FHI Biotechnology Approaches



Personnel

- Germplasm (material for clonal testing, transformation)
 - Fred Hebard (The American Chestnut Foundation, TACF)
 - Sandra Anagnostakis (Connecticut Agr Expt Station, CAES)
 - Jerre Creighton (Virginia Dept of Forestry, VDF)
 - Gary Griffin (Virginia Tech & Am Chestnut Cooperators' Foundation, ACCF)
- Breeding (crosses for resistance gene mapping)
 - Fred, Sandra
 - Sara Fitzsimmons (TACF & Penn State Univ)
- Testing (phenotyping, mechanisms of resistance)
 - Blight (Fred, Sara), Resistance mechanisms (new post-doc with Fred)
 - Early screening (SUNY Team & Josh Bronson (USFS, Resistance Screening Center)
 - Phytophthora (Joe James, Steve Jeffers) with Clemson Univ
 - Field (developing now and year 3)
- Mapping (genotyping, gene discovery, marker selection)
 - Tom Kubisiak(left USFS in Feb), Dana, SIFG lab (Chuck, Casey, Thomas, Kristel)
 - Clemson Team, Bert Abbott's Lab and CUGI
 - Bode Olukolu (leaving now for NC State)
 - Meg Staton, Ali Barakat, Eric Feng

Objectives/Deliverables

- Germplasm
 - Provide clonal and transgenic teams with plant material
 - Workhorse lines, experimental controls
 - Diverse Hybrids & Large Surviving Americans (LSAs) for clonal testing
 - Advanced backcross hybrids for clonal increase
- Breeding & Testing (phenotyping)
 - Develop informative populations for trait/gene mapping
 - Collaborate on early disease screening development
 - Collaborate on field testing; clones and transgenics
- Mapping (genotyping)
 - Fine map resistance genes and quantify their effects
 - Collaborate on integrating maps for candidate gene (CG) selection
 - Develop Marker-assisted Selection (MAS) for breeding

Germplasm

- The American Chestnut Foundation (TACF)
 - Germplasm Agreement negotiated & signed with Univ Georgia
 - under MOU between FHI and TACF
- Connecticut Ag Exp Station (CAES)
 Crosses from CAES's extensive, long-term program
- Virginia Department of Forestry (VDF)
 Crosses in VDF's operational program
- Am Chestnut Cooperators' Foundation (ACCF)
 - Crosses from ACCF's public domain trees

Breeding & Phenotyping

- Blight resistance mapping resources
 - TACF/NSF experimental 'Mahogany' F2 population
 - Blight resistance phenotyping summer 2011
 - TACF/Meadowview advanced backcross lines
 - Operational blight resistance phenotyping
 - Pennsylvania TACF operational BC3 population
 - Blight resistance phenotyping complete spring 2011
- Phytophthora (ink disease) resistance mapping
 - TACF with Clemson

Blight resistance QTLs

• Genetic map— 'Mahogany' F2 cross, 463 markers, 686 cM (92% genome coverage)

- Two isolates, quantitative scoring

- Three blight resistance QTLs confirmed (LG-B, LG-F, LG-G), with a fourth one likely (LG-E)
 - Three account for 70% of the genetic variation
 - FHI allows linkage to new, high density Chinese chestnut map, gene sequences and genome sequence
- 38 Candidate Genes identified for evaluation in transgenics

Ink Disease Resistance QTLs

(Phytophthora cinnamomi)

- Genetic map– 'Nanking' BC1 family, 203 SNPs, 575 cM (about 80% genome coverage)
 - markers from Am chestnut, not as efficient for mapping
 - scored for Ink Disease resistance
 - Two major QTLs mapped on LG-E
 - Maternal map: at 12-15 cM, LOD=4.42, R²=0.35
 - Paternal map: at 46-62 cM, LOD=5.39, R²=0.40
 - 4 Candidate Genes identified and are being tested in transgenics

Chinese Chestnut Integrated Consensus Linkage Map G

н

Α

в

С

D

Е

F

J

1

L

к

0.0 CmSR0385 0.0 CmSR0385 1.1 CmSR0385 0.0 CmSR0385 3.5 CmSR0385 0.0 CmSR0385 3.5 CmSR0385 0.0 CmSR0385 3.5 CmSR0385 0.0 CmSR0385 3.5 CmSR0385 0.0 CmSR0385 3.6 CmSR0385 0.0 CmSR0385 3.6 CmSR0385 0.0 CmSR0385 3.6 CmSR0385 0.0 CmSR039110 3.4 CmSR0497180 1.3 CmSR0497180 3.4 CmSR0497181 1.3 CmSR0497181 3.4 CmSR0497181 2.0 CmSR0497181 3.4 CmSR0497181 2.0 CmSR0497181 3.5 CmSR0497181 2.0 CmSR0497181 3.6 CmSR0497181 2.0 CmSR0497181 3.6 CmSR0498 2.0 CmSR0497181 3.6 CmSR0498 2.0 CmSR0497181 3.6 CmSR04988 2.0	0.0 CmSNP01141 0.1 CmSNP00761 CmSNP0077 1.1 CmSNP00797 CmSNP0077 CmSNP00797 1.2 CmSNP00798 CmSNP00798 CmSNP00798 1.3 CmSNP00798 CmSNP00798 CmSNP00798 1.4 CmSNP00798 CmSNP00798 CmSNP00798 1.5 CmSNP00798 CmSNP00798 CmSNP00798 1.5 <td< th=""><th>0.0 CmSNP01520 0.0 CmSNP01627 4 CmSNP0137 1.1 CmSNP01627 4 CmSNP0137 4.4 CmSNP01627 4 CmSNP0137 4.4 CmSNP01627 4 CmSNP0137 4.4 CmSNP0064 5 CmSNP0137 5.4 CmSNP0064 6 CmSNP0137 5.4 CmSNP0064 7 CmSNP0137 5.4 CmSNP0137 4 CmSNP0137 5.4 CmSNP0138 5 CmSNP0138 5.4 CmSNP01318 5 CmSNP0138 5.4 CmSNP01318 5 CmSNP0138 5.4 CmSNP01318 6 CmSNP0138 5.4 CmSNP01318 7 CmSNP0138 5.4 CmSNP0138 7 CmSNP0138 5.4 CmSNP0138 7 CmSNP0138 5.4 CmSNP0138 7 CmSNP0138 5.4 CmSNP0138 7 CmSNP0138 5.4 CmSNP0138</th><th>13.0 CmSNP0021 2.0 CmSNP0022 3.1 CmSNP0022 3.2 CmSNP0022 3.3 CmSNP0022 3.4 CmSNP0022 3.5 CmSNP0022 13.0 CmSNP0022 13.0 CmSNP0022 24.0 CmSNP0022 24.1 CmSNP0022 24.2 CmSNP0022 24.3 CmSNP0022 24.4 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112</th><th>0.0 CC-BSP001497 2.5 CC-BSP0015 2.5 CC-BSP0016 2.5 CC-BSP00111 2.5 CC-BSP00111 2.5 CC-BSP0134 2.5 CC-BSP0134 2.5 CC-BSP0134 2.5 CC-BSP0134</th><th>CmSNP00000 0.0 - CmSNP0007 CmSNP00050 1.1 - CmSNP0007 CmSNP00500 1.2 - CmSNP0007 CmSNP00500 1.2 - CmSNP00700 CmSNP00150 1.2 - CmSNP00700 CmSNP0015 1.2 - CmSNP007015 1.2</th><th>CnSNP00994 0.0 CnSNP00993 CnSNP0099 CnSNP009 CnSNP009</th><th>CmS0799 0.0 CmS0799 0.0 CmS0799 0.0 CmS079 0.0 CmS07 0.0 CmS07</th><th>803 304 305 305 305 305 305 305 305 305</th></td<>	0.0 CmSNP01520 0.0 CmSNP01627 4 CmSNP0137 1.1 CmSNP01627 4 CmSNP0137 4.4 CmSNP01627 4 CmSNP0137 4.4 CmSNP01627 4 CmSNP0137 4.4 CmSNP0064 5 CmSNP0137 5.4 CmSNP0064 6 CmSNP0137 5.4 CmSNP0064 7 CmSNP0137 5.4 CmSNP0137 4 CmSNP0137 5.4 CmSNP0138 5 CmSNP0138 5.4 CmSNP01318 5 CmSNP0138 5.4 CmSNP01318 5 CmSNP0138 5.4 CmSNP01318 6 CmSNP0138 5.4 CmSNP01318 7 CmSNP0138 5.4 CmSNP0138	13.0 CmSNP0021 2.0 CmSNP0022 3.1 CmSNP0022 3.2 CmSNP0022 3.3 CmSNP0022 3.4 CmSNP0022 3.5 CmSNP0022 13.0 CmSNP0022 13.0 CmSNP0022 24.0 CmSNP0022 24.1 CmSNP0022 24.2 CmSNP0022 24.3 CmSNP0022 24.4 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0022 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0122 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112 24.5 CmSNP0112	0.0 CC-BSP001497 2.5 CC-BSP0015 2.5 CC-BSP0016 2.5 CC-BSP00111 2.5 CC-BSP00111 2.5 CC-BSP0134 2.5 CC-BSP0134 2.5 CC-BSP0134 2.5 CC-BSP0134	CmSNP00000 0.0 - CmSNP0007 CmSNP00050 1.1 - CmSNP0007 CmSNP00500 1.2 - CmSNP0007 CmSNP00500 1.2 - CmSNP00700 CmSNP00150 1.2 - CmSNP00700 CmSNP0015 1.2 - CmSNP007015 1.2	CnSNP00994 0.0 CnSNP00993 CnSNP0099 CnSNP009 CnSNP009	CmS0799 0.0 CmS0799 0.0 CmS0799 0.0 CmS079 0.0 CmS07	803 304 305 305 305 305 305 305 305 305
 A Section of the secti	4.4 - CmSNP00493 64.5 - CmSN90498 5.6 - CmSNP00494 55.7 - CmSNP00496	Creative Cre	ese ch 26 ma 26 ma 26 ma 26 ma 26 ma 26 ma 20 mk d	isease	ut Ge overin Ls fror QTL fr	eneti g 743 n F2 m om B1	Constraints Constr	3

Candidate Gene List (1/3)

order C	Ccontig	Uniprot BestHit	Linkage_ Group	cDNA status	BinaryVector	BV status	TransPipe
1 C	Call-contig8901_v2	beta-1 3 glucanase	?	Cloned&sent	pFHI-B13Gluc	received	SUNY-ESF
20	Call-contig2586_v2	CBS domain protein	?	Cloned&sent	pFHI-CBS1	received	SUNY-ESF
3C	Call-contig11269_v2	UDP glucosyltransferase	B, G	Cloned&sent	pFHI-UDP	received	UGA

- Genes selected on multiple sources of information
 - trait mapping (QTL regions of genome)
 - including comparative analysis with Peach
 - gene expression analysis (genes on/off at site of blight infection)
 - gene sequence matching with other resistance-like genes
- Genes are going to Transgenic pipeline for testing
- Markers for these genes will be used for Marker-aided breeding

2 J14 CC454 contig41915_v2 Allene oxide cyclase (AOC) HI, Annual Meetworking

working

TBD

New Phenotyping Resources

- Expanded 'Mahogany' F2 population in VA
 - >50% increase in progeny number
 - Provides higher resolution mapping
- Large 'Mahogany-Graves' BC3 population in PA
 - 6x larger than F2 population (even higher resolution)
 - Evaluate in independent environment and genetics
- Operational B3F2 populations in VA
 - Test markers in operational materials

New Genotyping Resources

- 5000 SNP Chip
 - Using scalable, high-density, high-throughput tech
 - Includes validated SNPs from previous chips
 - Chinese (964 SNPs) and American (224 SNPs)
 - Designed new SNPs from Chinese chestnut gene sequences (~3900 SNPs)
 - Higher density markers necessary to take full advantage of larger, higher resolution mapping populations -> more precise gene mapping

Summary

- Our goals and progress are aligned:
 - High-quality candidate genes for transgene (cisgene) testing
 - High-quality DNA markers for marker assisted selection
 - High-quality germplasm for clonal testing and experimental materials
 - Emphasis on early, reliable screening for blight and Phytophthora resistance