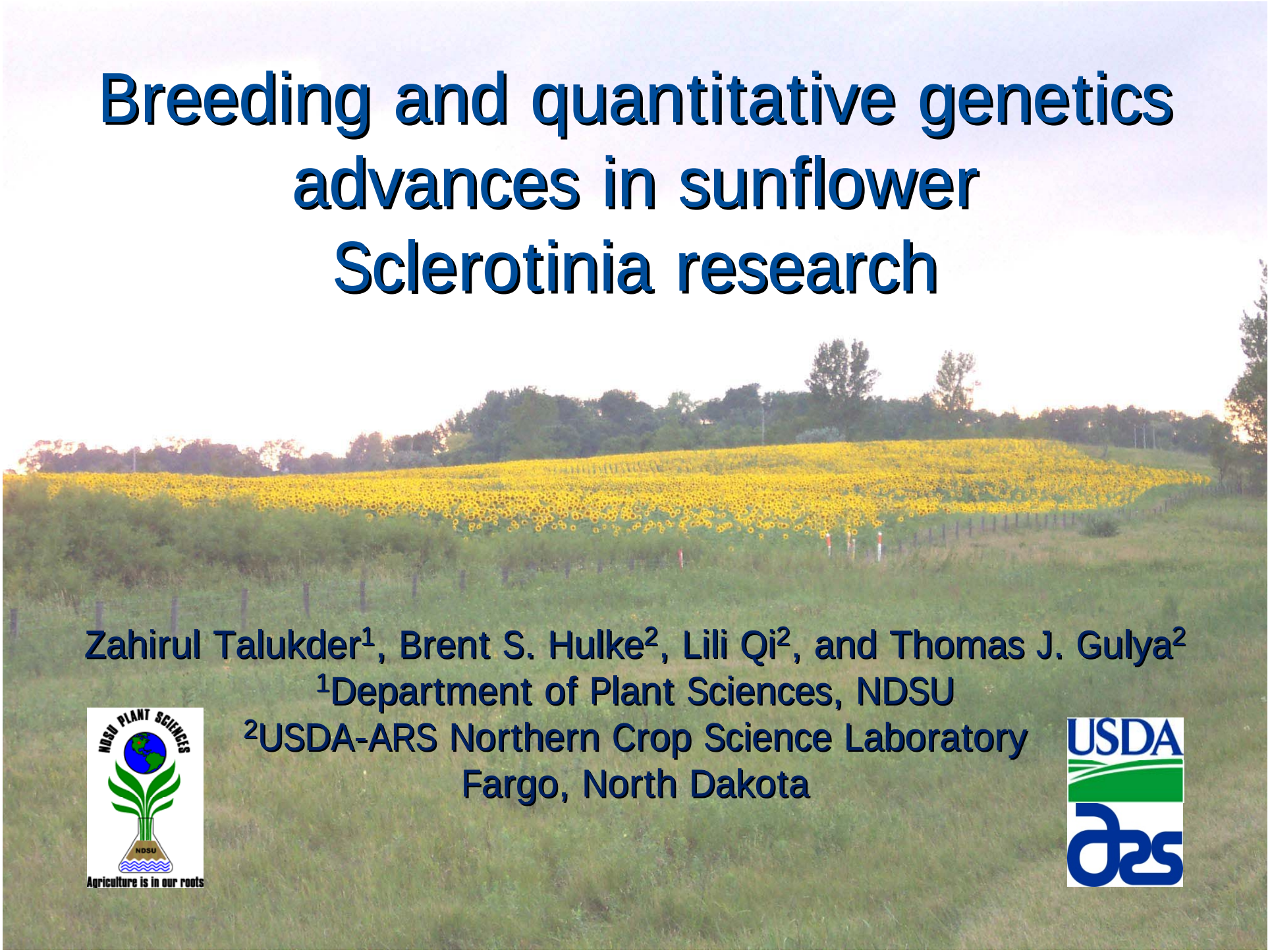
Breeding and quantitative genetics advances in sunflower Sclerotinia research



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Sunflower and Sclerotinia

- Sclerotinia causes two distinct diseases in sunflower:
  - Stalk rot: Underground infection. Sclerotia germinate into mycelia and infect the roots, unique to sunflower
  - Head rot: Above ground infection. Apothecium forms ascospores which fall and germinate on susceptible plants and cause infection
- Sclerotinia stalk rot is usually the most economically serious disease of sunflower
- Under favorable environmental conditions, Sclerotinia head rot can be more damaging too

Resistance to *S. sclerotiorum*

- *Sclerotinia sclerotiorum* has an extremely wide host range
- No known complete resistant sources
- Resistance is polygenic in nature
- Genetics of Stalk rot and Head rot resistance is different
- Resistance breeding relies on incorporating genetic factors from various partially-tolerant breeding lines

Research Efforts

Two major research efforts are discussed in this presentation

- Pyramiding Sclerotinia head rot resistance into elite sunflower breeding lines with the aid of DNA markers
- Association mapping of Stalk rot resistance in domesticated sunflower population using the candidate gene approach

Background

- Sixteen QTLs for head rot resistance have been mapped in two USDA-released lines, HA 441 and RHA 439 (Yue et al., 2008)
- Molecular markers (TRAP & SSR) linked to head rot QTLs are available to aid in introgressing QTLs into elite background
- Molecular mechanisms of host resistance to *S. sclerotiorum* have been reported in *Arabidopsis* (Guo and Stotz, 2007)
- Two hundred sixty domesticated *Helianthus annuus* plant introductions (PIs) were examined for stalk rot resistance in 2008 and 2009 in multi-location trials

Methodology-1

- Pyramiding Sclerotinia head rot resistance into elite sunflower breeding lines with the aid of DNA markers
  - Resistant donor parent:
    - A single F_6 RIL from the original HA 441 x RHA 439 mapping population
  - Recurrent parents:
    - CONFSCL R5 -confection type, and RHA 464 - oilseed type
  - Segregating backcross populations:
    - 50 CONFSCL R5 BC_1F_1 progeny lines, and 200 RHA 464 BC_1F_1 progeny lines
  - Targeted QTLs:
    - Seven QTLs in 4 linkage groups
  - Flanking DNA markers:
    - 9 TRAP markers - T123-R20-380, T47-R03-180, T02-R23-225, T08-R13-528, T36-R03-390, T05-R21-610, T36-R13-460, T61-R23-360 and T36-R03-670
    - 1 SSR marker - ORS 749
  - Both the BC_1F_1 populations were genotyped following the procedure described by Yue et al. (2008)

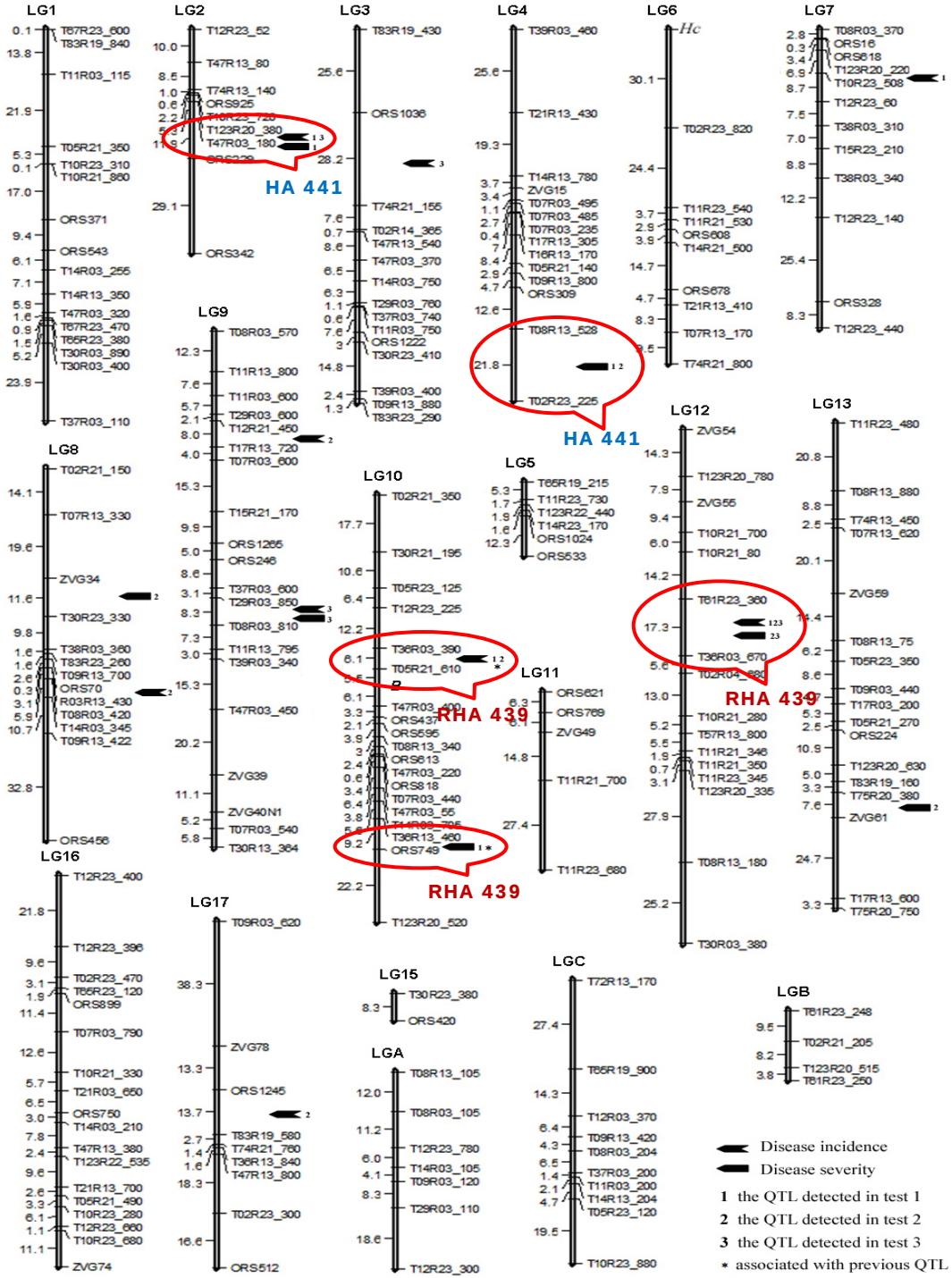


Fig. 1. Location of all 16 QTLs for head rot resistance detected in the HA 441/RHA 439 F2:3 population. Yue et al. (2008)

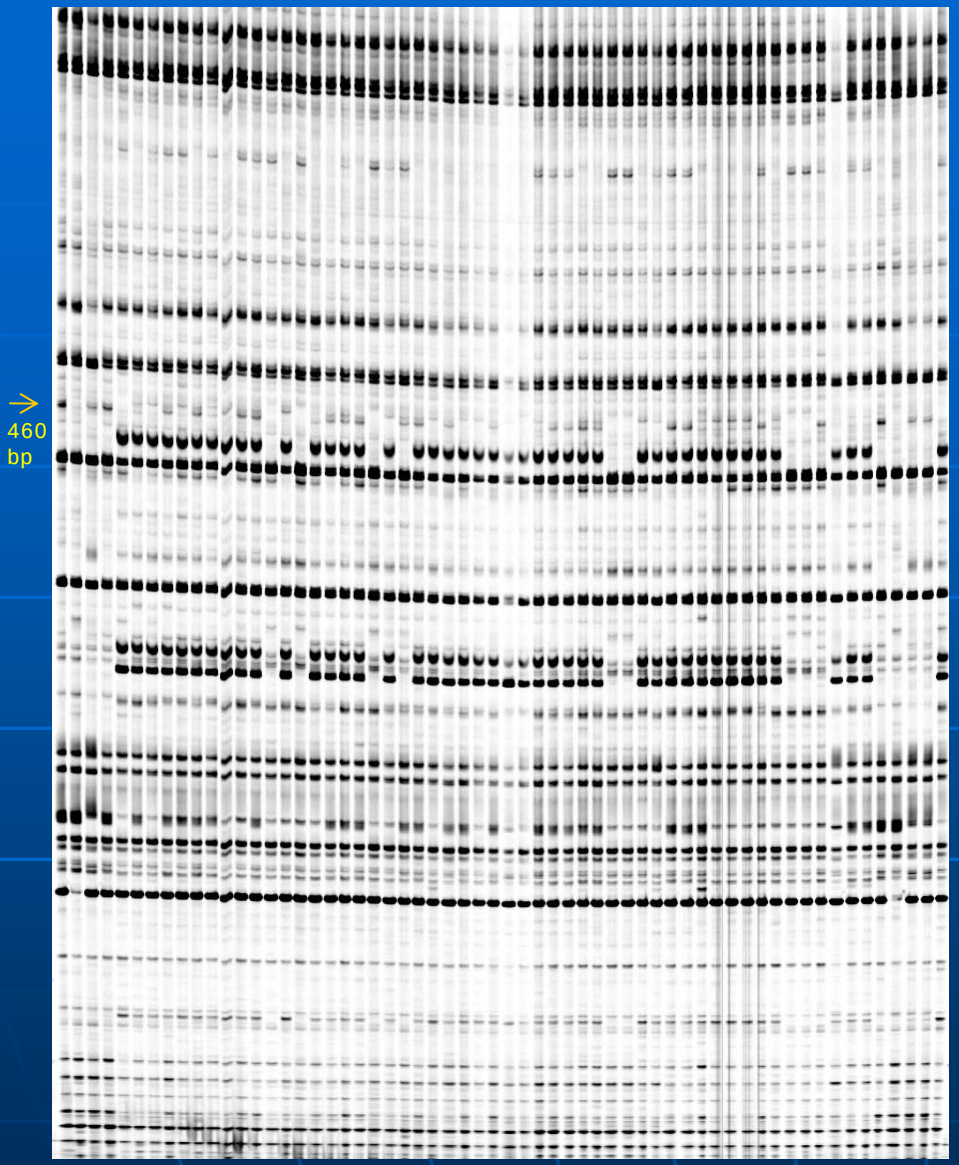
Three tests:
test 1, Carrington in 2006
test 2, Carrington in 2007
test 3, Fargo in 2007

Results

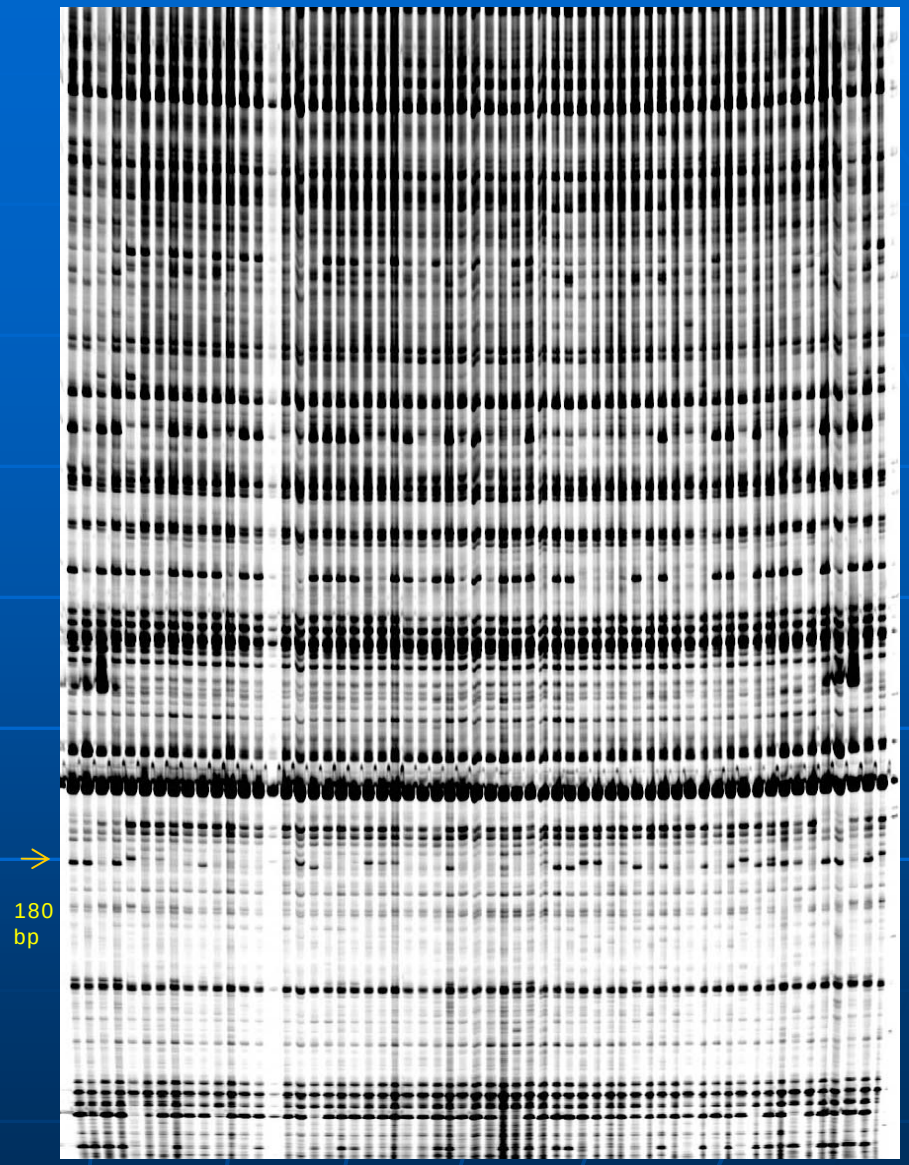
Selected QTLs for head rot resistance in the HA 441/RHA 439 mapping population with flanking markers and their distribution within the donor and recurrent parents, CONFSCL R5 and RHA 464

LG	QTLs	LOD	R^2	Flanking Markers	Distance (cM)	Resistance Source	Donor RIL	Recurrent Parents	
								CONFSCL R5	RHA 464
LG2	*QDi1 (2[a])* *QDs1 (1)*	4.4 2.9	16.2 12.8	T123-R20-380 T47-R03-180	5.3	HA441 (+) HA441 (+)	+ +	+ -	+ +
LG4	*QDs2 (2)*	6.9	24.7	T02-R23-225 T08-R13-528	21.8	HA441 (+) HA441 (+)	- +	+ +	- +
LG10	*QDi3 (2)**	3.4	13.6	T36-R03-390 T05-R21-610	6.1	RHA439 (+) RHA439 (+)	- +	+ +	+ +
LG10	*QDs3(1)**	11.8	34.5	T36-R13-460 ORS 749 (ssr)	9.2	RHA439 (+) RHA439 (+)	+ +	- -	+ +
LG12	*QDi4 (3)* *QDs5 (2)*	4.4 3.7	21.6 22.8	T61-R23-360 T36-R03-670	17.3	RHA439 (+) RHA439 (+)	+ +	- +	- +

QDi = Disease incidence QTL, *QDs* = disease severity QTL, * = QTL identified in other studies,
[a] = numbers of test sites the QTLs were identified, R^2 = amount of phenotypic variance (%) explained by QTL



ConfScl_BC1F1_T36-R13



ConfScl_BC1F1_T47-R03

Example of TRAP markers amplified from the CONFSCL R5 BC_1F_1 population along with the donor and recurrent parents and parents of the QTL mapping study, HA 441 and RHA 439

Results

- Based on marker segregation data, we have selected:
  - 8 progeny plants from CONFSCL R5 BC_1F_1 and
  - 30 progeny plants from RHA 464 BC_1F_1 population for further backcrossing

- Future activity: Complete two backcrossing and genotyping in 2011 and make testcrosses to confirm resistance to Sclerotinia head rot in our marker-assisted selection population.

Methodology-2

Association mapping of stalk rot resistance in domesticated sunflower population using the candidate gene approach

- Candidate genes (*Arabidopsis thaliana* defense genes) :
  - *ABI1* (ABA Insensitive 1), and *ABI2* (ABA Insensitive 2) -involved in abscisic acid (ABA) signal transduction
  - *EIN2* (Ethylene Insensitive 2) - central regulator of ethylene signaling
  - *LACS2* (Long-chain Acyl-CoA Synthetase 2) - involved in cutin biosynthesis pathway
  - *DET3* (De-Etiolated 3) - involved in oxalic acid signaling, and
  - *COI1* (Coronatine Insensitive 1) jasmonate receptor
  - *NPR1* (Nonexpresser of PR genes 1) - controls the onset of the SA-mediated systemic acquired resistance (SAR) pathway
  - *PAD3* (Phytoalexin Deficient 3) - encodes an enzyme required for biosynthesis of camalexin

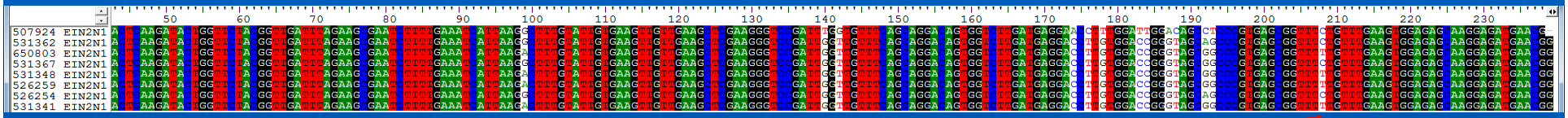
Methodology-2

- Primer design:
  - Nucleotide sequences of the candidate genes were used to BLAST search against the NCBI EST database for sunflower EST sequences
  - Sunflower EST sequences with high score and e-value were then selected for each gene, and searched for contig assembly sequences in the Compositae Genome Project database (http://cgpdb.ucdavis.edu/)
  - The contig sequences were reverse BLAST against Gene bank to confirm the gene identity
  - Multiple overlapping primer pairs were designed from contig sequences using the Primer3 software
  - *NPR1* and *PAD3* orthologs were not found in a BLAST search of sunflower ESTs

Methodology-2

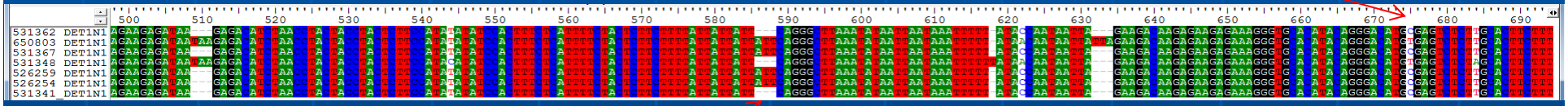
- Association mapping population:
  - 249 domesticated plant introductions PIs
  - 11 elite USDA lines
  - 2 hybrid checks, susceptible (Car 270) & resistant (Croplan 305)
  - examined for stalk rot resistance in 2008 and 2009 in multi-location replicated trials
- DNA extraction, PCR amplification & sequencing:
  - DNA extracted from 1040 individuals of 260 PIs
  - PCR conditions optimized for each pair of primers
  - Subset of 8 PIs selected, 4 each from susceptible & resistant groups for test sequencing
  - Cleaned PCR amplicon sent for sequencing to Genomics and Bioinformatics Research Unit at USDA-ARS, Stoneville, MS

Example of aligned sequences generated using primers developed from Sclerotinia resistance candidate gene sequences in the genetically distinct sunflower individuals of the association mapping population



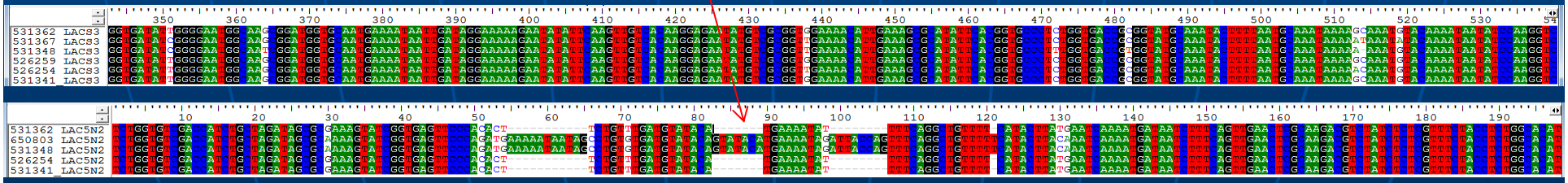
Arabidopsis thaliana EIN2 (Ethylene Insensitive 2) homologus gene sequences in cultivated *Helianthus annuus* lines

SNP



Arabidopsis thaliana DET3 (De-Etiolated 3) homologus gene sequences in cultivated *Helianthus annuus* lines

INDEL



Arabidopsis thaliana LACS2 (Long-Chain Acyl-CoA Synthetase 2) homologus gene sequences in cultivated *Helianthus annuus* lines

Summary

- The genetic factors contributing towards Sclerotinia head rot resistance derived from the mapping parents, HA 441 and RHA 439 has already been fixed to a great extent in the USDA sunflower breeding lines
- The PIs exhibited a wide range of Sclerotinia stalk rot reaction in the field, and initial data of the candidate gene sequences showed a great deal of variation at the genomic level
- Association mapping study might reveal potential sources of different resistant genes than found in USDA inbreds, and also would diversify the genetic basis of the USDA breeding material

Future Plans

- Complete two backcrossing and genotyping in 2011 and make testcrosses to confirm resistance to Sclerotinia head rot in our marker-assisted selection population
- Complete resequencing of amplicons from genetically distinct individuals of 260 association population
- Development of SNP markers for genotyping of the association population, and begin analysis

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Thanks for your
attention!