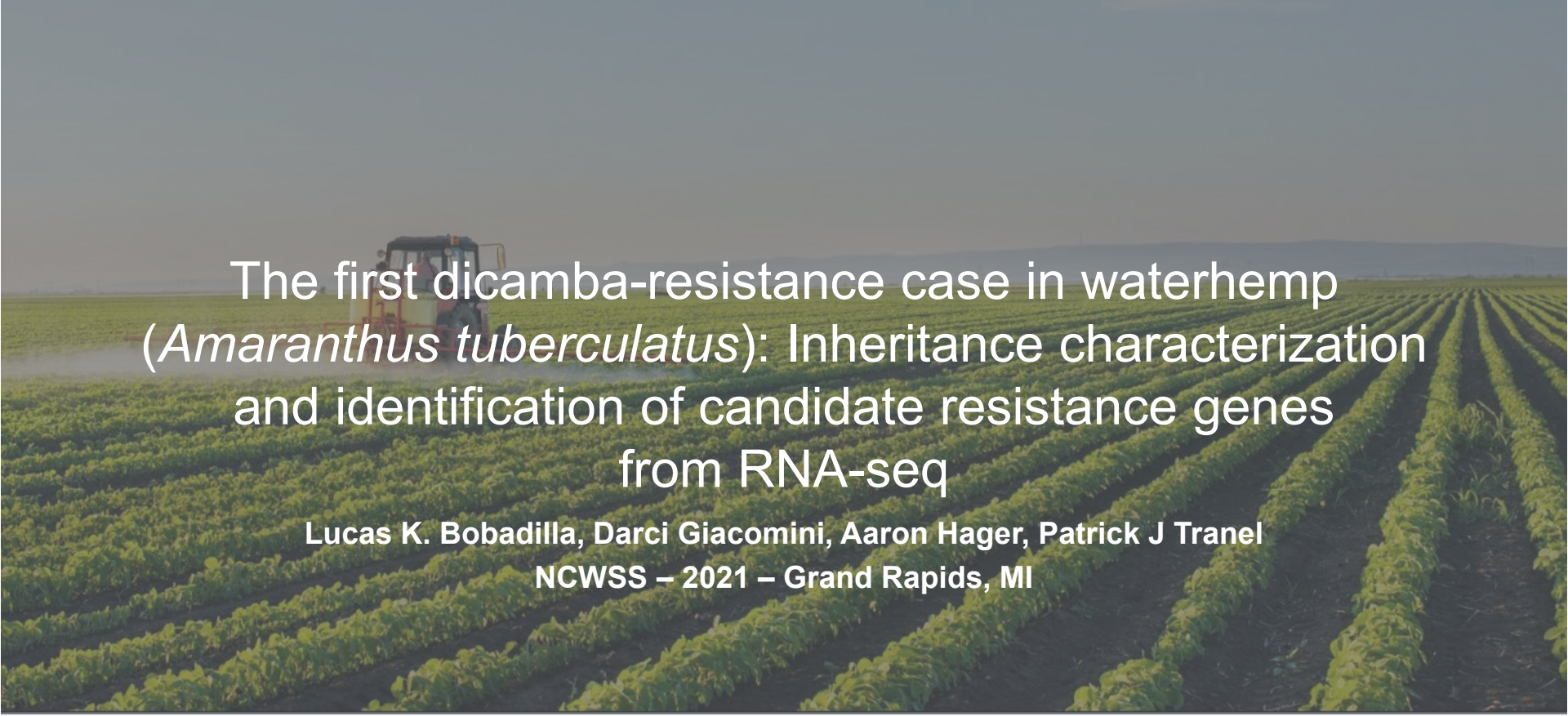

The first dicamba-resistance case in waterhemp (*Amaranthus tuberculatus*): Inheritance characterization and identification of candidate resistance genes from RNA-seq

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ILLINOIS
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Outline

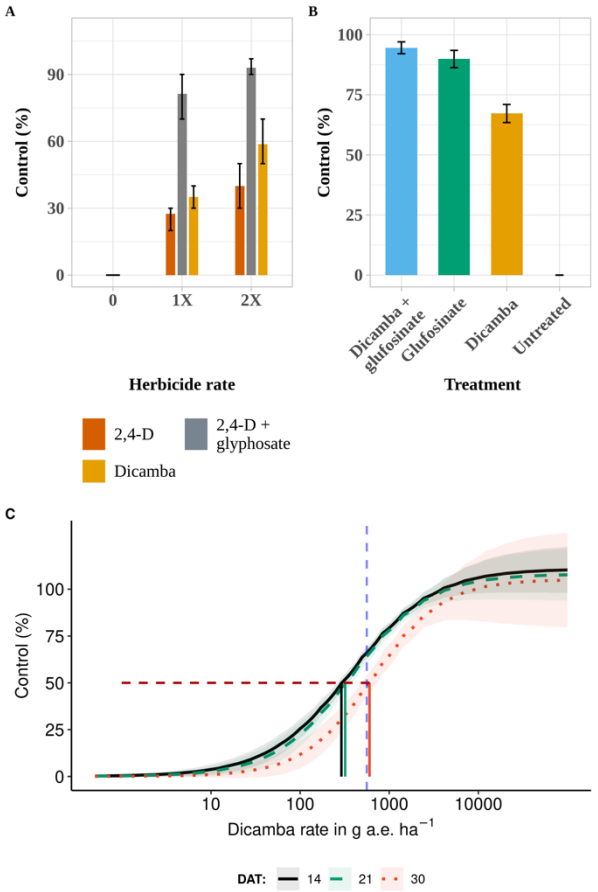
- Background
- Methods
- Results & discussion
- Conclusions & future studies

Background

- Waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer) is one of the most troublesome agronomic weeds in the midwestern US.

- **CHR population:**
  - Identified in 2012 in Champaign County, IL
  - Resistant to 6 sites of action (WSSA groups 2, 4, 5, 14, 15 and 27)
  - No history of dicamba or 2,4-D application in CHR field
  - Ineffective control of CHR was observed in the field after dicamba application

- Preliminary field experiments show a reduction in the effectiveness of dicamba -> **dicamba resistance evolution**



Objectives

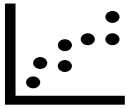
1. Quantify the dicamba resistance level and investigate its inheritance in CHR

2. Identify putative candidate genes involved with dicamba resistance via RNA-seq

Methods – Experiments

Population development

- Resistant (R) plants selected from original CHR field (after 560 g ae ha^{-1} of dicamba)
- RxR crosses for R parental line
- Select susceptible parent (S)
- Reciprocal crosses for F_1 generation
- Pseudo- F_2 and Backcross generations



Dose response

- Experimental design: RCBD + 6 reps/rate + 9 rates + 2 experiment replications
- Dicamba rates: 0, 1.18, 3.92, 11.8, 39.2, 118, 392, 1,180 and 2,350 g ae ha^{-1}
- Populations used: Parental (R and S) and F_1 lines
- Analysis: DRC and Dominance degree calculation in R
- Define delimiting rate for segregation analysis

Segregation analysis

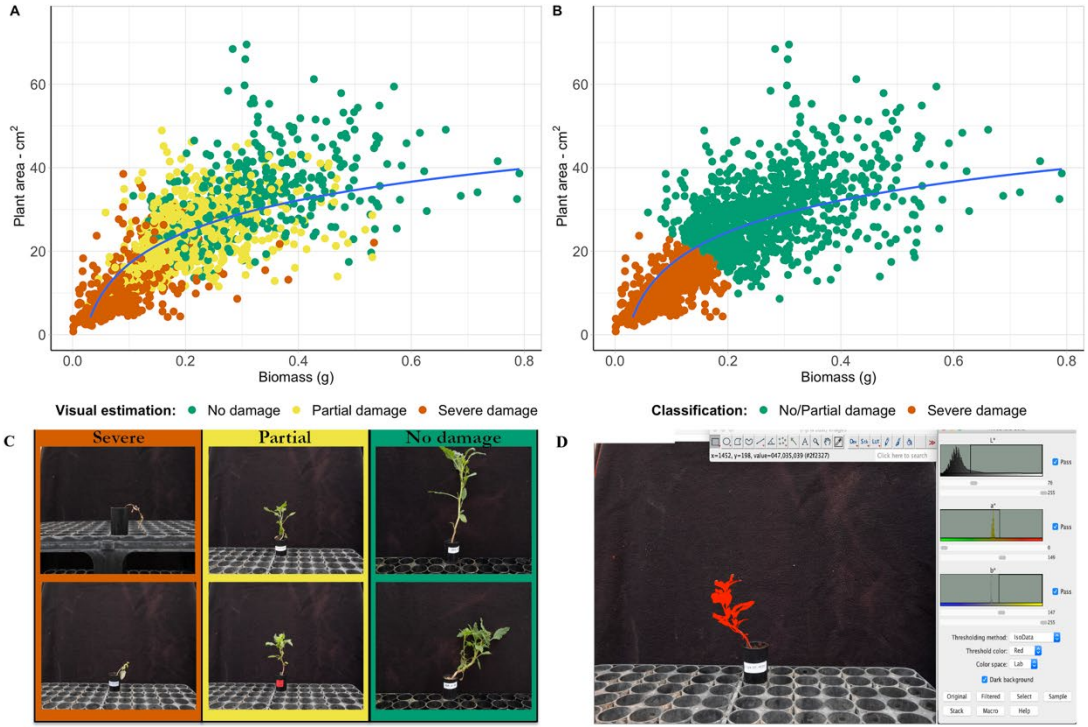
- Quantify dicamba resistance inheritance pattern
- Populations: All populations
- Dicamba damage: Using a delimiting rate damage quantification via machine learning
- Analysis: chi-square (x^2) and broad sense heritability in R
- Hypothesis: Dicamba resistance is caused by a single gene

RNA-seq

- RNA extraction (T = 0h)
- Dicamba - 560 g ae ha^1
- Phenotyping
- Apply machine learning model to selected plants
- Sequencing
- Giacomini et al. 2019

Methods – Dicamba damage estimation

- Image analysis: ImageJ + Python
- Analysis based on:
  - Plant area
  - Biomass
  - Visual estimation
- Unsupervised machine learning: Bayesian random forest model to classify samples
- 2,000 samples used as training dataset for the model
- 85% accuracy / 88% specificity



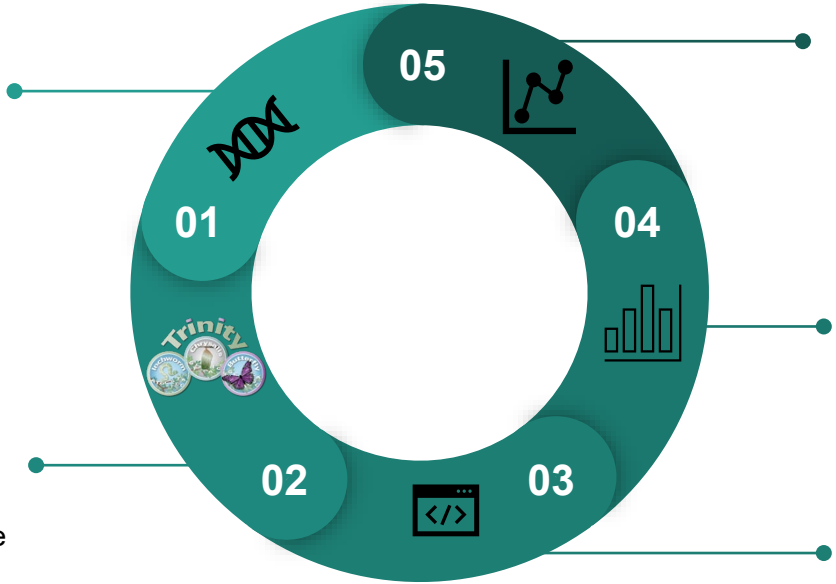
Methods – RNA-seq

Sequencing & data processing

- Illumina NovaSeq 6000 - 1x100bp
- 16 samples (8 R and 8 S)
- Adapters trimmed and rRNA removed

Transcriptome assembly

- Reads mapped to waterhemp genome using STAR
- Genome guided transcriptome assembly using Trinity



RT-qPCR confirmation

- RNA-seq candidate genes tested via qPCR
- Two housekeeping genes
- $2^{\Delta\Delta ct2}$ method for relative expression estimation
- 36 F_2 individuals tested (Including individuals used for RNA-seq)

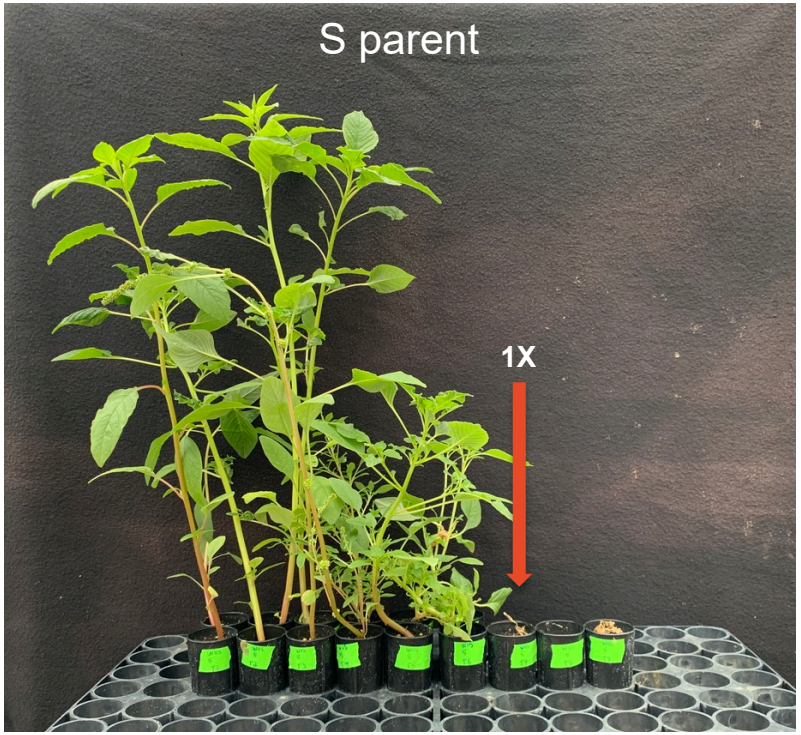
Differential expression analysis

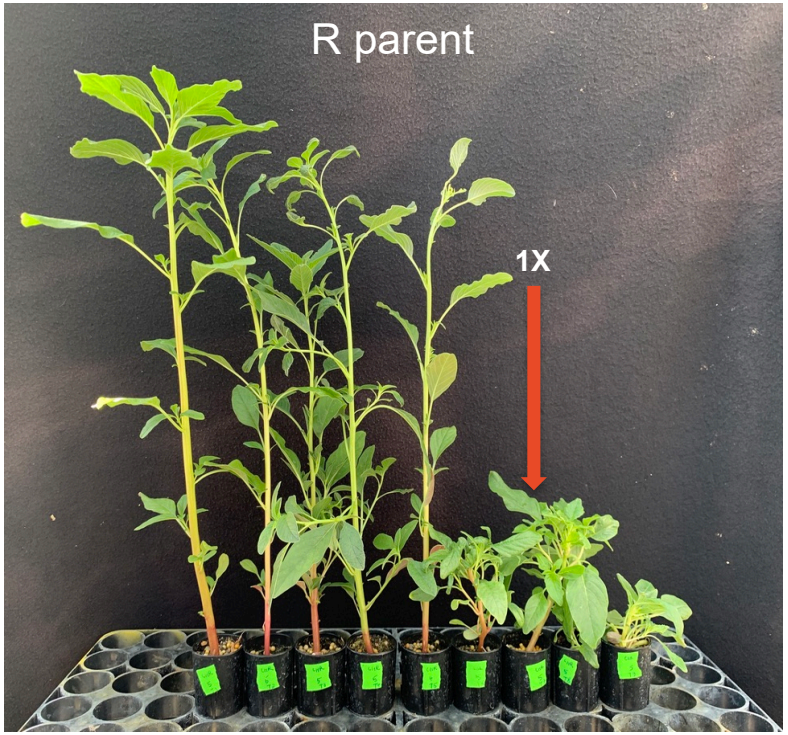
- Analysis conducted using the Sleuth and EdgeR for transcript and gene level, respectively.

Expression count

- Expression count done via pseudo-alignment using the software Kallisto.

Dose-response



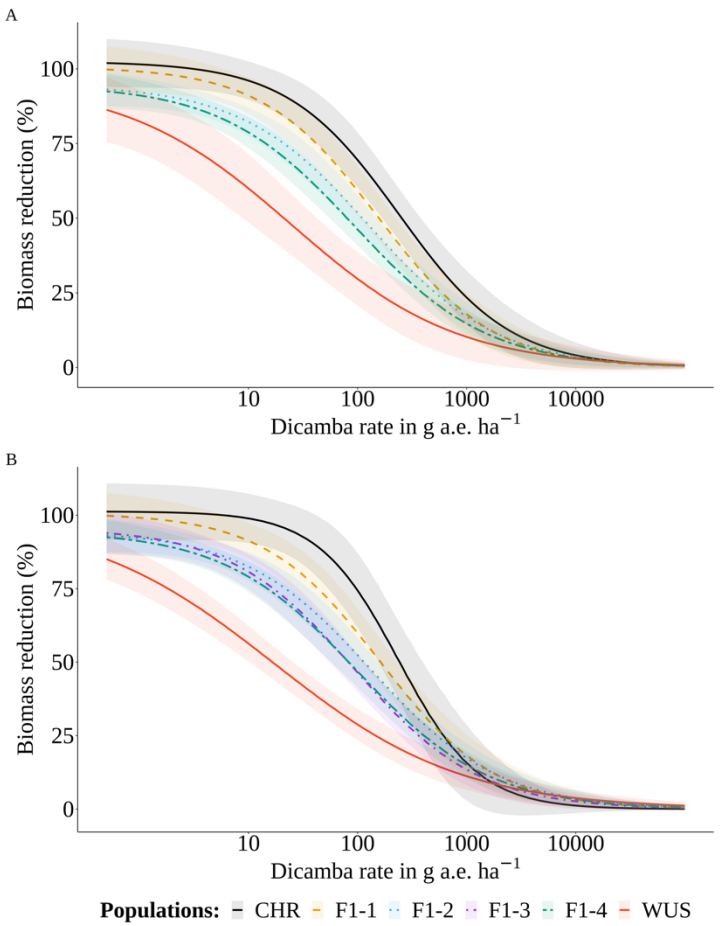


Dose-response

- 3 parameter Log-logistic model
- Two experiments
- R/S = 5 -10
- Degree of dominance = 0.25
- **Incomplete dominant trait**

$$Degree\ of\ dominance = \frac{(2W_3 - W_2 - W_1)}{(W_2 - W_1)}$$

$W_x = ED_{50}$
1,2,3 = S, R, F_1

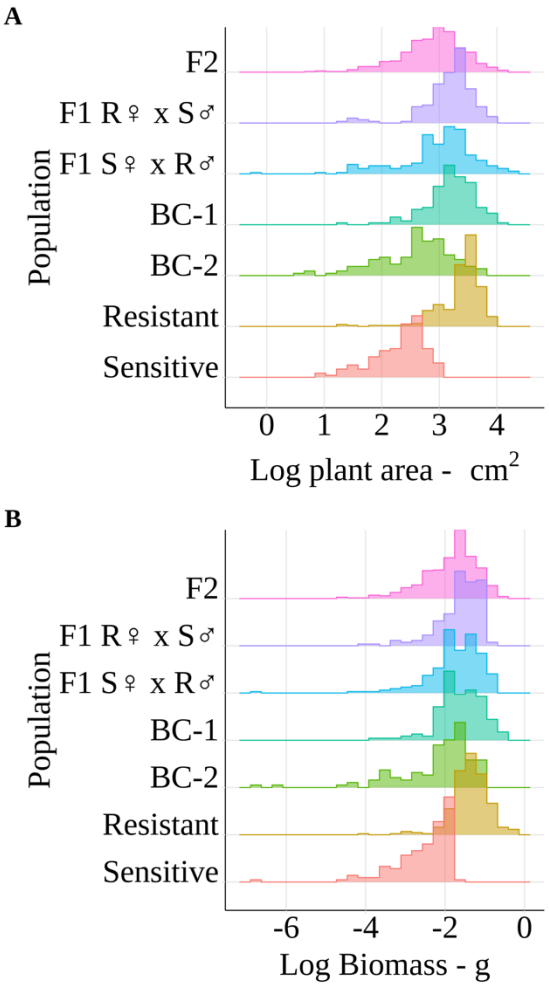


Segregation analysis

		Observed		Expected		Chi-square	
Population	# plants	No/partial damage	Severe damage	No/partial damage	Severe damage	x^2	p-value
F_2	431	247	183	**282**	**149**	**22.74**	**< 0.001**
BC-1	147	93	54	**72**	**75**	**10.34**	**<0.001**
BC-2	110	68	42	**54**	**56**	**6.14**	**0.01**
F_1 - R♀xS♂	165	140	25				
F_1 - S♀xR♂	199	134	65				
R parent	148	132	16				
S parent	131	18	113				

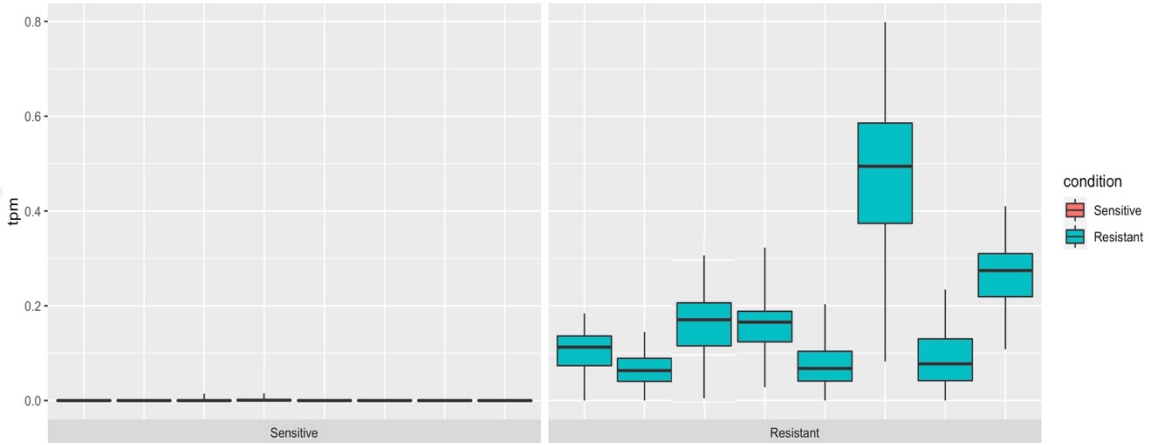
Reject 3:1 ratio (single gene)

- Moderate heritability
- **Dicamba resistance: Multi-genic trait**



RNA-seq – Transcript level

- 45 DE transcripts
- 1 major candidate:
  - **Auxin efflux gene (TIR3)**

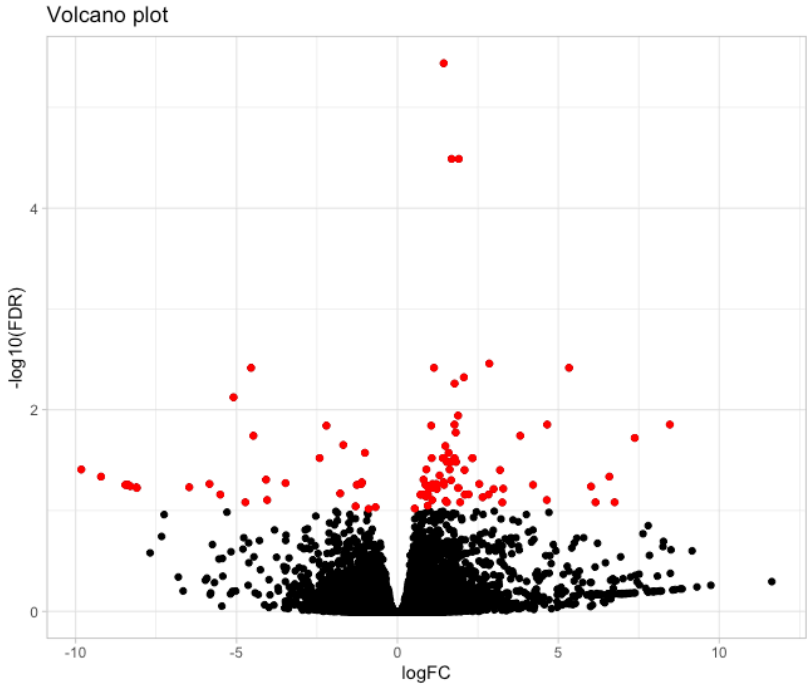


- Aggregation of transcripts:
- 2 major candidates:
  1. **Transcription activator for cytokine response (ORR)**
  2. **Auxin induced protein (IAA)**

RNA-seq – Gene level

- 103 DE genes
- 7 major candidates:

FC	
4.08	1. Auxin responsive protein SAUR
2.25	2. Peroxidase
1.98	3. GST C terminal domain
1.69	4. GST N terminal domain
1.5	5. UDP glycosyltransferase 85A
1.81	6. ABC transporter
3.25	7. MLP – ABA regulator



Upregulated

Down regulated

Candidate genes dicamba resistance

- Candidates:
  1. **SAUR –** First Auxin responsive protein
  2. **Peroxidase –** Auxin Catabolism and ROS detoxification
  3. **MLP –** ABA regulator when down regulated
  4. **TIR3 –** Auxin efflux gene
  5. **IAA induced protein –** Auxin response transcriptional factors that repress early auxin responses
  6. **UDP-glycosyl transferase –** Glycosylation of hormones and exogenous compounds
  7. **Glutathione-S-transferase –** conjugation of exogenous compound and hormones. Can also work as a peroxidase.
  8. **ABC transporter –** Transportation of secondary metabolites

RT qPCR – Gene confirmation

- 2 housekeeping genes (GAPDH and EF1α)
- 36 F_2 plants
- $2^{\Delta\Delta ct2}$ method
- Pairwise t-test

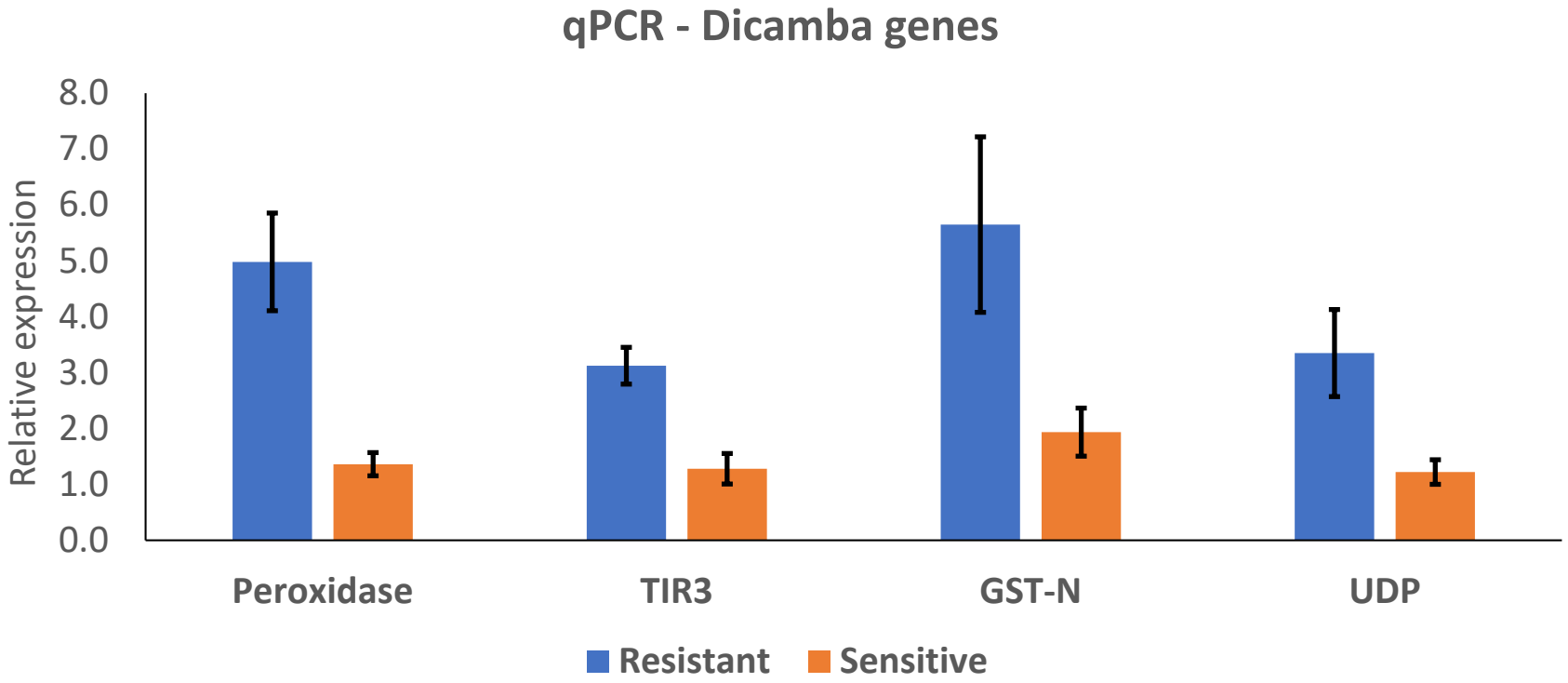
Gene	P-value
PEROX	**< 0.001**
PIN3	**0.0032**
GST-N	**0.005**
UDP	**<0.001**
SAUR	NS
GST-C	NS
ABC	NS
MLP	NS
IAA	NS
ORR	NS

F_2 plants

Resistant

Susceptible

RT qPCR – Gene confirmation

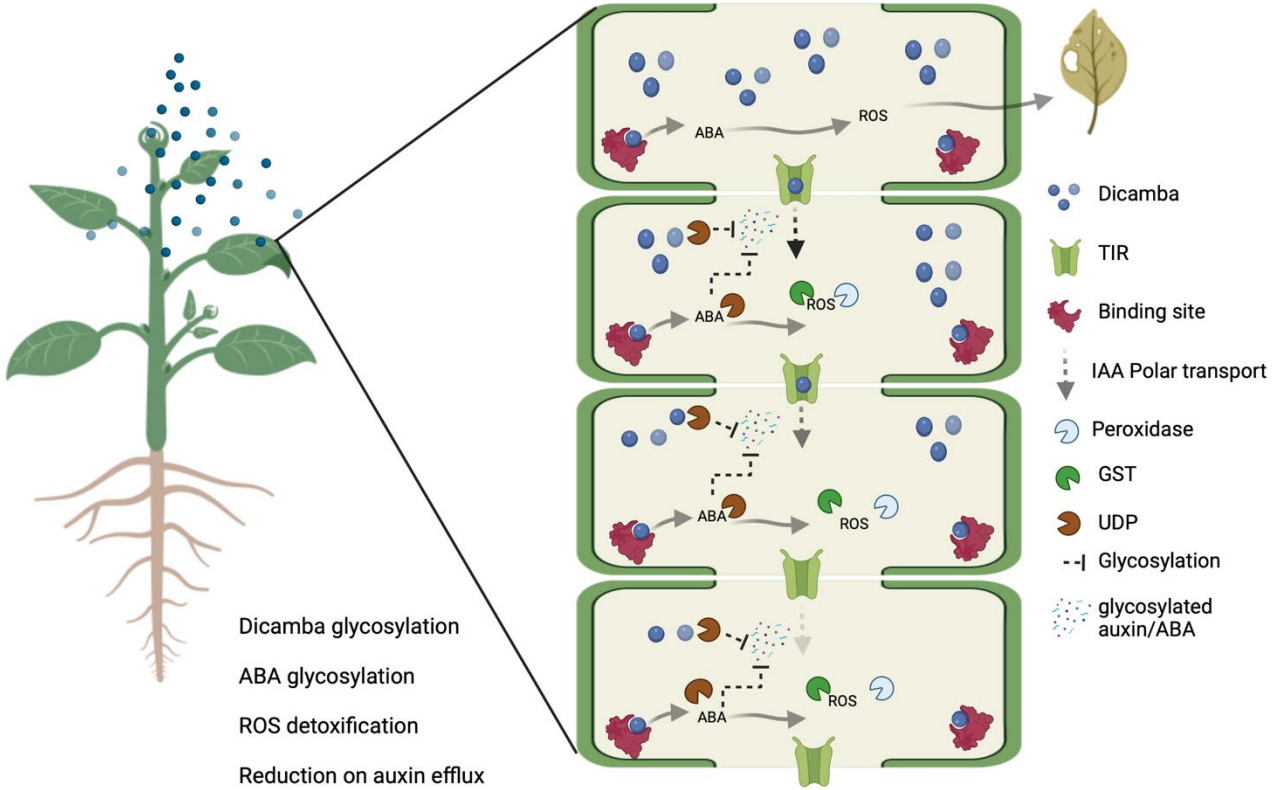


Hypothesis: Mechanism of resistance

Initial Auxin damage

Recovery

Hypothesis: mechanism of resistance



Conclusions

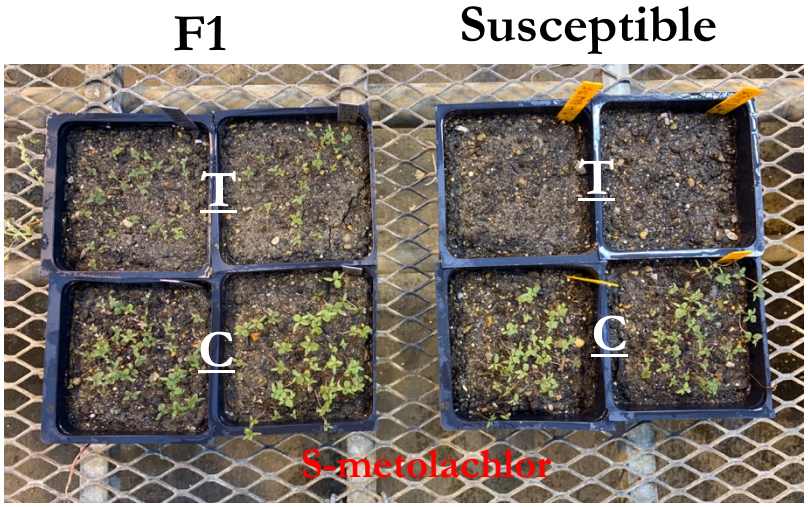
- Dicamba resistance:
  - Incompletely dominant trait
  - Moderate heritability
  - Multi-genic trait

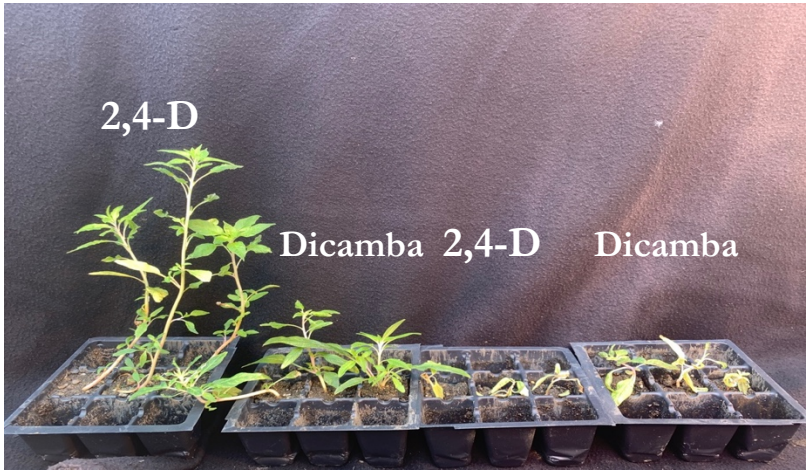
- Multiple candidate genes identified including Glutathione-S-transferases and UDP-glycosyl transferase

- Hypothesis of mechanism of resistance:
  - ROS detoxification via GST and peroxidase
  - Glycosylation and conjugation of ABA and dicamba
  - Changes in dicamba efflux

Future studies

- Physiology studies
- Expression variation after dicamba treatment
- Functional validation of genes
- 3-way resistance QTL mapping:
  - 2,4-D
  - S-metolachlor
  - Dicamba





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