Association mapping of Sclerotinia stalk rot resistance in domesticated sunflower plant introductions

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Background

- *Sclerotinia sclerotiorum* causes two serious diseases in Sunflower
  - Stalk rot and Head rot, genetics of resistance is different
  - Resistance is polygenic, no major resistant gene is known
  - **Resistance breeding relies on incorporating genetic factors from various partially-tolerant breeding lines**

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Quantitative disease resistance mapping

- Two approaches
  - Linkage analysis based QTL mapping, traditional tool
    - need to develop bi-parental mapping population by controlled crosses with known ancestry
    - few opportunities for recombination, resulting in low mapping resolution
    - QTLs that differ between two parental lines can only be detected in each mapping population

Quantitative disease resistance mapping

- Linkage disequilibrium (LD) based Association Mapping, new tool
  - no need to develop mapping population
  - exploits historical and evolutionary recombination events at the population level
  - mapping resolution is higher than bi-parental mapping population of the same size
  - suffer from risk of incurring false positives due to population structure and kinship among individuals

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Association Mapping

- Two approaches
  - Genome-Wide Association Mapping
    - whole genome may be scanned to identify markers that are associated with a particular phenotype
  - Candidate-Gene Association Mapping
    - alleles at a few selected functional candidate genes thought to be involved in controlling the trait of interest may be tested for association
- Candidate-Gene Association Mapping study is more hypothesis-driven than a Genome-Wide study

Candidate-Gene AM of Sclerotinia stalk rot resistance in sunflower

Methods

- AM population = 260 sunflower lines
  - 249 domesticated plant introductions (PIs)
  - 11 elite USDA inbred lines
  - 2 hybrid checks, susceptible (Car 270) & resistant (Croplan 305)
  - examined for stalk rot resistance in 2008 and 2009 in multi-location replicated trials
  - 52 genotypes with best resistant response, and 52 most susceptible genotypes were sequenced for selected candidate genes

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- Six 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
- 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 GenBank to confirm the gene identity
  - Multiple overlapping primer pairs were designed from contig sequences using the *Primer3* software

- DNA extraction, PCR amplification & sequencing:
  - DNA extracted from 260 individuals of AM population
  - PCR conditions optimized for each pair of primers
  - Cleaned PCR amplicons sent for sequencing to Genomics and Bioinformatics Research Unit at USDA-ARS, Stoneville, MS
- Sequence analysis and SNP survey
  - *DnaSP* v.5.1 software and web-based tool *SNiPlay* were used for sequence analysis and SNP survey within the gene sequences
- Population structure and Kinship analysis
  - SNP markers develop by NSA were used for both population structure and kinship analysis. Structure analysis was done with 750 randomly chosen SNP markers using *Structure* v.2.3.3 software and, kinship analysis was performed with 5244 SNPs using *SPAGeDi* v1.3a software
- Linkage disequilibrium and Association mapping
  - *Haploview* v.4.2 and *TASSEL* v.3.0 software were used for LD and association mapping analysis, respectively

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Results

Summary table of sequence analysis for eight candidate gene sequences

Sequence name	Length (bp)	LG	Map position (cM)	SNP	InDel	Coding region			Non-coding region		
						Length	SNP†	Freq.	Length	SNP	Freq.
ABI1N1	279	-	-	12	4	171	$1_{(0)}$	1/171	108	11	1/10
ABI2N2	189	10	44.9	5	0	189	$5_{(4)}$	1/38	0	-	-
COI24	668	14	50.9	23	1	668	$23_{(5)}$	1/29	0	-	-
COI57	660	14	7.0	12	0	660	$12_{(1)}$	1/55	0	-	-
DET1N1	808	16	68.3	61	24	192	$7_{(3)}$	1/27	616	54	1/11
EIN1	483	14	30.1	15	3	403	$13_{(2)}$	1/31	80	2	1/40
EIN2N1	239	-	-	3	0	239	$3_{(0)}$	1/80	0	-	-
LAC1N2	465	12	47.1	18	6	174	$2_{(0)}$	1/87	291	16	1/18
Total	3791			149	38	2696	$66_{(15)}$	1/41	1095	83	1/13

† number of SNP that led to changes in amino acid codons are shown in the parenthesis

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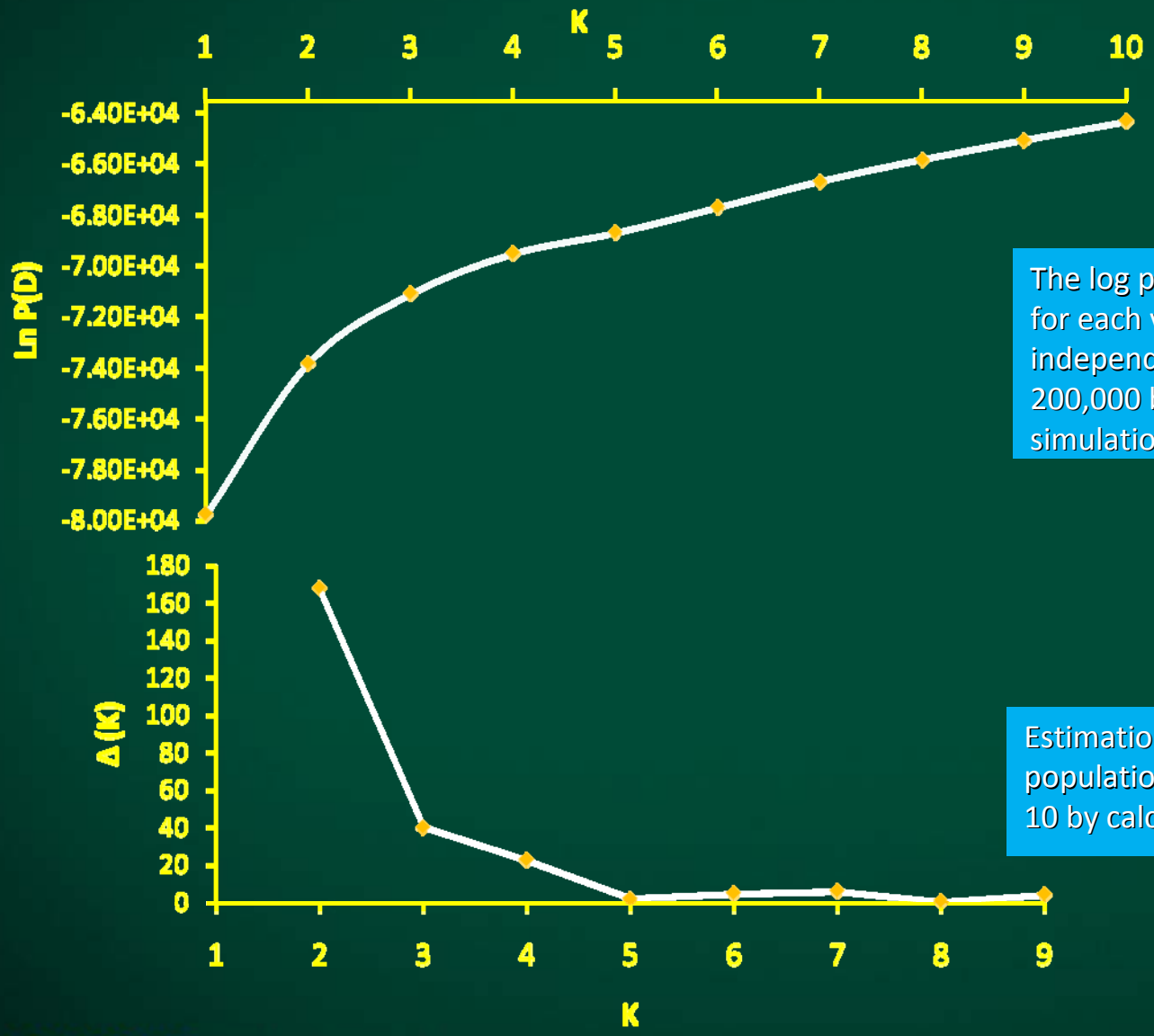
Estimation of number of sub-populations in the CG-AM population



The log probability of data, Ln P(D) for each value of K averaged over 3 independent runs of structure with 200,000 burn-in steps and 200,000 simulation steps.

Estimation of the number of populations for K ranging from 1 to 10 by calculating delta K values.

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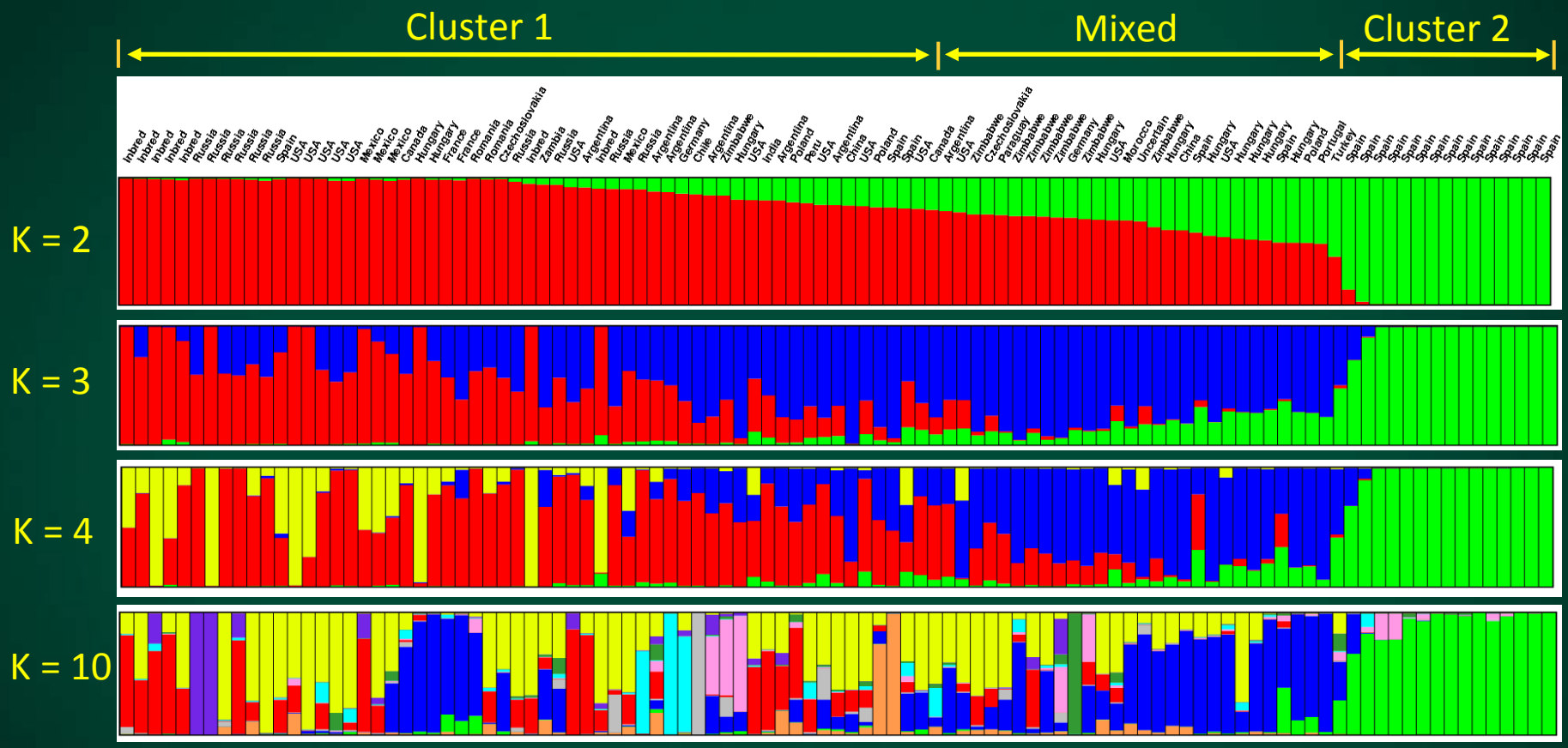
Bar plot of the STRUCTURE analysis



Each of the 104 genotypes is represented by a vertical bar, which is partitioned into K colored segments that represent the individual's estimated membership to the K clusters. The separation of cluster 1 and cluster 2 was done by the threshold for membership coefficient, Q ≥ 0.75.

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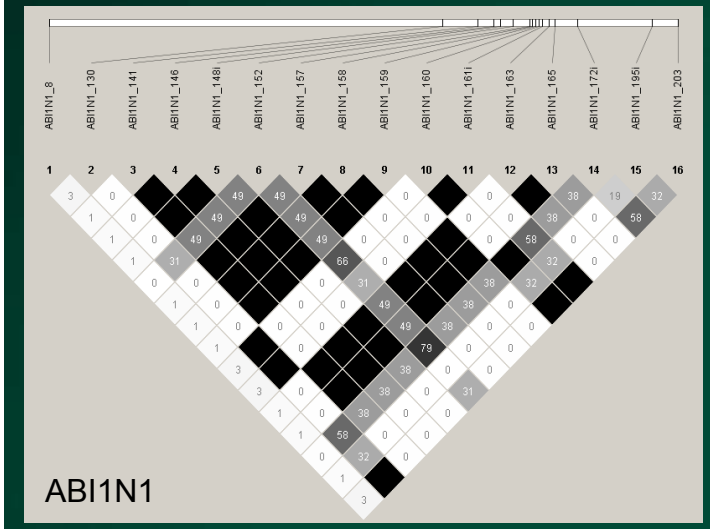
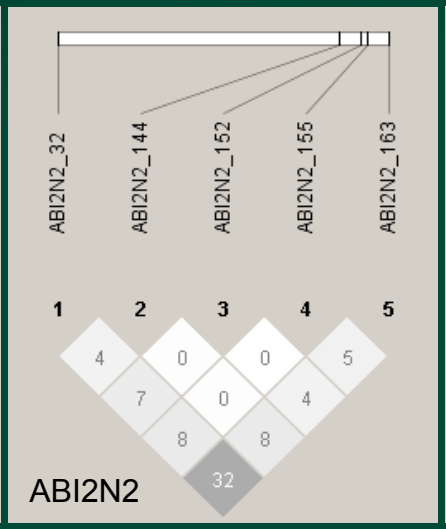
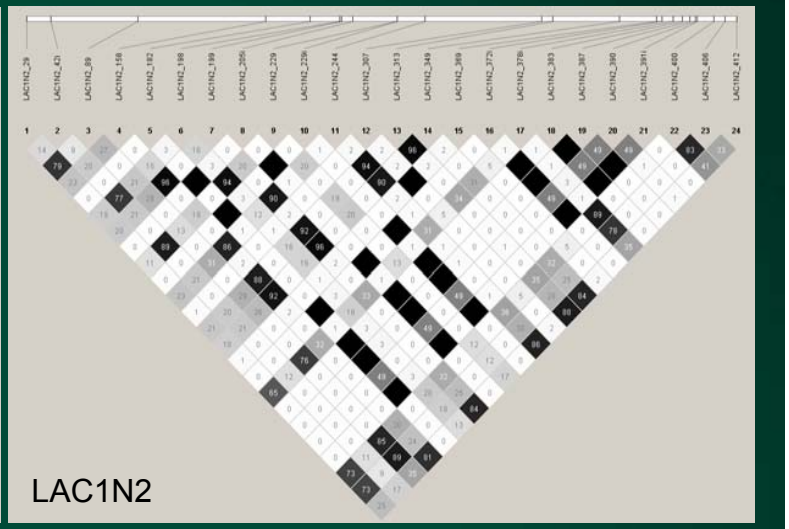
Patterns of linkage disequilibrium (LD) for eight candidate gene sequences

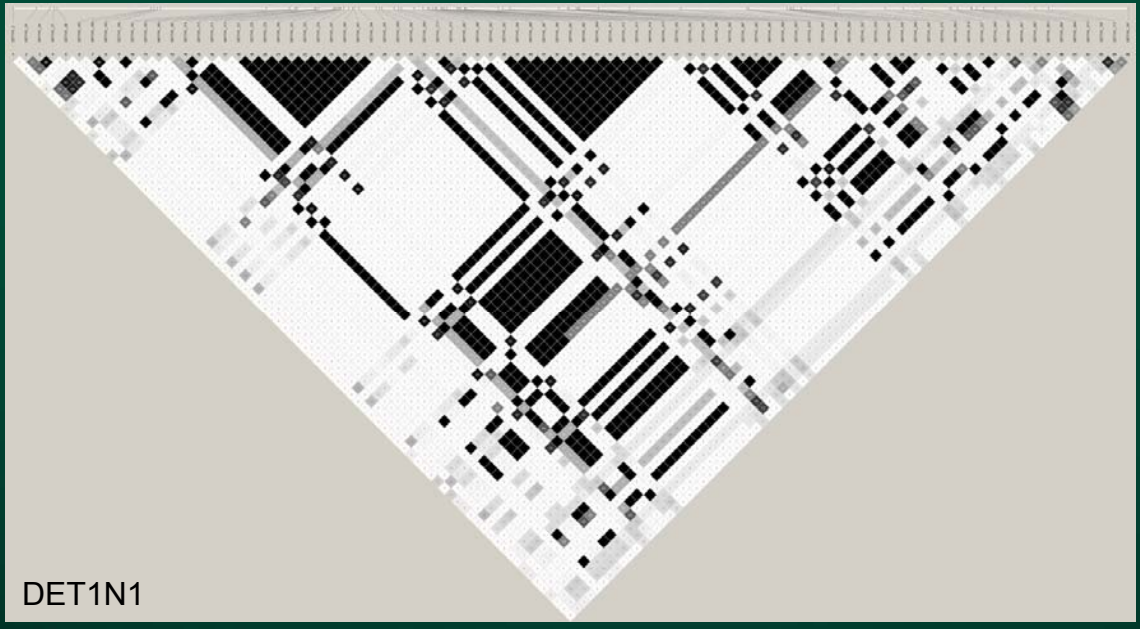


ABI1N1



ABI2N2


LAC1N2


DET1N1

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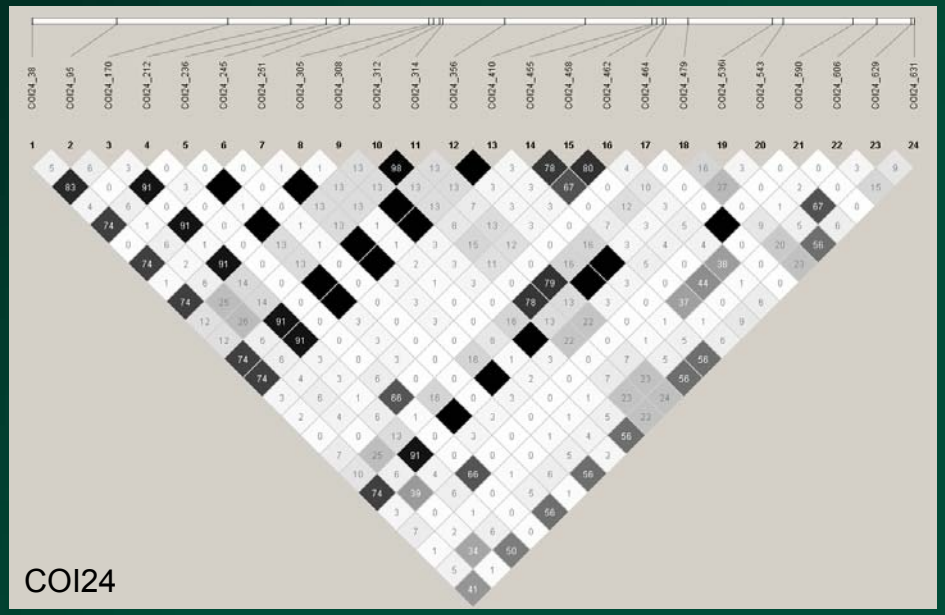

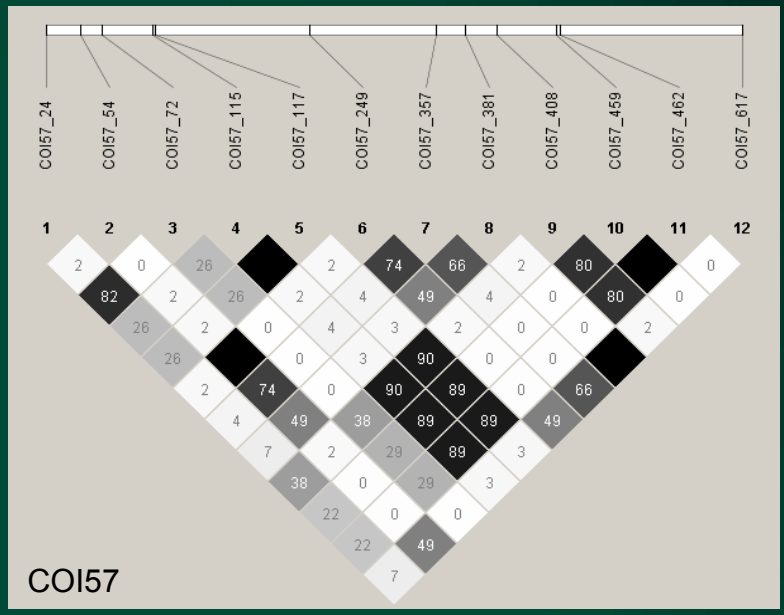

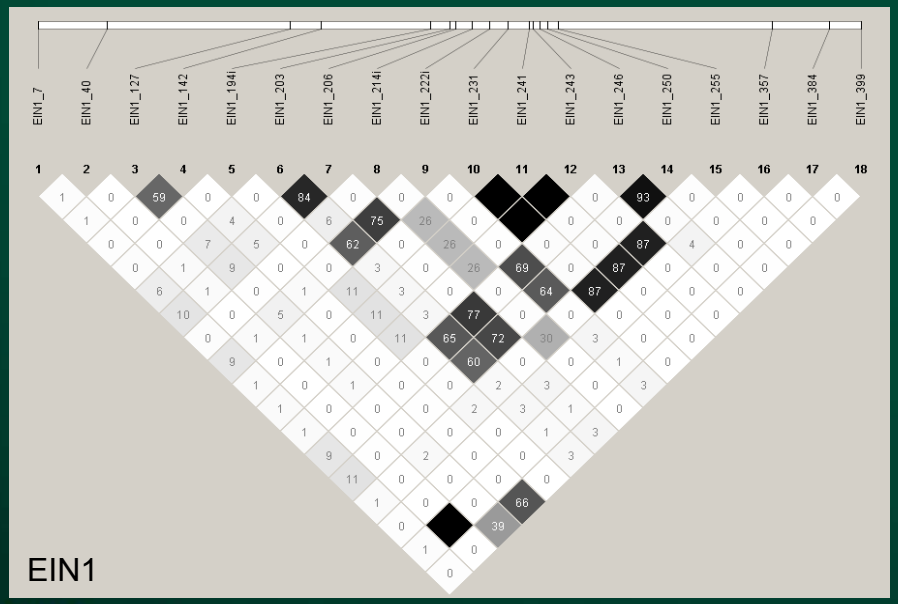

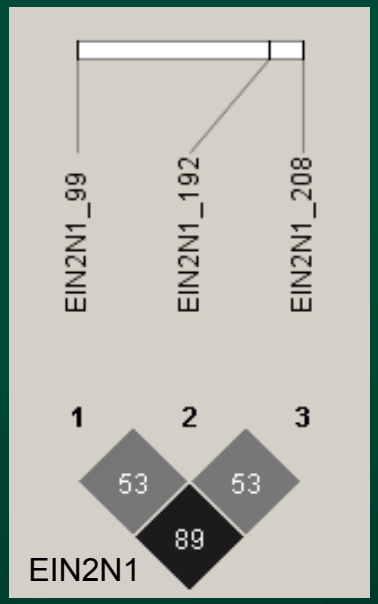

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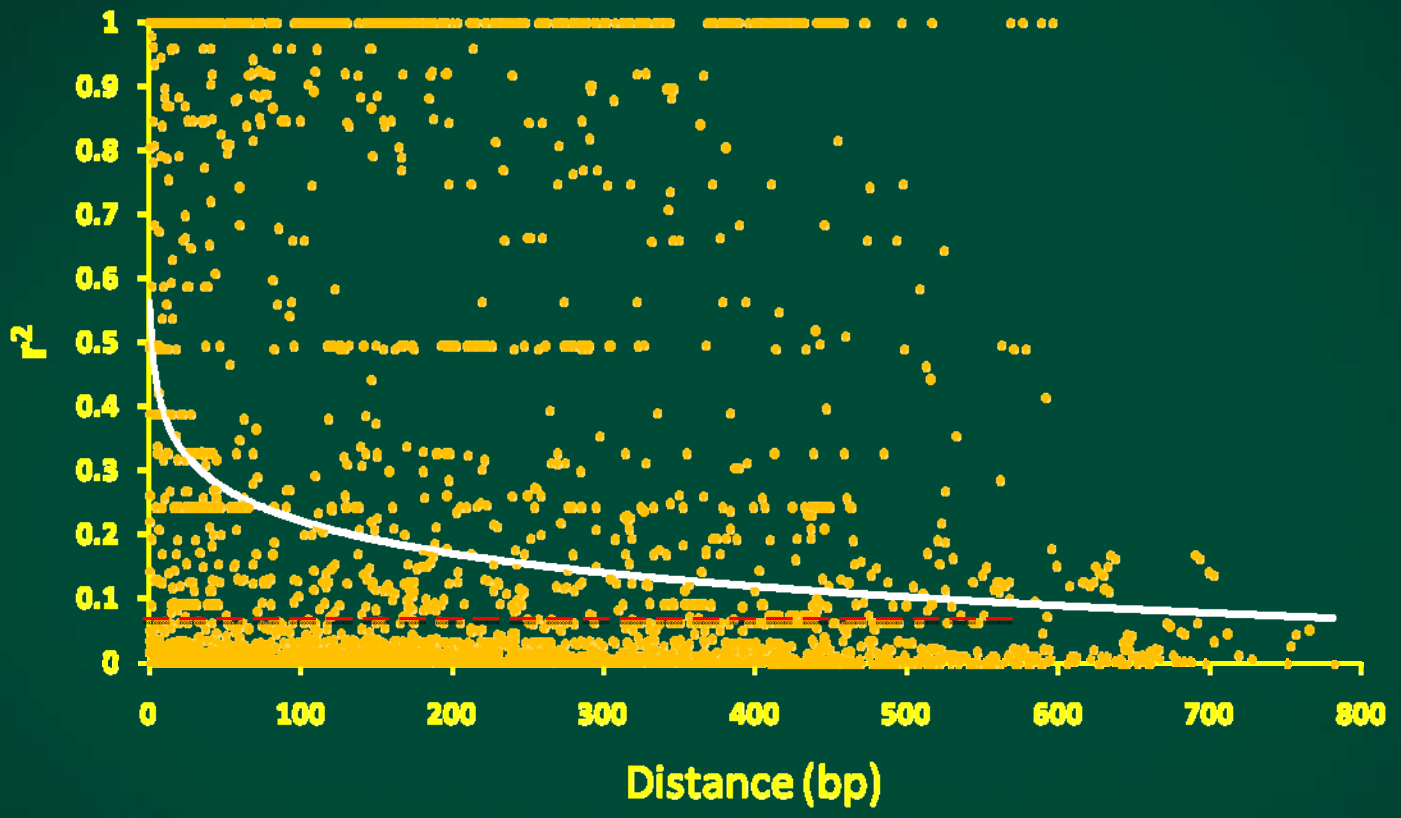
Linkage disequilibrium (LD) decay of eight candidate genes



LD decay of eight candidate genes was measured by plotting squired correlations of allele frequencies (r^2) against genetic distance (bp) between pairs of polymorphic sites. Inner fitted trend line is a nonlinear logarithmic regression curve.

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Significant association between candidate gene SNP markers and Stalk rot resistance in sunflower AM population using four statistical models

Gene	Significant SNPs	Tag SNP	3 Environment mean				Davenport			
			GLM	GLM +Q	MLM +K	MLM +K+Q	GLM	GLM +Q	MLM +K	MLM +K+Q
COI1	COI57_459, COI57_462, COI57_115, COI57_117 & COI57_408	COI57_408	***	**	**	**	***	**	*	*
	COI57_24 and COI57_72	COI57_72	***	ns	ns	ns	***	ns	ns	ns
	COI24_236, COI24_251, COI24_308, COI24_356, COI24_410, COI24_536i, COI24_170 & COI24_38	COI24_251	***	ns	*	ns	***	ns	ns	ns
	COI24_631	COI24_631	***	ns	ns	ns	**	ns	ns	ns
	COI24_606	COI24_606	ns	ns	*	ns	ns	ns	ns	ns
	COI24_312 & COI24_314	COI24_312	**	ns	ns	ns	**	ns	ns	ns
ABI1 and ABI2	ABI2N2_32	ABI2N2_32	***	ns	ns	ns	**	ns	*	ns
	ABI2N2_163	ABI2N2_163	**	ns	ns	ns	ns	ns	ns	ns
	ABI2N2_155	ABI2N2_155	**	ns	ns	ns	ns	ns	ns	ns
	ABI1N1_141, ABI1N1_146, ABI1N1_148i, ABI1N1_157, ABI1N1_158, ABI1N1_158, ABI1N1_158, ABI1N1_159, ABI1N1_163, ABI1N1_165 & ABI1N1_172i	ABI1N1_146	ns	ns	ns	ns	*	ns	ns	ns
EIN2	EIN1_246, EIN1_250, EIN1_222i & EIN1_206	EIN1_250	**	**	**	ns	**	ns	ns	ns
	EIN1_127	EIN1_127	ns	ns	*	ns	ns	**	*	*
DET3	DET1N1_614i	DET1N1_614i	**	ns	ns	ns	**	ns	ns	ns
	DET1N1_535,DET1N1_537, DET1N1_217, DET1N1_109, DET1N1_661i,DET1N1_103,DET1N1_428i, DET1N1_181, DET1N1_540i,DET1N1_660, DET1N1_677i, DET1N1_538 & DET1N1_202	DET1N1_217	ns	ns	*	ns	ns	ns	ns	ns
	DET1N1_394i	DET1N1_394i	ns	ns	*	ns	ns	ns	ns	ns
	DET1N1_25	DET1N1_25	ns	ns	ns	ns	***	ns	*	ns
	DET1N1_34, DET1N1_37, DET1N1_107i & DET1N1_105i	DET1N1_37	ns	ns	ns	ns	***	ns	ns	ns

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Progress in Genome-Wide AM of stalk rot and head rot resistance

- Population structure and Kinship analysis of AM population is complete
- Mapping of NSA SNPs in the sunflower linkage map are in progress
- Phenotypic evaluation of AM population
  - Stalk rot is complete (2 yrs x 2 locations)
  - Head rot is in progress (1 yr x 2 locations is done)

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Future Plans

- Genotyping of the remaining lines of the AM population with significant CG-SNPs, and complete CG-AM work
- Complete GW-AM of Stalk rot resistance
- Continue working on association mapping of Head rot resistance and Phomopsis resistance

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