INTERNATIONAL cotton genome INITIATIVE

ICGI research conference

CANBERRA 2010

conference proceedings

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Message of Welcome from the ICGI Chair

I would like to welcome everyone to the International Cotton Genome Conference on this, the 10th anniversary of our organization. ICGI grew out of an idea that originated at the Symposium on Molecular Markers in Cotton, sponsored by CSIRO in Canberra in 2000. Therefore it is very appropriate that we are back in Canberra with our original hosts in 2010. I believe we have seen large strides forward in our science, but also in our communication, cooperation and coordination in the past ten years. Among the many strides forward has been the initiation of several sequencing projects in cotton, which we will hear reports on in our conference. As our work has progressed, we have seen the distinctions between structural, functional, and evolutionary genomics begin to blur and our membership identification with the corresponding workgroups has begun to broaden. This is probably as it should be, and is being driven by science and good research. I look forward to many good presentations from our workgroups and appreciate the work their chairs have done to organize them. I thank the membership for their efforts to date to make this meeting a success and hope that all have a productive and stimulating experience.

Richard Percy
Chair
International Cotton Genome Initiative

CSIRO Plant Industry
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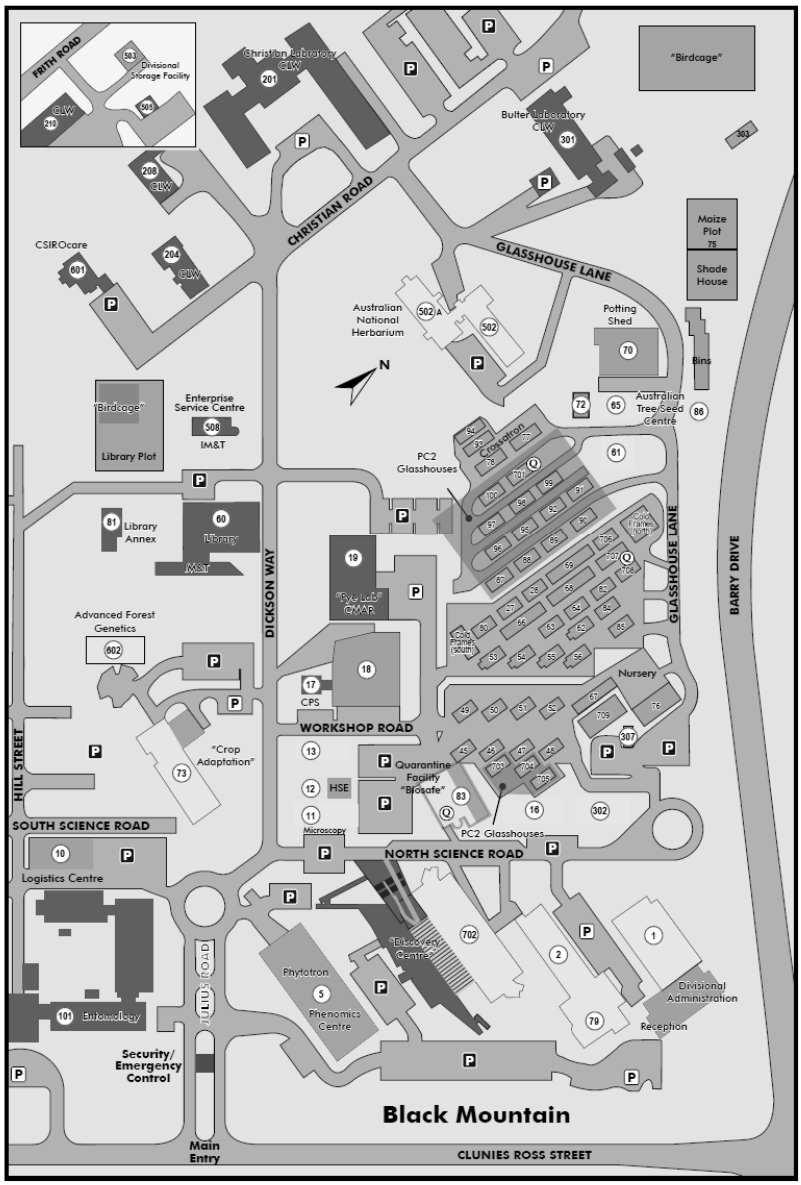


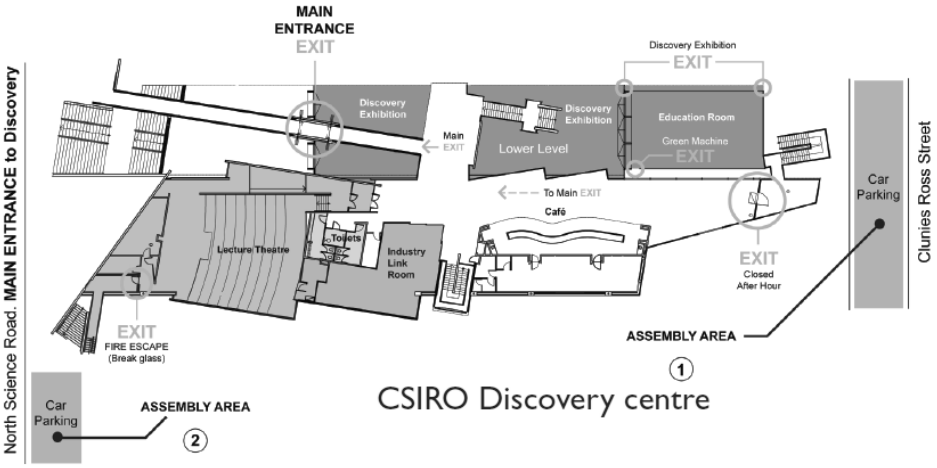
LEGEND

1	Cereal Laboratory / Divisional Administration and Reception
2/79	Genomics and Plant Development
702	Discovery Laboratories
5	High Resolution Plant Phenomics Centre and Phytotron Facility
10	Logistics Centre
11	Microscopy Facility
12	Health Safety and Environment Office
13	Seed Cleaning Facility
17	Property Services Office
18	Black Mountain Instrumentation Workshops
73	Crop Adaptation Laboratory and Analytical Chemistry Group
83	Quarantine Unit Laboratory and Biosafe
16	Wheat Breeding Annex
302	Soil Biology Laboratory
307	Chemical Waste Depot
503	Divisional Storage Facility
61	Pasture Research Laboratory
65 86	Australian Tree Seed Centre
70	Glasshouse Services
502	Australian National Herbarium
602	Advanced Forest Genetics
60	Black Mountain Library
508	Enterprise Service Centre
601	Early Childhood Centre

KEY

- Staff / Visitor Parking
- Fleet Parking
- Quarantine Facilities
- Roads
- Plant Growth Facilities
- Research Laboratories
- Administration / Services
- Other Business Units

CSIRO


MAIN ENTRANCE
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Discovery Exhibition
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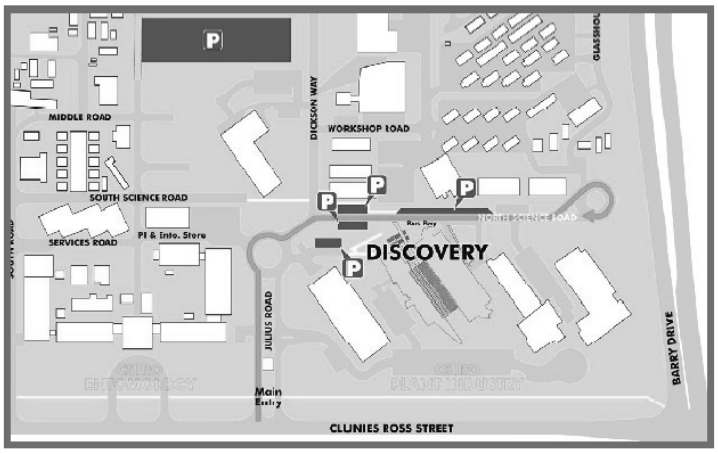
Discovery Exhibition
Discovery Exhibition
Education Room
Green Machine
EXIT
Main
EXIT
Lower Level
To Main EXIT
Café
Toilets
Lecture Theatre
Industry Link Room
EXIT
Closed After Hour
Car Parking
Clunies Ross Street
EXIT
FIRE ESCAPE
(Break glass)
ASSEMBLY AREA
1
North Science Road. MAIN ENTRANCE to Discovery
Car Parking
ASSEMBLY AREA
2
CSIRO Discovery centre


MIDDLE ROAD
SOUTH SCIENCE ROAD
SERVICES ROAD
PI & Into. Store
DICKSON WAY
WORKSHOP LOAD
DISCOVERY
JULIUS ROAD
Main Entry
CSIRO ENTOMOLOGY
CSIRO PLANT INDUSTRY
BARRY DRIVE
CLUNIES ROSS STREET
Legend
Foot paths
Roads
Vehicle access
Parking

DISCOVERY

ANU Campus

Conference Sessions

To City Centre

Conference Dinner ANU Functions Centre (Student Union)

University House

PROGRAM

Monday 20 Sept				
9:00AM - 5:00PM	Registration, poster setup			
	ICGI Steering Committee Meeting and facilities			
3:30 - 5:00PM	familiarisation			
5:00 - 7:00PM	Mixer (dinner not provided)		CSIRO Discovery Exhibition area	Lower floor Discovery Centre
Tuesday 21 Sept				
8:00 - 8:15AM	Conference Opening			
	Presentation of Outstanding Contribution to Cotton Genomics Award			
8:15 - 10:15AM	Keynote Session (Structural Genomics Session I)			
		K1	Prof. Andrew Paterson	Sequencing the cotton genomes, and beyond
		K2	Dr. Shuxun Yu	Progress on Upland cotton sequencing
		K3	Dr. Curt Brubaker	Looking beyond the genome sequences of cotton
		K4	Prof. Thea Wilkins	The genome sequence of the cotton ancestral species *Gossypiodes kirkii*
10:15 - 10:30AM	Group photo		Discovery stairs	
10:30 - 10:50AM	Coffee or Tea			
10:50AM - 12:30PM	Functional Genomics Session I			
		S1	Dr. Iain Wilson	Global gene expression responses to waterlogging in roots and leaves of *Gossypium hirsutum* L.
		S2	Prof. Yuxian Zhu	Comparative proteomics reveals important biochemical pathways during cotton fibre elongation
		S3	Prof. Ramesh Kantety	Deep transcriptome sequencing in *Gossypium* Spp.
		S4	Prof. Xiao-ya Chen	Functional analysis of a cotton BURP domain-containing protein
		S5	Dr. Sally-Ann Walford	GhMYB25-like: a key regulator of early cotton fibre development
		S6	Dr. Yves Al-Ghazi	eQTL analysis from microarray data of fibre expressed genes in an inter-specific *Gossypium hirsutum* x *G. barbadense* RIL population
12:30 - 7.00PM	Tidbinbilla Nature Reserve Lunch on the bus - BBQ dinner			
7:30 - 9:00PM	Germplasm and Genetic Stocks Session I			
		S7	Dr. Lucia Vieira Hoffmann	Genetic diversity of mocũ cotton (*Gossypium hirsutum* race *marie galante*) from the North-East of Brazil: implications for conservation
		S8	Dr. Richard Percy	Preliminary assessment of a candidate SSR core marker set to reveal genetic diversity within the U.S. Gossypium germplasm collection
		S9	Mr. Abdullahi Ibn Yahaya	Molecular characterization of Nigerian cotton using SSR markers
		S10	Prof. I.Y. Abdurakhmonov	Molecular diversity and population structure analysis in a global set of *G. hirsutum* exotic and variety germplasm resources and association mapping of the main fibre quality traits
		S11	Dr. C.D. Mayee	Current germplasm and species diversity in Indian cotton and its utilization
		S12	Dr. James Frelichowski Jr	Co-ordination and collaboration to document the global cotton germplasm resources
Wednesday 22 Sept				
8:00 - 10:00AM	Germplasm and Genetic Stocks Session II			
		S13	Dr. H. Benbouza	Identification of new chromosomal regions associated with segregation distortion of SSR markers and the genes controlling the low-gossypol seed & high-gossypol plant trait of *Gossypium sturtianum*
		S14	Dr. Jodi Scheffler	Methods to evaluate Host Plant Resistance
		S15	Ms. Wenxue Ye	Inheritance of cotton fibre initial development revealed by analysing of three fibreless mutants

		S16	Dr. Marc Ellis	Identification of the causal agent of the bunchy top disease of cotton
		S17	Dr. Fuzhen Li	Fine mapping of the *fg* gene using the inter-specific cross of *Gossypium hirsutum* X *Gossypium barbadense*
		S18	Prof. Nazira Bishimbayeva	Cotton improvement in Kazakhstan: breeding and biotechnology research
		S19	Dr. Zhu Shuijin	Effects of pigment glands and gossypol on growth, development and insecticide-resistance of cotton bollworm
		S20	TBA	To be advised
10:00 - 10:20AM	Coffee or Tea			
10:20AM - 12:30PM	Functional Genomics Session II			
		S21	Prof. Z. Jeffrey Chen	Genome-wide and functional analyses of microRNAs and transcription factors in the development of cotton fibres and Arabidopsis trichomes
		S22	Prof. Yong-Ling Ruan	Towards metabolic engineering of sugar transport and metabolism for improvement of cotton fibre development
		S23	Prof. Randy Allen	Functional characterisation of candidate genes for abiotic stress tolerance in cotton
		S24	Dr. Ananda Kumar Polumetla	Transcriptome analysis of cotton (*Gossypium hirsutum* L.) during boll development
		S25	Dr. Joshua Udall	A cotton reference transcriptome
		S26	Prof. Xuebao Li	Interactome analysis of the six cotton 14-3-3s that are preferentially expressed in fibres and involved in cell elongation
		S27	Dr. Todd Campbell	Discovering genes with potential abiotic stress tolerance applications in cotton
		S28	TBA	To be advised
12:30 -1:30PM	Lunch			
1:30 - 3:00PM	Bioinformatics, Evolutionary and Comparative Genomics Session I			
		S29	Prof. Tianzhen Zhang	Comparative analysis of 12A and 12D homoeologous chromosomes in allotetraploid cotton using integrated cytogenetic-linkage maps and homoeologous BAC sequences
		S30	Prof. Jinping Hua	Complete nucleotide sequences of six *Gossypium* chloroplast genomes: genome variation character and divergence time of *Gossypium* allotetraploids
		S31	Prof. Wangzhen Guo	Differential expression and domestication of fibre development genes in cultivated tetraploid cotton species
		S32	Dr. Junkang Rong	Functional and evolutionary characterization of six closely related sucrose synthase genes from diploid fibre cotton (*Gossypium arboreum* L.)
		S33	Dr. Jing Yu	The construction of a tetraploid cotton genome wide comprehensive reference map (CRM) and the comparison with cotton physical mapping information
		S34	Prof. Weihua Li	The effects of early generation selection on genetic gain of lint yield in upland cotton breeding program using QuLine simulation
3:00 - 3:20PM	Coffee or Tea			
3:20 - 5:30PM	Structural Genomics Session II			
		S35	Dr. David M Stelly	Inter-specific chromosome substitution lines as genetic resources for improvement, trait analysis and genomic inference
		S36	Mr. Muhammad Tehseen	Characterization of resistance gene analogs from *Gossypium arboreum* and their evolutionary relationships with their homologs from tetraploid cottons
		S37	Prof. Youlu Yuan	Molecular marker-assisted selection and pyramiding breeding of major QTLs for cotton fibre strength
		S38	Dr. David Fang	Association mapping of cotton fibre quality traits and yield using recombinant inbred lines derived from

				random mating
		S39	Dr. John Yu	Distribution of fibre genes and transcription factors between At and Dt sub-genomes in tetraploid cotton
		S40	Dr. Xinlian Shen	Fine mapping of a fibre length QTL on chromosome 1 by near-isogenic line
		S41	Mr. Muhammad Saeed Mian	Association mapping for salinity tolerance in cotton (*Gossypium hirsutum* L.)
		S42	Dr. Kunbo Wang	Cotton chloroplast genome sequence
5:30 - 6:30PM	Poster viewing			
6:30 - 9:00PM	Conference Dinner		ANU Functions Centre	
Thursday 23 Sept				
8:30 - 10:00AM	CSIRO facilities tours			
10:00 - 10:20AM	Coffee or Tea			
10:20AM - 12:30PM	Bioinformatics, Evolutionary and Comparative Genomics Session II			
		S43	Prof. Zhu Shuijin	New methods for QTL mapping and gene searching
		S44	Dr. Don Jones	Cotton marker database (CMD) for genetic and genome research
		S45	Dr. Robert Wright	Alternative respiration during cotton growth and development
		S46	Dr. P.K. Chakrabarty	Cloning of an unique chitinase gene in diploid cotton (*Gossypium arboreum*) showed enhanced expression of chitinase activity and delayed pathogenesis of *Myrothecium roridum*
		S47	Prof. Xianlong Zhang	Functional analysis of candidate genes for fibre quality based on expression profiles of fibre development in *G. barbadense*
		S48	Dr. Tuan-Ngoc Le	Role of RNA silencing in plant defence against Fusarium wilt disease
		S49	Dr. Qinxiang Liu	Pectin methylation during cotton fibre development differs between *G. hirsutum* and *G. barbadense*
		S50	Dr. David Fang	Phytohormonal and functional analyses of *Gossypium hirsutum* cellulose synthase catalytic subunit 4
12:30 -1:30PM	Lunch			
1:30 - 3:00PM	Concurrent workshop discussions			
3:00 - 3:20PM	Coffee or Tea			
3:20 - 5:40PM	ICGI Business Session			Budget report, workgroup activities, call for proposals for the 2012 Conference, Issues
5:40 -6:00PM	Conference Close			

Keynote Presentations

K1

Sequencing the cotton genomes, and beyond.

Andrew Paterson, (paterson@uga.edu), Distinguished Research Professor and Director Plant Genome Mapping Laboratory, University of Georgia, 111 Riverbend Road, Rm 228, Athens, GA 30602

The cotton genus (*Gossypium*) presents many novel opportunities to advance our understanding of the natural world. Sequencing of the respective cotton genomes in the next few years is likely to set the stage for the next few decades during which we reveal the genetic underpinnings of the unique features that adapt cotton species to a wide range of ecosystems in warmer, arid regions of the world, and account for the evolution of the 'lint fibre' that distinguishes cotton from all other plants and has made it of common interest to more than 80 countries as a crop. Since the endorsement by the international community of *G. raimondii* (a D-genome diploid) as the first sequencing priority, rapid progress has been made and will be updated here. The *G. raimondii* sequence will provide a foundation upon which to build the framework needed to investigate seminal questions in this botanical model and leading industrial crop - however, cotton evolution is a story of international partnership, and the post-genomic era will be no exception. Building the framework of enabling tools necessary to utilize the cotton genome sequences, and employing it to obtain meaningful answers to key questions, will best be realised as synergistic benefits of the diverse expertise of international partners who combine molecular and genomic skills with ecological and field-level knowledge of the spectrum of taxa in the genus, together with access to natural populations and the ability to conduct experiments in the range of environments to which the respective taxa are adapted.

Notes:

K2

Progress in Upland cotton sequencing.

Shuxun Yu, (yu@cricaas.com.cn), Cotton Research Institute, Chinese Academy of Agricultural Sciences (CRICAAS), Anyang, Henan, China.

Genome sequence analysis of a plant species provides detailed resources for genomics research, including structural, functional, and evolutionary genomic studies, significantly expanding the molecular foundation for improvement of its agronomic and biological Traits. To better understand the relevance of genome structure, genome size variation, and polyploidisation to cotton fitness and evolution, we screened 362 BACs, about 27.5Mb, from the libraries of *Gossypium hirsutum* L. based on DNA markers genetically mapped on chromosomes 12 and 26. We sequenced these clones and assembled the DNA sequences into contigs. We identified full length LTRs, analysed their phylogenetic relationship and found two times amplification of the major types of LTR, one that accrued before allopolyploidisation, the other after the allopolyploidisation. We investigated the co-linearity conservation between these cotton BAC sequences and with other model plant genomes, revealing that a high level component of TEs caused increased recombination in Upland cotton. The phylogenetic tree of single copy orthologues genes among cotton, Arabidopsis, Poplar, grape, rice and maize shows that poplar was a nearer relative to cotton than the others.

Notes:

K3

Looking beyond the genome sequence of cotton.

Curt Brubaker, (curt.brubaker@bayercropscience.com), Bayer CropScience, Technologiepark 38, B-9052 Gent, Belgium.
Jean Broadhvest, (jean.broadhvest@bayercropscience.com) Bayer CropScience, Technologiepark 38, B-9052 Gent, Belgium.

It is now clear that the publication of the cotton genome sequences is no longer a matter of if, but of when. As we look forward, eagerly, to this, it is perhaps useful to consider what we will do with our genome sequences. Genome sequences, like the genetic linkage maps and physical maps that came before, are not ends in themselves; they are enabling tools that will allow us to answer questions that were previously intractable. While genome sequences will certainly revolutionise how we reduce phenotypes to genetics, it will have an equally profound effect in how we approach plant breeding in the next decade. Selection, genetic or phenotypic, will be replaced by design.

Notes:

K4

The genome sequence of the cotton ancestral species *Gossypium kirkii*.

Thea Wilkins, (thea.wilkins@ttu.edu), Plant & Soil Science Department, Texas Tech University, Lubbock, TX, USA

Not available at time of printing

Notes:

Session Presentations

(Presenting author listed first and in bold)

S1

Global gene expression responses to waterlogging in roots and leaves of *Gossypium hirsutum* L..

Iain Wilson, (iain.wilson@csiro.au), CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia.
Jed A. Christianson, CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia
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Elizabeth S. Dennis, CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia

Waterlogging stress causes yield reduction in Upland cotton (*Gossypium hirsutum* L.). A major component of waterlogging stress is the lack of oxygen available in the rhizosphere. We assessed cotton growth responses to waterlogging and assayed global gene transcription responses in root and leaf cotton tissues of partially submerged plants. Waterlogging caused significant reductions in stem elongation, shoot mass, root mass and leaf number, and altered the expression of 1,012 genes in root tissue as early as 4 h after flooding. Many of these genes were associated with cell wall modification and growth pathways, glycolysis, fermentation, mitochondrial electron transport and nitrogen metabolism. Waterlogging of plant roots also altered global gene expression in leaf tissues, significantly changing the expression of 1,305 genes after 24 h of flooding. Genes affected were associated with cell wall growth and modification, tetrapyrrole synthesis, hormone response, starch metabolism and nitrogen metabolism. The implications of these results for the development of waterlogging-tolerant cotton are discussed.

Notes:

S2

Comparative proteomics reveals important biochemical pathways during cotton fibre elongation.

Yu-Xian Zhu, (zhuyx@water.pku.edu.cn), College of Life Sciences, Peking University, Beijing 100871, China
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Xiang Jin, (jinxiang@pku.edu.cn), College of Life Sciences, Peking University, Beijing 100871, China
Fangxing Jia, (jiafx@pku.edu.cn), College of Life Sciences, Peking University, Beijing 100871, China
Qin Li, (vargas@pku.edu.cn), College of Life Sciences, Peking University, Beijing 100871, China

Previously, we showed that very long chain fatty acids (VLCFA) regulate cotton fibre cell elongation by up-regulating transcription of genes important for ethylene biosynthesis. When applied in ovule culture medium, VLCFA (especially C24:0 and C26:0) promoted significant fibre cell growth while acetochlor, an inhibitor of VLCFA biosynthesis, abolished fibre elongation completely. The plant hormone ethylene nullified the ACE inhibition on fibre growth while C24 was inactive in the presence of the ethylene biosynthesis inhibitor, L-[2-aminoethoxyvinyl]-glycine (AVG), indicating that VLCFAs may act upstream of ethylene. Here we identified 93 preferentially accumulated polypeptides from 10 days-post-anthesis (dpa) wild-type cotton ovules using a proteomics approach in comparison with samples obtained from a fuzzless-lintless (fl) mutant. KOBAS analysis indicated that nucleotide sugar metabolism was the most significantly up-regulated biochemical pathway during fibre elongation. Seven protein spots potentially involved in pectic cell wall polysaccharide biosynthesis were specifically accumulated in wild-type samples both at the protein and the transcript levels. Protein and mRNA expression of these genes increased when either ethylene or lignoceric acid (C24:0) was added to the culture medium, suggesting that these compounds may promote fibre elongation by modulating the production of cell wall polymers. Quantitative analysis revealed that fibre primary cell walls contained significantly higher amounts of pectin, whereas more hemicellulose was found in ovule samples. Application of UDP-L-rhamnose (UDP-Rha) UDP-D-galacturonic acid (UDP-GalA) and UDP-D-glucuronic acid (UDP-GlcA) in cultured cotton ovules, which were readily incorporated into the pectin fraction of cell wall preparations, resulted in significant fibre growth. Our results suggest that ethylene and C24:0 may promote cotton fibre growth by activating biosynthesis of both UDP-L-Rha and UDP-D-GalA.

Notes:

S3

Deep transcriptome sequencing in *Gossypium Ssp.*.

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Sana Scherbakova, Agnes Chan, Yongli Xiao, Christopher Town, J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850

Cotton-reniform nematode interactions elicit complex plant responses at morphological physiological and biochemical levels that in turn are regulated by transcriptional, translational, and environmental signals. To help address such potentially complex phenomena, we characterized the response of selected *Gossypium spp* germplasm to reniform nematode and a multitude of biotic and abiotic stresses. Since the majority of publicly available expressed sequence tags (ESTs) originate from fiber tissues, we combined it with the root transcriptome data obtained from our studies and performed comprehensive analysis. Our primary objectives were to: 1) characterize the response of cultivated and wild cottons to the reniform nematode, *Rotylenchulus reniformis*; 2) analyse site-specific rhizogenic gene expression of various *Gossypium spp.* against the reniform nematode infection during both compatible and incompatible reactions; and 3) combine all public EST data and perform species/genotype/treatment-pair specific comparisons to identify novel functional genomic resources to study cotton-reniform nematode interactions and associated gene expression

signalling cascades. Response of several genotypes, susceptible, moderately tolerant, or resistant, belonging to *G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. longicalyx* were treated with nematode or other stresses and young root samples were collected for RNA extraction. RNA was isolated and cDNA libraries were constructed using the SMART^{TM} technique or Roche Transcriptor first strand synthesis procedure. The cDNA libraries were sequenced using Roche/454 GS FLX Genome Sequencer or Illumina Genome Analyzer and final assembly of the sequences were constructed based on the library, genotype, and pool of public and private sequencing resources. Over 2 Million 454 transcript sequences, ~375,000 publicly available ESTs, and 3 Gbp of Illumina data have been analysed in this study. This project enhances the knowledgebase in the cotton genome research, and plant biology, with potentially significant contributions to the definition of plant defence responses to nematodes, and to the biology of the root, the least understood plant organ. The data generated in this study has many applications including the development of genetic markers for plant breeding programs, development of comprehensive microarrays for functional genomic studies, the assembling and characterization of complex polyploid cotton genomes.

Notes:

S4

Functional analysis of a cotton BURP domain-containing protein

Xiao-Ya Chen, (xychen@sibs.ac.cn), National Key Laboratory of Plant Molecular Genetics, Institution of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P. R. China
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Cotton fibre development is a highly programmed process involving a large number of genes. *Gossypium hirsutum RD22-Like 1* (*GhRDL1*) gene, homologous to *Arabidopsis RD22*, was highly expressed in elongating cotton fibre cells (3~15 DPA). The *GhRDL1* ORF encodes a protein of 335 amino acids, with a 19-amino acid signal peptide at the N-terminal and a conserved plant-specific domain, BURP, at the C-terminal. In transgenic plants of *Arabidopsis thaliana* GhRDL1-VENUS fusion proteins were mainly accumulated in cell walls or the space between the cell wall and protoplasm. In a yeast-two hybrid system GhRDL1 interacted with cotton α-expansin, GhEXPA1, and the interaction was further confirmed by fluorescent co-localization and BiFC analysis.

As expansins were reported to play a role in cell wall loosening and cell wall acid growth, GhRDL1 may participate in this process. *GhRDL1* was then over-expressed in *G. hirsutum* under the 35S promoter. At least ten independent transgenic lines were generated, of which most showed increases in both fibre length and seed size. Analysis of the homozygous T_3 transgenic lines, 105, 117 and 119, showed that the fibre length was increased by 7%~15% and the seed weight by 13~17% against to the untransformed control. Interestingly, over-expression of either *GhRDL1* or *GhEXPA1* in *Arabidopsis* also led to a substantial increase in seed size. Furthermore, *GhRDL1* and *GhEXPA1* double-transgenes promoted stem elongation in *Arabidopsis* after bolting. Our results suggest that GhRDL1 plays a role in cotton fibre elongation, probably through co-operating with the α-expansin proteins.

Notes:

S5

GhMYB25-like: a key regulator of early cotton fibre development.

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Elizabeth Dennis, (liz.dennis@csiro.au), CSIRO Plant Industry, Canberra, Australia

MYB transcription factors have been implicated in the regulation of the development of ovule epidermal cells into the elongated seed fibres of cotton. An R2-R3 MYB, *GhMYB25-like*, identified from its reduced expression in a fibreless mutant of cotton (Xu142 *fl*) is here shown to play a key role in the very early stages of fibre cell differentiation. A *GhMYB25-like* promoter-GUS construct is expressed predominantly in the epidermal layers of cotton ovules before anthesis (-3 dpa), increasing in expression in 0 dpa ovules primarily in those epidermal cells expanding into fibres and then in elongating fibres at +3dpa, declining thereafter. This pattern was consistent with *GhMYB25-like* transcript abundance during fibre development. RNAi mediated suppression of *GhMYB25-like* results in cotton plants with fibreless seeds, but normal trichomes elsewhere, phenocopying the Xu142 *fl* mutant. Like Xu142 *fl* these plants had reduced expression of *GhMYB25* and *GhMYB109* normally expressed in developing fibres, indicating that *GhMYB25-like* is upstream from these MYBs. This finding is substantiated by the transcript levels of these MYBS measured in *GhMYB25*- and *GhMYB109*-silenced transgenic lines. Sequencing of the *GhMYB25-like* gene in the mutant and its parental wild-type identified a single point mutation in the DNA binding domain of the mutant gene that would likely impact on gene function. Transgenic cotton with an additional copy of the native gene had elevated expression of *GhMYB25-like* in ovules, but no obvious increase in fibre initial cells, suggesting that there are other components required to differentiate epidermal cells into fibre cells.

Notes:

S6

eQTL analysis from microarray data of fibre expressed genes in an inter-specific *Gossypium hirsutum* x *G. barbadense* RIL population.

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Microarrays provide a wealth of genome-wide gene expression data to characterise the biological variability between different plant genotypes, however it remains difficult to link such genomic results to a functional interpretation of how specific gene action determines a particular plant phenotype. Phenotypes themselves can be associated with specific regions of the genome by traditional QTL mapping, but most QTLs are very large physical regions that do not often allow the identification of specific underlying genes, particularly in species like cotton lacking a full genome sequence. Combining QTL and microarray analyses is a novel way to narrow down on subsets of candidate genes whose level of expression can be statistically correlated to regions of the genome, so-called eQTLs, and further correlated to specific phenotypes.

In this study we used a RIL population of 145 lines from an inter-specific cross between a *G. barbadense* Sea-Island accession (VH8) with very high fibre quality and a *G. hirsutum* cultivar of moderate fibre quality (Guazuncho II). The RIL population had been phenotyped for fibre quality traits at multiple sites and in most cases over multiple years and had also been genotyped. The consensus genetic map for the population contained over 600 SSR and AFLP markers and was used as the framework for both phenotypic QTL and eQTL mapping. Microarray analyses (approx. 24,000 genes per array) were carried out on 10 dpa fibre cDNA on 102 of the RILs and the individual gene expression values for each transcript treated as a quantitative trait and mapped to the genome. The distribution of eQTLs was not uniform across the chromosomes, with chromosomes 5 and 12 having more eQTLs than any others. A number eQTL hotspots were identified that may represent the locations of master regulators of fibre expressed genes. A selection of genes co-located with the phenotypic QTLs for fibre traits were also identified and validated by quantitative PCR. Some, but not all eQTLs identified from the microarray data were confirmed by Q-PCR. Large correlations tables of the expression of all the genes on the arrays and specific fibre traits like length, strength and fineness were developed and are also being used to identify potential candidate genes that may be causal in conferring the commercially useful properties of cotton fibres.

Notes:

S7

Genetic diversity of mocũ cotton (*Gossypium hirsutum* race *marie galante*) from the North-East of Brazil: implications for conservation.

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Mocu cotton (*Gossypium hirsutum* race *marie galante*) is a potential source of valuable alleles for breeding programs, mainly due to its great adaptability to semi arid conditions. With the aim of quantifying mocu cotton genetic variability, 187 plants collected in the North-East of Brazil were evaluated using 12 microsatellite markers. A total of 63 alleles were amplified, ranging from three to eight polymorphic alleles per locus. Total genetic diversity was high (0.52), and when measured on a per state basis, was of 0.37 on average. The population showed a low level of heterozygosity (HO=0.16), reflecting a high level of endogamy (FIS=0.69). Phylogenetic analysis using the neighbour-joining method revealed that plants sampled in different states tended to cluster according to their geographic origin, except for those collected in the states of Paraba and Rio Grande do Norte which grouped together. Plants from the state of Piau formed two groups, one with an apparent allelic contribution from *G. barbadense*, while the second group of plants was closer to those from the states of Paraba and Rio Grande do Norte. Despite the high genetic diversity that was observed in the remaining populations, urgent conservation efforts should be undertaken due to the high level of endogamy and accelerated extinction process that characterizes these populations. Such efforts should focus on the collection and *ex situ* maintenance of representative genetic diversity.

Notes:

S8

Preliminary assessment of a candidate SSR core marker set to reveal genetic diversity within the U.S. *Gossypium* germplasm collection.

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A comprehensive knowledge of the genetic diversity within the U.S. Gossypium Germplasm Collection is necessary to achieve the greatest efficiency in maintenance of the collection and its most effective utilization. To improve our knowledge of the collections diversity, a project was initiated in 2009 to characterize between 20 and 25 percent of the collection using a candidate core marker set of 105 SSRs. Labelled primers for the SSRs were created using FAM, HEX, or NED labels, creating 35 primer sets for multiplex PCR amplification. A small, 96 accession subset of the collection representing the tetraploid species and the A and D genome diploid progenitor species was created, and DNA was harvested for a pilot project to verify the efficacy of the multiplex primer sets, develop protocols for amplification, and develop data collecting bins for a data file. Difficulties were encountered with four primers that failed to amplify. The remaining 101 primers worked well, revealing considerable polymorphism and many alleles within and between the species of the 96 accession subset. On average, 9.5 variants or alleles per marker were observed. Within *G. hirsutum*, an average of 6.3 alleles per marker was observed in race stock accessions, whereas cultivars accessions only produced an average of 2.9 alleles per marker. Lower levels of variation were observed within *G. barbadense*. Species specific alleles could be identified, allowing one to identify species introgression within accessions. Overall, the candidate core marker set appears sufficient for the task of characterizing diversity within *G. hirsutum*, and may suffice for the larger collection.

Notes:

S9

Molecular characterization of Nigerian cotton using SSR markers.

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Cotton was the fifth major cash crop in Nigeria. The commercially grown cotton lines are known to be resistant to bacterial blight, with long and medium staple length (22mm-30mm). Forty (40) SSR markers are being used to assess the distinctiveness and diversity among 46 cotton cultivars including the released 6 commercial varieties developed by the National breeding program. This is intended to facilitate breeding of identified elite cotton lines in Nigeria to reposition its place among cash crops in the Country.

Notes:

S10

Molecular diversity and population structure analysis in a global set of *G. hirsutum* exotic and variety germplasm resources and association mapping of the main fibre quality traits.

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Cotton is the worlds leading cash crop, but it lags behind other major crops for marker-assisted breeding due to limited polymorphisms and a genetic bottleneck through historic domestication. This underlies a need for characterization, tagging, and utilization of existing natural polymorphisms in cotton germplasm collections. Here, we selected and conducted a genetic analyses in a global set of ~1000 *G. hirsutum* accessions from Uzbek cotton germplasm collection, representing, at least, 37 cotton growing countries and 8 breeding ecotypes as well as wild landrace stock accessions. The important agronomic (yield potential, lint percentage etc.) and fibre quality (fibre length and strength, micronaire, uniformity, reflectance, elongation etc.) traits were measured in two distinct environments of Uzbekistan and Mexico. A number of morphological traits including early-maturity were also recorded in Uzbekistan. These cotton accessions were genotyped with 100 core sets of SSR markers, evenly spaced throughout 26 chromosomes of cotton, with an average of 4 markers per chromosome. Molecular diversity, population structure, and linkage disequilibrium level were estimated using SSR markers. This study allowed us to design an association mapping (mixed liner model -MLM) study to find biologically meaningful marker-trait associations for important fibre quality traits in Upland cotton that accounts for population confounding effects. Several SSR markers associated with main fibre quality traits along with donor accessions were selected to be used for marker-assisted selection (MAS) programs. These donor genotypes and SSR markers should be useful for MAS breeding programs of cotton research community worldwide.

Notes:

S11

Current germplasm and species diversity in Indian cotton and its utilisation.

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Plant Genetic Resources (PGR) are the foundations of any crop improvement process. The National Gene Bank (NGB) on cotton was initially started by obtaining representative seed samples from genetic resources scattered within the country. Further collection expeditions in various parts of the country were organized by the Central Institute for Cotton Research (ICAR), Nagpur, which is the National Archive Germplasm site, in collaboration with the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Currently NBPGR and CICR are jointly working on cotton germplasm. A total of 8000 exotic accessions belonging to four cultivated and twenty six wild species of *Gossypium* from over 40 countries have been introduced. At present CICR has a collection of 10300 accessions (as of March 2009), 50% of which are exotic in origin.

The CICR has also maintained wild species, perennials and cytogenetic stocks in a field gene bank. Multi-location evaluation of germplasm is conducted in a phased manner. The data are recorded for various descriptors on cotton germplasm index cards designed by CICR. The descriptors prepared by IPGRI (FAO) were modified suitably for Indian situations. Augmenting the gene pool including the wild species, characterising and evaluating the existing germplasm using modern approaches, updating the cotton genetic resource catalogue, adopting improved conservation techniques has become extremely important at the present time.

Genetic resources held in NGB are made known to all through bulletins, catalogue, publications etc. They are supplied to Universities, research workers and organizations interested in utilization. Some important GR have been utilized to develop commercial cultivars of tetraploid and diploid cottons. Super okra, American Nectariless, 45 Red AK, Acala glandless, AET 5 and their morphological variants have been frequently used in basic, strategic and applied research. Large numbers of commercial lines have been bred. However, there is wide scope for untapped GR available. Registration of promising germplasm has also been initiated as per recent developments of IPR.

In diversified breeding programmes for drought tolerance, biotic stresses resistance, cytoplasmic-nuclear male sterility/fertility system, lint quality including colour, early maturity are of paramount significance. Introgressive hybridization has played a key role in transferring fibre quality, disease and insect resistance, drought tolerance, male sterility systems from various species of cotton. The introgression breeding efforts have made it possible to develop basic germplasm material enriched with rare useful genes from wild species. Efforts have been successful for improvement of diploid and tetraploid genotypes for different traits. The paper also discusses future strategies of improving germplasm and its utilization in view of impending new problems due to climate change, soil degradation, water deficiency, salinity issues. Germplasm also becomes the carrier of technology and helps in optimizing the benefits of the transgenic traits. Bt cotton being now cultivated on nearly 80 per cent of the area in India, germplasm diversity has become a key issue for crop improvement. Germplasm resources newly created have now been very well utilized by both private and public sectors.

Notes:

S12

Co-ordination and collaboration to document the global cotton germplasm resources.

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Co-ordinated efforts to collect and maintain cotton genetic resources have increased over the last 100 years to insure the worldwide economic value of cotton fibre and cotton by-products. The classified genetic resources of cotton are extensive and include five tetraploid species in the primary gene pool, 20 diploid species in the secondary gene pool, and 25 diploid species in the tertiary gene pool. There are at least eight major cotton collections worldwide and their status and contents are discussed. An overview of the collections suggest that there is a substantial coverage of the *Gossypium* genome but some recently identified species are not yet maintained and several species are underrepresented and threatened by loss of their natural habitat. Meeting the high demand for cotton genetic resources and increasing the coverage of the genus with decreasing budgets are a few of the challenges facing individual collections. These types of challenges and the opportunities for international collaboration that they create are discussed. One desirable outcome of co-ordinated efforts among collections would be finding gaps in the collections and sharing of the workload to conserve the genus. Multinational communication and collaboration are critical for the evaluation of rare and unique cotton germplasm and protection of the global cotton germplasm resources.

Notes:

S13

Identification of chromosomal regions associated with segregation distortion of SSR markers and the genes controlling the low-gossypol seed & high-gossypol plant trait of *Gossypium sturtianum*.

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Distorted segregation of DNA markers is commonly encountered, especially in inter-specific crosses. Our main objective in this study was to identify chromosomal regions consistently associated with segregation distortion in [(*G. hirsutum* x *G. raimondii*) x *G. sturtianum*] (HRS) hybrid. Segregation distortion skews the genotypic frequencies from their Mendelian expectations. In HRS progeny, chi square analysis ($P < 0.01$) showed significant skewed in all targeted linkage groups c2-c14, c3-c17, and c6-c25. Chromosomal region was regarded as being associated with skewed segregation, if three or more closely linked markers exhibited significant segregation distortion in one or more population(s). The targeted introgression regions in the tested population seem to be favourable for segregation distortion. Segregation distortion in HRS hybrid progenies differed in male and female gametes. Furthermore, the data indicated that the environment has strongly influenced the transfer of SSR markers through microspores. The consistent location of these chromosomal regions in selfed and backcross of HRS derivatives indicate probably the identification of segregation distortion regions (SDRs) in HRS hybrid. Comparison with results regarding the segregation distortion regions obtained in previous research by other authors and results we obtained regarding the absence of recombinations between BNL3436 and BNL1153 markers mapped on c6-c25 chromosome and spanned by 64 cM on the *G. hirsutum* map, after several generations of selfing, arise the question of the conservation of the gene order and spacing in *G. sturtianum*. Results showed that three SSR markers mapped on c6-c25 linkages groups were systematically transmitted in all selected progenies of the HRS tri-specific hybrid. Furthermore, the high percentages of loci with significant segregation distortion observed in this study suppose that a genetic mechanism may exist for preferential transmission of alien chromosomes segments. High heterozygosity frequencies (+/- 80%) were observed for all conserved *G. sturtianum* SSR markers, after several generations of backcrossing and selfing, which indicate that the cytogenetic and genetic conditions for obtaining homozygotes at high frequency are not met.

Notes:

S14

Methods to evaluate Host Plant Resistance.

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Cottonseed contains high quality protein that could be used in animal feeding rations, provide additional income for farmers and potentially be a new protein source for humans. However, the seed is currently under-utilized as a protein source because it contains a terpenoid aldehyde called gossypol, which limits its use. Cultivars with decreased levels of seed gossypol have been developed using both genetic engineering and conventional methods. Using classic genetic methods supported by molecular and biochemical tools, germplasm lines have been developed that have low seed gossypol (> 90% in the plus form). It is now essential to determine if these lines are as effective for pest control as normal lines or those with high minus gossypol. A variety of methods have been evaluated including field and laboratory feeding studies using fresh leaf tissue and live heliothines, chemical analyses of the non-seed tissues for a range of terpenoid aldehydes and digital microscope observations of feeding behaviour.

Notes:

S15

Inheritance of cotton fibre initial development revealed by analysing three fibreless mutants.

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Segregating populations were developed to evaluate the inheritance of lintless and fuzzless seed phenotype of upland cotton. One normal fibre line (TM-1) two fuzzless lines (*N1* NSM and *n2* NSM) and three fibreless lines (SL1-7-1, XZ142 FLM and MD17) were used to study the inheritance of fibre. A total 17 BC_1 families, 15 F_2 generations and 11 $F_{2:3}$ families deriving from the crosses of them were constructed. According to evidence of this research, we proposed a genetic model that contained six gene loci which can well explain the inheritance of fibre in each cross. Especially, lintless was the precondition of fibreless. The lint inheritance existed complementary effect. The inheritance of lint was mainly controlled by five different gene loci: At least three different lint loci were dominant, phenotype showed lint, or else lintless. The fuzz was mainly controlled by two different loci: the dominant fuzzless seed allele *N1*, the recessive fuzzless seed allele *n2*. The gene of *N1* can inhibit fuzz initiation, and have dominant epistasis effect on *N2*.

Notes:

S16

Identification of the causal agent of the bunchy top disease of cotton.

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The cotton bunchy top disease (CBT) has caused important yield losses in Australia, and is now largely controlled by pesticide eradication of its vector, the cotton aphid (*Aphis gossypii*, Glover). Because of its symptoms, mode of transmission and other clues, CBT has been suggested to be a viral disease, most likely a Luteovirus, but so far the isolation of the causing agent has remained elusive.

We used degenerate primers to conserved regions of the genomes of Luteoviruses, and a nested (RT-)PCR approach, to successfully amplify viral cDNA fragments from CBT-infected cotton tissue that were not present in healthy tissues. This new virus bears some similarities to other members of the Luteoviridae, but is clearly novel. The coding region for the viral RNA-dependent-RNA-polymerase showed the greatest homology with the virus responsible for the cotton Blue Disease (CLRDV). Other parts for the virus such as the coat protein, however, showed greater similarity to Luteoviruses other than CLRDV, suggesting a "hybrid" nature to the new virus.

Having genome sequence for the CBT causing agent opens up the possibility of designing RT-PCR and antibody diagnostic tests to enable the detection of the virus in cotton, weedy hosts for CBT and aphids. The ability to determine whether aphids are viruliferous may help farmers to make informed decisions as to whether insecticidal sprays are required.

The possibility of engineering CBT resistance through the expression of "hairpin" constructs targeted against the virus is also being explored.

Notes:

S17

Fine mapping of the *fg* gene using the inter-specific cross of *Gossypium hirsutum* x *Gossypium barbadense*.

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Frego (*fg*) bract is an important agronomic trait in tetraploid cotton, which has been widely introduced into several cotton varieties or lines in the past several years. In order to help us further understand the underlying molecular mechanism of frego bract development, a map-base cloning strategy was used to localize the *fg* locus. An F_2 population which comprised of 290 *fg* individuals derived from a cross of the multiple-marker line T582 (*G. hirsutum*, carrying the *fg* gene) with Hai7124 (*G. barbadense*) was constructed. Genetic linkage analysis was carried out to map of the *fg* locus with SSR and EST-SSR markers in tetraploid cotton. Genetic linkage analysis showed that the *fg* locus was flanked by the marker NAU3016 and NAU3172 on the long arm of chromosome 3, with the genetic distance of 0.3 cM and 4.7 cM, respectively. The information of *fg* locus provided the basic information for the final isolation of this important gene in tetraploid cotton, these marker information could be used in marker-assisted selection in cotton.

Notes:

S18

Cotton improvement in Kazakhstan: Breeding and biotechnology research.

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Cotton is the one of major agricultural crops in Kazakhstan that has an export importance. Cotton producing region of Kazakhstan is the most northern zone of world cotton sowing and characterized by the of warm days limit, water deficit, the significant spread of verticillium and fusarium wilt, bacterial blight, black root rot and other diseases and insects. Therefore the main task of cotton improvement in Kazakhstan is the creation of new local varieties with complex of economically important traits: fast ripening, high productivity and fibre quality, adaptive capacities to the low temperatures, disease and pest resistance.

Genetic-breeding investigations of cotton are carried out in the Kazakh Cotton-growing Research Institute (KCRI), recently founded on the site of the Makhtaaral breeding station (Mirzakent, South Kazakhstan). New cotton varieties with traits of fast-ripening, high-yielding, high fibre quality, resistance to the complex of diseases (verticillium wilt, bacterial blight, and black root rot), tolerance to salinity and water deficit have been produced by the use of statistical programs for overall combinative capacity (OCC) and specific combinative capacity (SCC). Overall, 7 new varieties has been created in KCRI, four of them has been introduced into cotton growing regions. On their main valuable characteristics (yield, pod size, fibre strength, fibre length, fibre yield, verticillium-resistance) they exceed traditionally growing cultivars, which were considered as elite for middle-fibred cotton in Kazakhstan. Various approaches for the development of genetic transformation techniques for the elite Kazakh cotton varieties are being investigated in the Institute of Plant Biology and Biotechnology (Almaty). Pollen transformation has been optimized for the local variety Maktaaral-4005. Pollen was transformed by an Agrobacterium strain containing a plasmid with the GUS reporter gene and selective nptII gene. From 140 pollinated flowers 19 bolls were produced; 600 seeds were obtained from these bolls and 25 of them gave rise to plantlets with positive reaction to histochemical GUS-assay. Molecular evidence of GUS and nptII genes insertion has been obtained for the 12 plants of their T_1 progeny, yielding a 2.0% transformation rate. Pollen transformation has been used for the transformation of Kazakh cotton varieties with the Lactoferrin gene responsible for resistance to broad spectrum of bacterial, fungal and virus diseases. Seeds of 52 lines have been produced from 200 transformed plants. Screening of T_0 generation of these lines showed insertion of target gene in 14 of them, which is equal to 7%. Further elaboration of various systems for the transformation of local Kazakh varieties through meristems, hypocotyls, and callus tissues are in progress.

Notes:

S19

Effects of pigment glands and gossypol on growth, development and insecticide-resistance of cotton bollworm.

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Cotton bollworm (*Heliothis armigera*) is a major pest of cotton and other crops. It is important to understand the mechanisms of insecticide tolerance of cotton bollworm on cotton cultivars with host plant resistance to this insect pest. The objectives of this study were to investigate the effects of cotton pigment glands and their gossypol on the growth, development and insecticide tolerance of cotton bollworm. Three pairs of cotton isogenic lines with glanded versus glandless leaves, as well as artificial diets with 5 levels of gossypol, were used to raise cotton bollworm larvae for five generations. The growth, development and insecticide tolerance of larvae were studied. The results indicated that the cotton pigment glands and higher levels of gossypol resulted in a significant decrease in larval weights and eclosion rates and delayed the development of larvae and pupae. Larvae that fed on glanded cotton leaves were significantly more tolerant to two insecticides, cyhalothin and monocrotophos, than those fed on glandless cotton leaves. LD50 values were only increased where they were in amount per unit body weight, and not where were calculated in amount per individual. Also the insecticide tolerance of cotton bollworm larvae increased as the gossypol content was raised from 0 to 0.225% in artificial diets. Meanwhile, the activities of two detoxifying enzymes, carboxylesterase and glutathione s-transferase, were significantly higher in the larvae fed on glanded cotton leaves than those fed on glandless cotton leaves. The activities of two enzymes also increased greatly with the increase of gossypol content from 0 to 0.225% in artificial diets. Across 5 generations of feeding and investigation, the significant inhibition effect on larval growth and larval tolerance to two pesticides were found to be only associated with the feeding by current generation, but were not related to previous diets. The activities of two detoxifying enzymes in larvae were also not enhanced significantly when they were fed continuously on glanded cotton leaves or artificial diet with high gossypol. These results indicated that pigment glands and higher levels of gossypol not only inhibited the growth of cotton bollworm larvae but also enhanced their insecticide tolerance. However, the inhibition effect and enhanced insecticide tolerance were inducible but could not be accumulated or inherited. These results will help us better understand the interaction and co-evolution of insecticide tolerance in larvae of cotton bollworm and host chemical components, and also has provided useful information on cotton bollworm management in cotton production, especially in glandless cotton.

Notes:

S20

Topic to be advised.

Speaker to be advised,

S21

Genome-wide and functional analyses of microRNAs and transcription factors in the development of cotton fibres and Arabidopsis trichomes.

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Each cotton fibre is a single-celled seed trichome or hair, and over 20,000 fibres may develop semi-synchronously on each seed. The molecular basis for seed hair development is unknown but is likely to share many similarities with leaf trichome development in Arabidopsis. Using laser capture microdissection and microarray analysis, we found that many cotton genes encoding putative MYB transcription factor and structural genes were differentially expressed in fibre and non-fibre tissues. *Gossypium hirsutum* MYB2 (*GhMYB2*) and its putative downstream gene (*GhRDL1*) were highly expressed during fibre cell initiation. GhRDL1, a fibre-related gene with unknown function, was predominately localized around cell walls in stem, sepals, seed coat, and pollen grains. Over-expression of GFP:GhRDL1 and GhMYB2:YFP in Arabidopsis wild-type and try mutant plants showed additive effects of *GhRDL1* and *GhMYB2* as well as synergistic effects of *GhRDL1* and *GhMYB2* with try on Arabidopsis seed hair and trichome development. Using next-generation sequencing, we found enrichment of siRNAs in ovules and fibres, whereas many miRNAs are repressed in fibres, which correlate with up-regulation of a dozen validated miRNA targets encoding transcription and phytohormone response factors. Further analysis of cotton miRNAs and their target genes showed functional diversification between homoeologous genes on trichome formation. The data collectively suggest that cotton fibre genes can program seed hair development in Arabidopsis, providing a novel system for discovering cotton genes that control cell fate and development.

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S22

Towards metabolic engineering of sugar transport and metabolism for improvement of cotton fibre development.

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Cotton fibre development is characterized with extraordinary magnitude of cell elongation and cellulose biosynthesis, two major determinants of cotton yield and quality. We aim to unravel the underlying mechanisms for designing better approaches to ultimately enhance cotton fibre elongation and cellulose synthesis. Sugars are crucial signalling molecules controlling the expression of a wide range of genes including many transcription factors and are key osmotic solutes and substrates for fibre elongation and cellulose biosynthesis, respectively. Therefore, we focus on elucidating (i) the molecular and cellular basis of sugar import to, and utilization within, cotton fibres and (ii) the impact of altering identified sugar genes on fibre development. By using a combination of cell biology and gene expression approaches we discovered a remarkable transient closure of plasmodesmata (PD) during cotton fibre elongation. The PD closure, probably mediated by callose deposition, coincided with high expression of sucrose transporters (SUT) and rapid cell expansion. To understand how imported sucrose is utilized in fibre cells to power and regulate their elongation and cellulose biosynthesis, we examined the role of sucrose synthase (Sus) and invertase (Inv), the two enzymes that degrade sucrose into hexoses or their derivates in higher plants. In this context, reverse genetic and biochemical analyses demonstrated the critical role that Sus plays in fibre elongation, whereas over-expression of Sus increased early fibre elongation and seed weight. Notably, a significant amount of sucrose must also enter into vacuoles as indicated by high activity of vacuole Inv (VIN) during fibre elongation. Indeed, VIN activity correlated with fibre elongation both developmentally and genotypically. The VIN activity appears to be conferred largely by GhVIN1 that localizes to vacuole and function as VIN *in planta* based on complementation studies in Arabidopsis. Importantly, fibres transformed with *GhVIN1* RNAi or over-expression constructs reduced or enhanced cotton fibre elongation, respectively. At the onset of cellulose biosynthesis stage, plasma-membrane and cell wall association of Sus protein appears to be required for rapid cellulose synthesis. Furthermore, fibre PD became increasingly branched towards the secondary cell wall stage, which may function as a molecule sieve for tight control of macromolecule trafficking into fibres to sustain intensive cellulose synthesis.

An integrative model on regulation of fibre cell elongation and cellulose synthesis by PD, SUT, Sus and VIN will be presented and possibilities of engineering these candidates for improvement of cotton fibre development will be discussed.

Notes:

S23

Functional characterization of candidate genes for abiotic stress tolerance in cotton.

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Abiotic stresses associated with harsh environmental conditions are the primary limiting factor for crop productivity world-wide. In most places where cotton is grown, drought is a persistent problem and, with anticipated changes in temperature and rainfall patterns and depletion of freshwater resources for irrigation, this problem will continue to become more serious. Identification and exploitation of new genetic resources for improved stress tolerance is a critical need for the cotton industry. With this in mind, we have undertaken the development and characterization of transgenic cotton plants with altered expression of a collection of candidate genes that regulate plant stress responses. These genes include transcription factors, protein kinases and ubiquitin ligases. Transgenic plants that express these genes exhibit a range of stress tolerance phenotypes and, through ongoing physiological analyses, we have begun to identify the stress tolerance mechanisms that are affected in each line. Constitutive expression of several of these genes is well-tolerated but others cause severe secondary effects when over-produced and require the use of specialized regulatory strategies. Using this approach, we have initiated a stress tolerance candidate gene pipeline. Although the throughput of this pipeline is limited by the relatively tedious cotton transformation and regeneration procedure, this can be partially overcome by maintaining a steady pace of transformations. Other critical restrictions arise due to the slow generation time, large size, and need for containment of transgenic cotton plants, which can quickly fill all of the available greenhouse and growth chamber space. In addition, reliable and quantitative methods for physiological evaluation of transgenic cotton lines during early stages of development that provide data relevant to field production are still needed.

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S24

Transcriptome analysis of cotton (*Gossypium hirsutum L.*) during boll development.

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Cotton boll development is the result of a genetically programmed process. Sequence information derived from advanced EST sequencing is an essential resource for functional genomics studies which is necessary for understanding such processes. Transgenic technology necessitates the availability of novel promoters that are constitutive, regulated and are native to the plant system. The present study was undertaken to understand the boll wall development process and with an objective to identify and isolate promoters that are consistently expressed during all the stages of boll wall development. ESTs for three crucial boll developmental stages viz. bud, ovary wall (0 dpa) and boll wall (10 dpa) were constructed by employing PCR select cDNA subtraction using leaf as a driver and a cDNA macroarray. It resulted in the development of 170, 289 and 238 ESTs individually for bud, ovary wall (0 dpa) and boll wall (10 dpa) stages and assembled in to 64, 122 and 120 contigs. They were further annotated by using Blast2Go *in silico* analysis and functionally annotated by the available GO terms. Further, candidate genes were identified from the SSH library sequences of 0 dpa ovary wall and 10 dpa boll wall by a combination of *in silico* analysis and quantitative PCR analysis. Three genes viz, putative senescence associated protein, glycosyl transferase protein and a putative zinc finger family protein were identified as candidate genes. A promoter sequence from putative senescence associated protein gene was isolated and analysed *in silico*.

Notes:

S25

A cotton reference transcriptome.

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Approximately 300,000 mostly Sanger-based ESTs have been generated from various cotton species, including diploid *G. arboreum* (A genome) and *G. raimondii* (D genome) and tetraploid *G. hirsutum* and *G. barbadense* (AD genome). To deepen this database for the cotton transcriptome and to generate a more global assembly, we generated over 4,000,000 new ESTs using 454 sequencing. Because of low inter-specific and inter-genomic sequence divergence in coding regions and significant overlaps in long reads, it was possible to combine ESTs into a single, de novo assembly that represents a reference transcriptome for cotton. Through homology searches, we estimate that the reference transcriptome includes >90% of the genes present in the cotton genome. Using 454 and Illumina reads from the diploids, approximately 34,000 pairs of homoeologous loci have been identified. The assembly and individual EST sequence are publicly available from our project website (http://cottonevolution.info).

We expect that this large collection of ESTs and their corresponding reference assembly will be useful for the eventual annotation of the cotton genome sequence.

S26

Interactome analysis of the six cotton 14-3-3s that are preferentially expressed in fibres and involved in cell elongation.

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Proteins of 14-3-3 family regulate a diverge set of signalling pathway in all eukaryotic organisms. In this study, the several cDNAs encoding 14-3-3 proteins were isolated from cotton fibre cDNA library. The Gh14-3-3 genes share high sequence homology at nucleotide level in the coding region and at amino acid level. Real-time quantitative RT-PCR analysis indicated that the expressions of these Gh14-3-3 genes were developmental-regulated in fibres, and reached their peak values at the stage of rapid cell elongation of fibre development. Furthermore, over-expression of *Gh14-3-3a*, *Gh14-3-3e* and *Gh14-3-3L* in fission yeast promoted atypical longitudinal growth of the host cells. Yeast two-hybrid analysis revealed that the interaction between cotton 14-3-3 proteins is isoform-selective. Through yeast two-hybrid screening, 38 novel interaction partners of the six 14-3-3 proteins (Gh14-3-3a, Gh14-3-3e, Gh14-3-3f, Gh14-3-3g, Gh14-3-3h and Gh14-3-3L), which are involved in plant development, metabolism, signalling transduction and other cellular processes, were identified in cotton fibres. Given these data together, we propose that the Gh14-3-3 proteins may participate in regulation of fibre cell elongation. Thus, the results of this study provide novel insights into the 14-3-3 signalling related to fibre development of cotton.

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S27

Discovering genes with potential abiotic stress tolerance applications in cotton.

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In the face of changing climatic conditions, drought, as it relates to crop water usage, is one of the most challenging agricultural issues limiting sustainable crop production. This is particularly true for crops, such as cotton, that are primarily grown in rainfed agricultural areas. In spite of its economic importance, studies on drought-resistant cotton are limited. In our laboratory, we are using two primary approaches to study drought and cotton at the molecular level. The first approach is narrow and focuses on the role a specific gene family plays in drought. Alternatively, the second approach is very broad and focuses on identifying a genome-wide suite of genes involved in drought. In this report, we will update our progress on both approaches to study cotton and drought at the molecular level. We have identified the large aquaporin gene family in cotton and characterized gene expression patterns in various plant tissues in response to drought conditions. We have also identified a suite of genome-wide genes showing differential expression patterns in response to drought. The genes described in this report offer potential targets for improving cotton water use efficiency under drought and well-watered environmental conditions.

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S28

Topic to be advised

Speaker to be advised

Notes:

S29

Comparative analysis of 12A and 12D homoeologous chromosomes in allotetraploid cotton using integrated cytogenetic-linkage maps and homoeologous BAC sequences.

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Cotton is a model system for studying polyploidisation, genomic organization, and genome size variation because the allotetraploid was formed 1-2 million years ago, which is old enough for sequence divergence but relatively recent to maintain genome stability. In spite of characterizing random genomic sequences in many polyploidy plants, the cytogenetic and sequence data that decipher homoeologous chromosomes are very limited in allopolyploid species. Here we reported comprehensive analyses of integrated cytogenetic and linkage maps of homoeologous chromosomes 12A and 12D in allotetraploid cotton using fluorescence *in situ* hybridization (FISH) and a large number of bacterial artificial chromosomes (BACs) that were anchored by SSR markers in the corresponding linage maps. Integration of genetic loci into physical localizations showed considerable variation of genome organization, structure, and size between 12A and 12D homoeologous chromosomes. The distal regions of the chromosomes displayed relatively lower levels of structural and size variation than other regions of the chromosomes. The highest level of variation was found in the pericentric regions in the long arms of the two homoeologous chromosomes. Moreover, DNA sequence analysis of two pairs of homoeologous BACs derived from the low- and high-variable regions, respectively, indicated sequence conservation as well as rapid evolution of homoeologous regions of the chromosomes after formation of cotton allotetraploid. The genome size difference between A and D sub-genomes in allotetraploid cotton were mainly associated with uneven expansion or contraction between different regions of homoeologous chromosomes. In the pericentric region expansion of transposable elements including gypsy in the A-genome species prior to polyploidisation results in larger genome size of A-genome.

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S30

Complete nucleotide sequences of six *Gossypium* chloroplast genomes: genome variation character and divergence time of *Gossypium* allotetraploids.

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Cotton is the most important natural fibre crop in the world. The taxa cotton (*Gossypium* spp.) is totally comprised of 51 species, including five allotetraploid species and 46 diploids. While the species which is the cytoplasm donor of allotetraploid hybrids remains uncertain. The likely ancestors of allotetraploid *Gossypium* are A-genome lineages as the source of maternal and D-genome lineages as the paternal origin. In this research, we have sequenced six Gossypium chloroplast genomes of *G. herbaceum* var. *africanum* and *G. arboreum*, which are the A-genome lineages, *G. raimondii*, which is the D-genome lineage, and *G. tomentosum*, *G. mustelinum* and *G. darwinii*, which are the allotetraploid lineages. The sizes of the genomes range between 160,161bp (*G. raimondii*) and 160,433 (*G. tomentosum*). The eight genomes, included those chloroplast genomes of *G. hirsutum* and *G. barbadense* sequenced previously, is identical to the others with the identity >98%. The chromosomes display the typical tetrapartite genome organization as found in most other higher plants, including a large single copy (LSC) region, a small single copy (SSC) region and two inverted repeats (IR) region. But the eight genomes encode nearly the same set of 112 unique genes with the same gene order too. The genomes include 78 protein-coding genes, 25 of which are completely identical among the eight species in amino acid level. The genomes of A-genome lineages are very similar with the allotetraploid species, while the *G. raimondii* is genetically distinct relatively. Introns appear in 18 genes, all of which suffer from variation. There are 20 out of 131 intergenic sequences completely conserved in nucleotide level. The gene *ndhF* changed greatest, while the intergenic region *psbZ/trnG*-GCC varied sharpest. Moreover, the longest indel appears in *petN/psbM* which has a 51 bp insertion in *G. raimondii*. Phylogenetic trees are generated based on the 77 protein-coding genes, showing that: (1) The five allotetraploid species have a common maternal source (2) A-genome *Gossypium* species are the source of maternal and the pioneer divergence of allotetraploid species originates from about 0.443 MYA (3) The divergence of the two A-genome species tracing from ~ 0.083 MYA, is posterior to the appearance of allotetraploid cottons (4) the divergence time between *G. raimondii* and A-genome comes of 2.576 MYA. Key Words: the chloroplast genomes, allotetraploid species, the cytoplasm donor, *Gossypium*.

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S31

Differential expression and domestication of fibre development genes in cultivated tetraploid cotton species.

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Although *G. hirsutum* and *G. barbadense* probably originated from a single hybridization event between A- and D- diploid species, the two cultivated tetraploid cotton species have very different agronomic and fibre quality characters. In this study, 19 fibre development related genes were individually cloned and sequenced from *G. hirsutum* acc. TM-1 and *G. barbadense* cv. Hai7124, and their two living models of diploid progenitors, *G. herbaceum* and *G. raimondii*, further, their structures, tree topologies, chromosomal location and transcripts were studied, to elucidate the inter-specific divergence of fibre development genes in the two cultivated tetraploid cotton species. In all orthologous loci of 19 studied genes, the sequence and structure of 68.42% were conservative with the same exon length and numbers in different cotton species and 31.58% were diverse. Gene tree topologies showed that 16 genes were independent evolution between A- and D-subgenome in the allopolyploid after polyploid formation, while 3 evolved different degrees of colonization. Based on the sequence divergence between TM-1 and Hai7124, 26 duplicated genes were located on our backbone genetic map, in which at least one fibre quality QTL previously reported was detected in the interval. The molecular evolutionary rates among orthologs revealed that D-sub-genome of allotetraploid had rapid differentiation in the evolutionary history, and selection had acted on the tetraploid level. Compared with TM-1, Hai7124 may have a closer relationship with their progenitor. The genes expression profiles showed that at fibre initiation and early elongation period, most genes had greater transcripts in Hai7124 than in TM-1, however, at fibre elongation period, most genes transcripts, except for *CelA3* and *HOX3*, were the same or greater in TM-1 than in Hai7124 with an exception at 8DPA, and at primary-secondary transition period, expression peak of transcripts in most genes was earlier in TM-1 than in Hai7124. The genome-specific expression profiles showed that 73.7% genes had the same expression patterns in TM-1 and Hai7124, while 26.3% expressed differently between them. The combined analysis of the structure and expression patterns of these studied genes further indicated that agronomic selection play important role in altering the molecular evolutionary and expressional patterns of genes related with fibre development between *G. hirsutum* and *G. barbadense*.

Notes:

S32

Functional and evolutionary characterization of six closely related sucrose synthase genes from diploid fibre cotton (*Gossypium arboreum* L.).

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Sucrose synthase (Sus) is widely considered as the primary key regulator in carbohydrate metabolic pathway in plants and is also well characterized to play a pivotal role in the development of cotton fibre. Multiple paralogous genes with high sequence similarities predicted to encode sucrose synthase have been identified in several plant species, but the evolutionary and functional roles of these genes are not well understood. Here we report the genomic organization, evolution and expression profiles of six closely-related cotton (*Gossypium arboreum* L.) Sus genes (*GaSS1*, *GaSS2*, *GaSS3* and three newly isolated paralogs, *GaSS4*, *GaSS5* and *GaSS6*), of which *GaSS2* and *GaSS3* were mapped tandemly on chromosome A3. Phylogenetic analyses from the deduced amino acid sequences showed that GaSS1, GaSS2, GaSS3 GaSS4 and GaSS5 could be well clustered into a dicot group, while GaSS6, together with the known poplar Sus and citrus Sus, are defined into another group, suggesting that GaSS1, GaSS2, GaSS3 GaSS4 and GaSS5 might have evolved through duplication of a single ancestral Sus gene following the divergence of cotton and poplar. Unlike the other five paralogs containing 11~12 introns, *GaSS6* shows a distinctive arrangement of molecular structure with only nine introns. Interestingly, RT-PCR analysis showed that the expression profiles of these genes were more diverged between than within the two Sus groups: *GaSS1-5* are expressed not only in cotton fibre, but also to some extent in the source leaves, flowers, stems and even roots, while the transcript of *GaSS6* could be only detected in stems and fibres. Taken together, these results will provide new insights into the evolution and sub-functional divergence of the six closely related Sus paralogs in the regulation of carbon partitioning and translocation in plants.

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S33

The construction of a tetraploid cotton genome wide comprehensive reference map (CRM) and the comparison with cotton physical mapping information.

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The integration of multiple genomic maps provides a higher density of markers and greater genome coverage, which not only facilitates the identification and positioning of QTLs and candidate genes, but also provides a basic structure for the genome sequence assembly. However, the diversity in markers and populations used in individual mapping studies limits the ability to fully integrate the available data. By concentrating on marker orders rather than marker distances, published map data could be used to produce a comprehensive reference map (CRM) that includes a majority of known markers with optimally estimated order of those markers across the genome. In this study, a tetraploid cotton genome-wide CRM was constructed from 28 public cotton genetic maps. The initial CRM contained 7,424 markers and represented over 93% of the combined mapping information from the 28 individual maps. Further, the CRM is improved through the comparison with cotton FPC contigs and other physical mapping or sequence information.

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S34

The effects of early generation selection on genetic gain of lint yield in Upland Cotton breeding programs using QuLine simulation.

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Early generation testing as a breeding procedure for autogamous crops consists of testing heterogeneous families, followed by selection homozygous lines from superior families. Although early generation testing has been compared with other breeding methods, there have been few comparisons of different early generation testing procedures. Different generations have been proposed as early generation in which families can be derived for testing in the first phase. Testing of genotypes in an adequate sample is probably the most expensive feature in most plant breeding programs. Relatively little published research is available, however, to guide breeders toward optimum allocation of resources in multistage testing programs. We used computer simulation to investigate the effectiveness of early generation selection for such economic important traits as lint yield and fibre strength. The QU-GENE approach to simulation of plant breeding programs involves two stages. In the first stage, the genotype-environment system is specified and the starting population of genotypes is generated by the QU-GENE engine. In the second stage, the application module QuLine manipulates the starting population according to the breeding and selection strategy specified in the module. Results from the analysis of variance showed that inheritance model (IM), recombination frequency (RF) and two-way interaction between inheritance model and recombination frequency (IM*RF) were the three main contributors to the overall variation of yield gain, followed by Heritability, breeding strategy (BS) and two-way interaction between recombination frequency and breeding strategy. Among the 4 genetic models highest genetic gain of lint yield was obtained from epistasis model after one breeding cycle, least genetic gain yielded from additive model. Higher genetic gain was achieved when genes were more tightly linked recombination frequency of which being 0.001, followed by weak linkage, lowest genetic gain was achieved when genes were independently inherited Starting selection at F_2 and F_3 without fibre strength selection (SN,TN) resulted in higher genetic gains of yield, increased 11.06% and 9.55% gains respectively as compared with starting selection at F_6 without fibre strength selection (XN), followed by starting selection at F_4 without fibre strength selection (FN)and starting selection at F_2 with fibre strength selection (SF), less gains were obtained from starting selection at F_3 with fibre strength selection (TF) and starting selection at F_4 with fibre strength selection (FF),starting selection at F_6 with and without fibre strength selection (XF and XN) produced the least genetic gain. Each breeding strategy without fibre strength selection produced more genetic gains as compared with its counterpart with fibre strength selection. On the other hand, average genetic gains of fibre strength differed from yield in many aspects. All the gains were negative, the only exception was that obtained from 0.500 recombination frequency level.

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S35

Inter-specific chromosome substitution lines as genetic resources for improvement, trait analysis and genomic inference.

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Three of the research areas likely to very significantly impact genetic improvement of cotton are inter-specific introgression, genetic dissection of complex traits and sequencing of [AD] genomes. Inter-specific introgression is expected to increase genetic diversity of breeding germplasm, creating opportunities for novel and faster rates of genetic improvement. Genetic dissection of complex traits is expected to render the improvement process more amenable to various types of marker-assisted selection. Sequencing and assembly of the [AD]1 genome and other *Gossypium* genomes will usher forth a new paradigm for research and breeding. Here, we will describe a collaborative inter-specific chromosomes substitution program, in which we are developing quasi-isogenic substitution lines of Upland cotton (recurrent parent) for specific chromosomes of *G. barbadense*, *G. tomentosum* and *G. mustelinum* (donors). After briefly reviewing the breeding process to create cotton chromosome substitutions and the complementarity of this breeding method to other means of inter-specific introgression, we will discuss the longer-term goals, including genomic analysis, genetic dissection and genetic improvements. A number of the inter-specific F_1 hybrid hypoaneuploids have been used to localize a number of markers to specific chromosomes or arms. The most advanced CS line efforts involve *G. barbadense* as donor, and have already led to the development and release of a number of BC_5Sn 'CS-B' lines. These and various types of derived hybrid progenies from them have been used in replicated testing to associate specific stocks with important effects on fibre and agronomic traits. We have used the existing CS-B lines to create quasi-isogenic F_1, F_2 and F_3 generations and to begin dissecting genetic effects quantitatively, in a chromosome-targeted manner. We created top-crosses with five cultivars, and demonstrated their usefulness for discovery of breeding-relevant cultivar-to-cultivar variations. Using a partial diallele mating scheme, we've begun to dissect the 'holy grail' of inter-specific introgression, epistasis. We've also initiated chromosome-specific dissection of quantitative trait loci (QTLs), by initiating the development of chromosome-specific recombinant inbred lines (CS-RILs) for higher resolution mapping. The anticipated completion of chromosome substitution line development for *G. tomentosum* ('CS-T lines') and *G. mustelinum* ('CS-M lines') will further expand the breeding and research opportunities, in terms of breeding, genetics and genomics. All of these materials will provide additional tools in genomic analysis and genetic improvement of Upland cotton, and help expedite the organization of genomic resources into chromosome-specific bins; creating smaller target areas will facilitate

sequence assembly and organization of certain genome assembly tasks. An important subsequent application of these advances is likely to be the implementation of trait-targeted genome selection to wide-cross introgression populations using high-throughput genome-wide genotyping methods.

Notes:

S36

Characterization of resistance gene analogs from *Gossypium arboreum* and their evolutionary relationships with their homologs from tetraploid cottons.

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Four cotton species belonging to the genus *Gossypium* produce spinable fibre. The two diploid species of Asiatic origin namely *Gossypium arboreum* and *G. herbaceum* have been largely replaced by tetraploid cotton of the New World. However, these species are rich source of genes particularly for resistance against biotic and abiotic stresses. As a first step towards understanding of resistance, twenty four RGAs from *G. arboreum* were characterized and compared. The majority of the RGAs were homologous to those isolated from *G. hirsutum* and *G. barbadense*. The cloned RGAs from *G. arboreum* were examined in A and D genome species. Most of RGAs are conserved in the A genome *G. arboreum*, suggesting a bias toward the A genome. These are useful not only to serve as probes for identification of full-length resistance genes but also for confirmation of inter-specific hybrids involving *G. arboreum*.

Notes:

S37

Molecular marker-assisted selection and pyramiding breeding of major QTLs for cotton fibre strength.

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With the development of molecular biology and molecular marker technology, more and more QTLs for cotton fibre quality had been identified, which provide an opportunity for molecular marker-assisted breeding in cotton fibre quality. Owing to small effect for single QTL to fibre quality, it is necessary to pyramid a few QTLs of fibre quality trait to improve cotton fibre quality. TG41 and sGK156, two commercial cotton cultivars (lines) and HS427-107235 and 0-153, three elite fibre quality germplasm lines were used as parents to develop three double-cross combinations, (sGK156HS427-10)(0-1537235)(Pop1), (TG41HS427-10)(0-1537235) (Pop2)and (sGK1560-153)(sGK156HS427-10)(Pop3). Six SSR markers, NAU1262, BNL1521, MUSS034, TMK19, CM67 and MUCS616, which were tightly linked with four QTLs of fibre strength, were used to select in the three combinations to study the effect of molecular marker-assisted selection and pyramiding breeding, and the donor gene derived from 7235, HS427-10 or 0-153. As a result, when we selected plants with single marker, it showed significant differences for the fibre strength between with and without marker in the 3 combinations, respectively. This indicated that the effects of the four QTLs for fibre strength were stable in different genetic backgrounds and different generations. When we selected the plants with two markers randomly, its fibre strength was higher than that of the plants with only one marker, and the difference could reach to the level of significance for some times, especially in Pop2. This indicated the effect of QTL could be added for fibre strength. When we study the pyramiding effect for 3 and 4 QTLs, it showed that the more QTLs for fibre strength were pyramided in the cotton, the higher was the fibre strength for the plants with the markers, and the fibre strength was the highest when four QTLs were all pyramided in the plants. In conclusion, it is effective to increase fibre strength through molecular marker-assisted selection and the effect can be increased when pyramid more QTLs in plants. In addition, the three double-cross populations have very complicated genetic playground, and most of QTLs exist in heterozygous genotype. But the significant effects were identified for single QTL and combination of QTLs in the populations, which indicated the genetic effect could be identified in the early populations for breeding selection with heterozygous genotype for most of plants. It is necessary to develop stable recombinant inbred lines of advanced generations which pyramid several fibre-related genes to study the effect for pyramiding more genes.

Notes:

S38

Association mapping of cotton fibre quality traits and yield using recombinant inbred lines derived from random mating.

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The overall objective of this research is to identify molecular markers associated with quantitative trait loci controlling fibre quality traits and yield through an association mapping strategy, and eventually apply the identified markers in breeding. Recombinant inbred lines (RILs) derived after 6 cycles of random mating of 11 parental varieties (Acala Ultima, Coker 315, DP90, FM966, HS26, M240RNR, PSC355, SG747, STV825, STV474, and Tamcot Pyramid) were previously developed at USDA-ARS, Mississippi State. A total of 792 simple sequence repeat (SSR) markers were selected based on their mapped positions. An average of 30 markers was chosen from each chromosome. These markers were analysed among 11 parental varieties, and 572 (72%) markers were found polymorphic among the varieties tested. The 572 markers revealed 1204 loci. Of them, 636 are polymorphic loci that covered all 26 chromosomes, and the average marker locus interval is about 5 cM. Two hundred thirty-three RILs were analysed with the polymorphic markers. They were also planted in Starkville, Mississippi in 2009, and 2010 to obtain fibre quality and yield data. Marker selection, 6-marker multiplex PCR, marker allele flow from parents to the RILs, and preliminary marker-trait associations will be reported in the conference.

Notes:

S39

Distribution of fibre development genes and transcription factors between A_t and D_t sub-genomes in tetraploid cotton.

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As the worlds leading natural material used in the manufacture of textiles, cotton fibres are important seed trichomes derived from individual cells of the epidermal layer of the seed coat. Cotton fibre development is determined by large numbers of genes and transcription factors. However, little is known about how these genetic elements are distributed or organized between A_t and D_t sub-genomes of the tetraploid AD genome. An integrated genome map of fibre genes was constructed to investigate the organization and evolution of such genes whose functions were previously verified and confirmed. A total of 535 cotton fibre genes, including 103 fibre transcription factors, 259 fibre development genes, and 173 SSR-contained fibre ESTs, were analysed at the sub-genome level. A total of 499 contigs were assembled and they covered about 151 Mb in physical length which represented about 6.7% of the AD cotton genome. Among them, 397 contigs were anchored into individual chromosomes. Results indicate that more fibre development genes were from sub-genomes A_t than D_t while more transcription factors from sub-genomes D_t than A_t. It is suggested that after merging of two diploid *Gossypium* genomes that sub-genome A_t, functioning similarly as its probable A diploid ancestor (*G. arboreum*) that by itself produces fibre, provides more genes for fibre development. On the other hand, sub-genome D_t, with its probable D diploid ancestor (*G. raimondii*) that by itself cannot produce fibre, provides more transcription factors that regulate the expression of the fibre genes in the sub-genome A_t. The resulting integrated map of fibre genes would provide a framework to study individual full-length fibre genes and their dynamic gene networks that are interacted during the process of fibre development in the tetraploid cotton.

Notes:

S40

Fine mapping for fibre length QTL on Chromosome 1 in cotton by Near-isogenic Introgression Lines (NILs).

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Earlier analysis of a backcross-self mapping population derived from a cross by *Gossypium hirsutum* cv. Tamcot 2111 and *G. barbadense* cv. Pima S6 resulted in the identification of a number of QTL for fibre quality. A significant QTL (qFL-chr1) for fibre length was identified on the chromosome 1 with stable and major effect. Three plants from BC_3F_2 population carrying the target QTL in heterozygous condition were selected to construct three BC_3F_2-derived populations to confirm qFL-chr1 effect and develop a set of near-isogenic lines (NILs) for the target QTL qFL-chr1. One of these NILs (R01-40-08) was evaluated for its genetic background similarity and fibre quality performance. The results showed NIL qFL-chr1, R01-40-08, has about 96.7% of recurrent genome composition. Fibre length of qFL-chr1 NIL was significantly higher than that of recurrent parent Tamcot 2111 in Tifton, GA ($P<0.01$ level) and in Nanjing, China ($P<0.05$ level). Fine mapping was carried out with a total of 1679 F_2 individual from a cross of NIL R01-40-08 and recurrent parent Tamcot 2111. The linkage analysis with 23 PCR-based markers show that qFL-chr1 was mapped to a 0.6 centimorgans (cM) interval flanked by SSR marker NAU3384-BNL2921.These results further elucidate the genetic fine structure of the qFL-chr1 locus and unravel the genetic architecture of fibre quality trait.

Notes:

S41

Association mapping for salinity tolerance in cotton (*G. hirsutum L.*).

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Salinity is a major worldwide abiotic stress limiting agricultural production. Identification of genomic regions involved in salinity tolerance will be helpful in future molecular breeding strategies. In our present study, we design an experiment to assess the marker-trait associations under high salinity conditions. We planted 109 cotton (*Gossypium hirsutum* L.) cultivars from China and USA under the green house conditions and phenotypic data were recorded at the seedling stage (30 DAS). The cultivars were sown in polythene bags containing vermiculite. The polythene bags were arranged according to randomized complete block design with three replications with three treatments (Control, 100mM NaCl and 200mM NaCl treatments). Phenotypic data were recorded for shoot length, root length, plant length, fresh shoot weight, fresh root weight, fresh plant weight, dry shoot weight, dry root weight, dry plant weight and root-shoo ratio. The relative values of these traits were also calculated. Considerable variability was found among cotton cultivars for salt tolerance. Genotyping of cotton cultivars was done by using a set of 112 SSR primer pairs with ~3-5 primers/chromosome. The genotypic data obtained from SSR screening was analysed by software STRUCTURE2.2 for estimating subgroups in population. The phenotypic data and information about the ancestry of individuals obtained from STRUCTURE2.2 software was put in the software TASSEL to determine marker-trait associations. Six markers (TMH05, BNL3590, BNL3089, NAU483, JESPR135 and NAU458) were found to be associated with all three treatments i.e., control, 100mM and 200mM NaCl. These markers were located on D11 (Chr. 21), A2 (Chr. 2), A4 (Chr. 4), A3 (Chr. 3), A11 (Chr. 11) and D1 (Chr. 15) respectively. NAU483 (A3) was associated with maximum number of traits under each treatment, and, especially, it was associated with more number of traits under stress conditions (both 100mM and 200mM NaCl) than under control treatment. So it is a good candidate marker for future MAS in molecular breeding programs for salinity tolerance. TMH05 (D11) was found to be associated with more number of traits under control treatment than under stress treatments. JESPR135 (A11) was found to give highest phenotypic variance (R^2) value of 17.3% under control treatment than under stress conditions. 41 markers were found to be associated with 200mM NaCl treatment. NAU483 (A3) was found to be associated with maximum number of traits (8) under high salinity (200mM NaCl) treatment. BNL3103 was associated with 7 traits. NAU1167 (A3) was associated with highest phenotypic variance explained (R^2) value of 13.1%. Seventeen markers were found to be exclusively associated with 200mM NaCl treatment and these were not associated with either control or 100mM NaCl treatment. Markers which gave relatively higher phenotypic variance explained (R^2) values were NAU1042 (A5), NAU980 (A11) and NAU2503 (D5). Eleven markers were common in control and 200mM NaCl treatment. These markers are of special concern while considering crop improvement both under normal and high salinity stress conditions. This is the first ever report of association mapping for salinity tolerance in cotton.

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S42

Cotton chloroplast genome sequence.

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The first complete chloroplast DNA sequences were reported in tobacco in 1986 and liverwort and since then the entire sequence of the chloroplast DNA has been determined from various plants species. The sequenced taxa cover the main groups in plants with a total of 157 species submissions of sequenced data to the three main databases up to the end of 2009. The data increased significantly, over two third of the total submissions, since 2005, which benefited mainly from improvements in sequencing technology and decreased costs. Chloroplast genome sequencing in the genus *Gossypium*, included the two cultivated tetraploid species *G. hirsutum* and *G. barbadense* and were submitted in 2006. Last year the chloroplast genome of one diploid species was sequenced completely by a laboratory from China Agricultural University. This year we finished complete sequencing another seven diploid species and data from one of them has been submitted. Data for the remaining species are expected to be submitted in the next 3 months. We are now working on chloroplast genome sequencing with another eleven *Gossypium* species, which are expected to be finished within the next two years. The laboratory at the China Agricultural University is also working with another three or more species and these will be soon finished. We have collaborated with the laboratory in China Agricultural University in this study. Therefore we believe chloroplast genome sequences from half or more of the *Gossypium* taxa will be soon available. The chloroplast genome structures of *Gossypium* are similar to most land plants and are composed of a large single copy (LSC), a small single copy (SSC) and two identical inverted repeats (IR) regions. Chloroplast genome data for almost all sequenced *Gossypium* species show little variation in genome size among the species, all of which are about 160Kb. The sizes of the four regions are all conserved among the different species. But we found expansion or contraction in the boundary of the Inverted Repeat regions with the result of some gene(s) insertions and deletions. The degree of conservation of the four regions is different between species, and the IR is the most conserved. The gene numbers of chloroplasts are similar among the different species. All sequenced cotton species have the same gene orders in their chloroplast genomes, but *G. hirsutum* showed a special gene order with the inversion of its SSC. This large inversion may be related to its two inverted repeat regions and happened after the speciation.

Notes:

S43

New Methods for QTL mapping and gene searching.

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Most important agronomic traits of crops, such as crop yield and quality, are complex traits affected by multiple genes with gene x gene interaction as well as gene x environment interaction. Conventionally, the genetic properties of traits can be revealed by partitioning the total variation into component variation due to specific genetic effects. With recent advances in molecular genotyping and high-throughput technology, great opportunity is provided for detecting the genetic architecture of complex traits by analysing quantitative trait loci (QTL). Improvement of complex traits can also be achieved by pyramiding of individual QTLs. We have developed some statistical methods of QTL mapping which can analyse the QTL x QTL interaction (QQ) as well as QTL x environment interaction (QE), and their applications in crop breeding for complex traits.

The QTL mapping approach of mixed-model based composite interval mapping (MCIM) was proposed by Zhu (1998). On the basis of the analysis framework of MCIM, Wang et al. (1999) proposed a genetic model of QTL mapping for DH populations and RIL populations, the corresponding software developed for QTL analysis being named as QTLMaper (http://ibi.zju.edu.cn/software/qtlmapper/). In the procedure of MCIM, a two-dimensional searching strategy is adopted for scanning paired QTLs involved in epistasis. There is a lot of computation, especially for calculating the inverse of many (nxn) matrixes.

Yang et al. (2007) proposed a new approach of QTL mapping for complex traits in the form of an optimized MCIM by using no matrix inverse, but just the Henderson method III and the Bayesian method via Gibbs sampling. Based on this new approach of MCIM, a new software named QTLNetwork (version 2.0) was developed, which can be downloaded from http://ibi.zju.edu.cn/software/qtlnetwork/ (Hu et al. 2007; Yang et al. 2008). It is noteworthy that QTLNetwork can analyse the data of DH, RIL, IF_2 populations, as well as any advanced population (BCxFy) derived from the two parents by backcrossing x times and selfing y times. The software also provides the potential superior QTL design for improving complex traits in the light of QTL main effects and QTL x environment interaction effects (Yang and Zhu, 2005).

Most QTL mapping methods can only analyse QTLs in one genome. In order to completely understand the genetic basis of host-parasite interaction, a genetic model was proposed that integrates genetic information from both that of the host and the parasite genomes (Yang et al. 2008). QTLNetwork can be used for mapping quantitative trait loci (QTLs) conferring the interaction between host and parasite and detecting interactions among these QTLs.

The newly released QTLNetwork version 2.1 has many useful features, such as mapping multiple traits and multiple populations shared common parents. We also developed QTLNetworkR which is an R package that aims to provide a user-friendly and platform-independent tool to visualise quantitative trait loci (QTL) mapping results.

Notes:

S44

Cotton Marker Database (CMD) for genetic and genome research.

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The primary objective of the Cotton Marker Database (CMD) project is to develop and maintain a comprehensive cotton molecular data resource for the cotton research community (http://www.cottonmarker.org). The total number of cotton SSRs currently displayed through CMD is 12,250 (including 312 SSR-containing RFLPs). The first cotton SNP project data provided by Allen Van Deynze (UC Davis) is displayed through the CMD on the Downloads page. The updated results of the SSR primer redundancy analysis are available on CMD in the Primer Redundancy section (View and Search) and on the Downloads page (2,570 redundant primer sequences of 18,002 sequences total, or 14.2%). The CMap Viewer on CMD has been updated by adding 13 new and previously published cotton genetic maps, with the total number of 26 maps available for comparative view. These cotton genetic maps present a comprehensive collection of the major maps with mapped cotton SSRs. In addition, the information about the CMD SSRs linked to a number of agriculturally important traits in cotton was collected, annotated and analysed. The initial results include annotation of 29 agriculturally important traits in cotton and 142 SSR markers associated with those traits and located on 15 different genetic maps. The information has been uploaded into the new sections View and Search Traits and is now publicly available at www.cottonmarker.org/cgi-bin/cmd_search_trait_index.cgi. The continued expansion of the CMD database continues to serve the cotton research community as well as provides direct benefit to the cotton industry.

Notes:

S45

Alternative respiration during cotton growth and development.

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The inability of cotton (*Gossypium* spp.) cultivars to reach their genetic potential is largely due to late season cool weather. Cool temperature during boll and fibre maturation greatly slows the deposition of cellulose in the secondary cell wall. The ultimate result can be immature fibres and lower yield. In voodoo lily (*Sauromatum guttatum*) an increase in temperature of the floral tissue serves to volatilize aromatic compounds that attract pollinators. This stress response is regulated by the alternative oxidase enzyme (AOX). Unlike the lily, cotton does not need to attract pollinators, but an increase in temperature in the boll during exposure to low temperature would serve to provide a more optimum and stable environment for fibre development. Agrobacterium mediated transformation was used to create transgenic lines that express the tobacco *Aox1* gene. The spatial and temporal expression of two lines verified as homozygous for a single copy of the transgene, was increased by 100 fold in stem, root and bolls (8-10 and 20-25 DPA) when compared to the null line. Transgenic lines appear to have improved growth characteristic when exposed to early seasonal cool temperatures.

Notes:

S46

Cloning of a unique Chitinase gene in diploid cotton (*Gossypium arboreum*) showed enhanced expression of chitinase activity and delayed pathogenesis of *Myrothecium roridum*.

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Chitinases are known to hydrolyse chitin and are effective against fungi having chitin content in their cell walls. Chitinases belong to group of PR proteins that constitute the second line of plant defence. Using conserved as well as degenerate primers a 1.3 kb novel class I chitinase gene was amplified and cloned from *Gossypium hirsutum* variety LRA5166. Analysis of sequence revealed the gene to be unique to upland cotton (GenBank #HM 125506). The gene-specific primers failed to amplify the sequence from *G. arboreum* lines including cultivars PA255, PA402 and RG-8. The forward and reverse primers were engineered with *Eco*RI sites flanking the initiation and termination codons, respectively. Using the primers the 1.3 kb chitinase gene flanked with *Eco*RI sites was amplified, cloned in pGemT (3.0 kb) and sub-cloned at *Eco*RI site in the binary vector pBInAR (11 kb). The gene was guided by 35S CaMV promoter and Nos terminator. The correct orientation of the cloned gene was checked and the resultant plasmid was transformed into Agrobacterium tumefaciens strain EHA105 by triparental mating. The *G. arboreum* cultivars PA255, PA 402 and RG8 were transformed with chitinase genes by direct-shoot organogenesis. Putative transformants were selected on MS supplemented with 50 ug/ ml Kanamycin. The genomic DNA isolated from the putative transformants (T_1) of cultivar PA 255, showed presence of 1.3 kb gene which was not amplified from wild type PA255. Integration of transgene was further confirmed by Southern hybridization using gene-specific probe. Bioassay of transgenic cotton against *Myrothecium roridum*, showed delayed pathogenesis.

Notes:

S47

Functional analysis of candidate genes for fibre quality based on expression profiles of fibre development in *G. barbadense*.

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Sea-Island cotton (*Gossypium barbadense* L.) is one of the most valuable cotton species due to its silkiness, luster, long staples, and high strength, so transferring the excellent fibre traits from *G. barbadense* to the vastly cultivated *G. hirsutum* is an attractive aim of breeders. We constructed a normalized fibre cDNA library (from C2 to 25 DPA) of 3-79 by saturation hybridization with genomic DNA and sequenced 12,175 cDNAs. The 10,937 high quality ESTs were assembled into 5852 consensus sequences (4360 singletons and 1492 contigs), of which 3788 were assigned to functional categories using gene ontology. After analysis the expression profiles of genes related to fibre development of *G. barbadense* and comparison of different development stages expression profiles between *G. barbadense* and *G. hirsutum*, some candidate genes for fibre quality were mined. Now we have produced RNAi and over-expression T_1 plants of 6 genes that were specifically expressed in fibre and related to the phytohormones, and RNAi T_1 plants of 3 genes related to secondary cell wall synthesis. We found that the repression of some genes, such as expansin, *GLP* and *TIP*, caused the fibre of T1 plants to be shorter. We also have cloned and analysed four promoters specifically/ preferentially expressed in fibre. They may provide novel target genes and promoters for genetic engineering for the enhancement of quality of the upland cotton fibre.

Notes:

S48

Role of RNA silencing in plant defence against Fusarium wilt disease.

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RNA silencing is a widespread mechanism of repressing gene expression in eukaryotes by means of small RNAs that are typically 20-24 nt in length. To study the role of RNA silencing in plant defence against Fusarium wilt, we have utilised the large collection of mutants available in the model plant Arabidopsis thaliana. Approximately ~30 Arabidopsis mutants, each carrying a different defective RNA silencing gene, were screened for their susceptibility (resistance) to the fungal wilt pathogen *Fusarium oxysporum* f. sp. *conglutinans* (Foc). Some mutant plants show few signs of fungal wilt symptoms (characterised typically by leaf chlorosis) after 16 days post-infection. These mutants carry defects in genes which have been shown to play a role in the biogenesis of small interfering RNAs (siRNAs) and are required for silencing transgenes derived from plant pathogens. We have also performed a microarray experiment to examine for differences in expression of >20,000 genes between the resistant and susceptible phenotypes. To further examine the role of siRNAs and other small RNAs in plant defence against Fusarium wilt, we performed deep sequencing of small RNAs from control and Foc-infected *A. thaliana* plants. Sequencing data revealed that, in general, there was an increase in the number of siRNAs in *A. thaliana* plants during Foc infection. Some of these siRNAs are mapped nearby to plant disease resistance genes within the A. thaliana genome. We are currently investigating whether these siRNAs negatively affect the expression of disease resistance genes in infected plants. In contrast to siRNAs, the number of microRNAs (miRNAs) decreased in infected plants compared to control plants. MiRNAs are important regulators of plant development and plant responses to biotic and abiotic stress. We are examining whether miRNAs also play a role in plant defence against Fusarium wilt. Our preliminary data suggest that RNA silencing may play a role in plant defence against fungal pathogens. The findings may have implications for the control of Fusarium wilt disease in cotton.

Notes:

S49

Pectin methylation during cotton fibre development differs between *G. hirsutum* and *G. barbadense*.

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Pectin is an important component of the primary cell wall. It is synthesized in the golgi, secreted into the cell wall as a methylester and subsequently de-esterified by pectin methylesterase (PME) to expose acidic residues. These free carboxyl groups can be cross linked by calcium to structurally stiffen the cell wall. PME is believed to be involved in regulating the extensibility of cotton fibre cell walls and so is likely to play a role in determining the physical dimensions of the fibre that are related to some fibre quality attributes. We have studied the differences in PME enzyme activity and gene expression between a high quality *G. barbadense* (Pima) and an average quality *G. hirsutum* (Upland) cotton variety. The degree of methylation of pectin extracted from cotton fibres during their development was also examined. Cotton has several PME genes that are expressed at different levels and times during development. The transcript levels of PME3, 4 and 8 genes increased during the early stage of fibre development, especially in Pima, while transcripts of the PME10 gene were more abundant at the end of fibre elongation in Pima. The total PME enzyme activity was highest during fibre elongation and was significantly different between the two species. The degree of methylation of pectin in the fibre sharply declined after 12 dpa and further decreased after 20 dpa in Pima fibres. In contrast, the change in methylation degree of the pectin in Upland cotton was not as pronounced. Immunolabelling studies using anti-pectin antibodies (JIM5 and JIM7) supported the chemical data showing that de-esterified pectin increased during fibre development, and was higher in Pima compared to Upland cotton. These results indicated that PME activity and the degree of pectin methylation might play key roles in fibre development and cotton fibre quality.

Notes:

S50

Phytohormonal and functional analyses of *Gossypium hirsutum* cellulose synthase catalytic subunit 4.

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Gossypium hirsutum cellulose synthase catalytic subunit 4 (*GhCesA4*) plays an important role in cellulose biosynthesis during cotton fibre development. At the transition from fibre elongation stage to cellulose biosynthesis stage in developing cotton fibres, *GhCesA4* transcript levels are significantly up-regulated. To understand molecular mechanisms involved in transcriptional regulation of *GhCesA4*, the *GhCesA4* promoter activity was studied in both cotton tissues and transgenic Arabidopsis. The promoter assay by determining a beta-glucuronidase activity regulated by full length *GhCesA4* promoter (2,574 nt) showed that the *GhCesA4* promoter was commonly functional in trichomes and root vascular tissues in both cotton and transgenic Arabidopsis. Promoter deletion assays and *in silico* analyses also showed that the common putative promoter motifs were positively or negatively involved in trichome and vascular tissue specific expression in cotton and transgenic Arabidopsis. Exogenous phytohormonal treatments on both cotton fibres and transgenic Arabidopsis revealed that auxin, brassinosteroids, and cytokinin may play important roles of differential regulations of *GhCesA4* during cotton fibre development. The potential regulation of cellulose biosynthesis through temporal and spatial regulations of *GhCesA* genes will be discussed.

Notes:

Poster Presentations
(presenting author in bold)

P1

Evaluations of Fusarium wilt resistance in Upland cotton from Uzbek cotton germplasm resources.

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Fusarium oxysporum f. sp. *vasinfectum* Atk. Sny & Hans (FOV), in combination with *Verticillium dahliae* Kleb, causes a wilt disease complex in cotton that significantly reduces yield. A highly virulent strain of FOV, No. 316, was isolated that caused up to 80% plant death in commercial cotton in Uzbekistan. We developed an experimental $F_{2:3}$ cotton population by crossing a Mexican cultivar, Mebane-1B, (wilt resistant) and the germplasm line No.11970 (susceptible) and assessed response to FOV in two studies. One study used an artificial inoculation of strain FOV No. 316 in a field known to be infested by FOV, and the second study used an artificial inoculation of FOV No. 316 in an uninfested field. In the evaluation including natural FOV infestation the observed phenotypic segregation pattern was about 3:1 (susceptible:resistant) whereas in the second study the segregation ratio was about 1:1. The observed segregation ratios in the two studies suggested a difference in response to natural and artificial infestations of FOV. These results are consistent with previous reports. We confirmed the presence of FOV isolate No. 316 from infected plants in the naturally infested field, but we also isolated a strain of Verticillium from the same field. Therefore, observed segregation ratios should be interpreted with caution. Molecular screening efforts with 526 SSR primer-pairs yielded 69 polymorphic SSRs between susceptible germplasm line No. 11970 and resistant cultivar Mebane-1. Phenotypic and marker data are being used to further investigate inheritance of resistance, the number of involved genes, and to construct associated genetic and QTL maps.

P2

Microsatellite markers associated with fibre length trait in Pima cotton from Uzbek cotton germplasm resources.

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Development of cotton cultivars with superior fibre properties is of great importance for the cotton breeders in cotton exporting and producing countries. Identification of molecular markers, associated with fibre length, helps for further improvement of cultivated cottons. We crossed two cotton cultivars Ash-161 and Ash-143 (*G. barbadense* L.), from Uzbek cotton germplasm collection, with short (≤33mm) and long (≥41mm) fibres and developed a bi-parental mapping population. Ninety nine F_3 individuals of this experimental population, widely segregating for fibre length were used for a QTL-mapping of fibre length trait. Phenotypic variation of fibre length in F_3 population ranged from 30,4 mm to 45,9 mm. Fifty five polymorphic loci were identified in parental genotypes (Ash-161 and Ash-143) out of 568 SSR markers tested from JESPR, BNL, TMB, GH, CIR and NAU collections. The marker trait correlations using single-marker analysis after permutation test identified determined 5 potential markers significantly (α=0.001) associated with fibre length. Interval mapping demonstrated that these five SSR markers with stable LOD threshold values have significant QTL effect. The most significant QTLs of fibre length exist between markers TMB0206_215 and GH247_155 (LOD 2.1 - 5.8; explained about 24.1 - 40% of the phenotypic variation of fibre length) and between GH247_155 and BNL1317_220 (LOD 5.3 - 5.46; accounted for 21.2 - 24.1% of the phenotypic variation). Multiple QTL mapping (MQM) revealed that GH247_155 significantly associated with fibre length QTL. Moreover, linkage analysis showed that markers GH247_155 and BNL1317_220 are tightly linked (2cM). It was shown that marker BNL1317 located on chromosomes 9 and 23, where a number of QTLs associated with fibre traits had been mapped by other research groups. Results should be useful for marker-assisted selection programs to improve superior fibre quality cultivars in future.

P3

Molecular evolution of clustered *MIC-3* (*Meloidogyne Induced Cotton-3*) multigene family of *Gossypium* species.

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Uniqueness, content, localization, and defence-related features of the root-knot nematode resistance-associated *MIC-3* multigene cluster in the genus *Gossypium* are of great interest for molecular evolutionary studies of duplicate genes in allopolyploids. Here we report molecular evolutionary rates of the *MIC-3* gene family in 15 tetraploid and diploid cotton genotypes. We observed independent, equal rate, accelerated, and concerted evolutionary patterns among members of the *MIC-3* gene family. However, synonymous (Ks) and non-synonymous (Ka) nucleotide substitution rates suggest that *MIC-3* genes are generally evolving by a birth-and-death process under strong purifying selection with positively selected copies. Our results suggest that a gene amplification mechanism has maintained duplicated copies in the genomes, which best fits with the bait and switch model of R-gene evolution. Comparative analysis showed that the second of the two exons of *MIC-3* genes is under strong positive selection pressure, while the first exon is under strong purifying selection to conserve function. Using silent nucleotide substitution rates, we estimated divergence time among allotetraploid genomes and their closest diploid progenitors. We find a *MIC-3* gene duplication pattern in which duplication events occur once in every 1 million years (MY) in allotetraploids, once in every ~2 MY in A/F-genome, and once in every ~8 MY in D-genome representatives. This duplication pattern might also be due to pathogen-mediated selection process of cotton genomes. These features of the *MIC-3* gene family seem to reflect evolutionary selection for increased functional stability, while also expanding the capacity to develop novel switch pockets for detecting diverse pest and pathogen genotypes. Such evolutionary roles are congruent with the hypothesis that this unique gene family provides fitness advantages in *Gossypium* as it combats certain pests and pathogens.

P4

Development of SNP markers in tetraploid cotton genomes by haplotype clustering.

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Single nucleotide polymorphism (SNP) markers have become markers of choice for marker assisted selection (MAS) in several crop improvement programs because of their higher abundance, amenability for automation and availability of high throughput genotyping platforms. Transcriptome and genome complexity reduction techniques combined with high throughput sequencing technologies enable rapid development of informative SNP markers. However, high genomic complexity, narrow genetic base, allotetraploid nature and lack of reference genome hinder development of candidate SNP markers in cultivated cotton species. To increase the efficiency of SNP detection from homologous sequences, and reduce the risk of high false positive rate due to homeologous genomes in cotton, we have implemented 'QualitySNP' program with additional modifications. Sequence contigs generated at high stringency were searched for haplotype information, allelic frequency in respective genotypes along with progenitor sequence information to discriminate paralogous/homeologous contigs from homologous contigs. Additional scripts were incorporated for the classification of identified SNPs into Type I (true SNPs from single locus); Type II (heterologous in one genotype and homologous in other genotype) and Type III (paralogous/homeologous SNPs with in each genotype). Type I and II markers can be used for genotyping and mapping purposes. The modified program was automated to parse the contig information and generate SNP markers with flanking sequence information for direct use in assay design with high throughput genotyping platforms such as Illumina Golden Gate and Infinium assays. This SNP detection pipeline achieved higher validation rate compared to other approaches due to its efficiency in classifying three different types of SNPs *in silico*.

P5

Validation and application of SNPs in cotton using competitive allele-specific PCR (KASPar) SNP genotyping system.

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The tetraploid nature and non-availability of reference genome sequence pose multiple challenges for the development of SNP markers in cotton. By making improvements to existing software, we have developed an efficient pipeline for *in silico* discovery and validation of SNPs based on haplotype clustering. In order to genotype the SNPs resulting from this pipeline and other efforts, we have evaluated different technologies most of which are high-throughput (HTP). While the HTP genotyping platforms are very useful for rapid screening of SNP markers, they are not economical for projects such as SNP validation, saturation of targeted regions and marker assisted selection for a region/locus of interest that utilize smaller number of SNP markers on varying number of samples. From this perspective and need, KBiosciences' (Hoddesdon, UK) PCR SNP genotyping system (KASPar®), a homogeneous fluorescent endpoint single-plex genotyping system, is a very attractive and cost-effective platform. In a pilot study, we have utilized KASPar® genotyping technology for validating in silico SNPs by designing assays for 150 candidate SNPs using PrimerPicker software (http://www.kbioscience.co.uk/primerpicker/). The assay consists of three unlabeled interrogation primers that include two allele-specific primers to target SNP and one common primer. KASPar data obtained from a FRET capable plate reader with relevant filters for FAM and VIC fluorescence were plotted to determine the genotype clusters. The genotyping results and efficacy of this cost-effective SNP genotyping system for marker development and marker-assisted selection will be discussed.

P6

Engineering gossypol biosynthesis and cotton resistance to insects.

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Cotton plants accumulate gossypol and related toxic sesquiterpene aldehydes to protect themselves against pathogens and insect herbivores. However, these secondary metabolites greatly limit the utilization of cottonseeds as a rich resource for human and animal consumption. Gossypol is synthesized via the sesquiterpene biosynthesis pathway. Enzymes that catalyze three consecutive steps in gossypol synthesis were identified in our laboratory, including farnesyl diphosphate synthase (FPS), (+)-delta-cadinene synthase (CAD) and (+)-delta-cadinene-8-hydroxylase (CYP706B1). Reducing gossypol content specifically in the cotton seed will increases its value for utilization, while not affecting the content of sesquiterpene aldehydes in the rest tissues will maintain the plant's defense functions. For this purpose, a seed-specific promoter was used to drive the expression of double-stranded RNA (dsRNA) targeting *CYP706B1*, and this construct was transformed into cotton. We found that *CYP706B1* transcript levels and gossypol content were drastically reduced in seeds of some transgenic lines.

Gossypol and its derivates are toxic to insects and monogastric animals, however, cotton bollworm (*Helicoverpa armigera*) shows tolerance up to a certain concentration of gossypol. During investigations of bollworm detoxification mechanisms, we identified a bollworm cytochrome P450 gene, *CYP6AE14*, which has a high expression level in midgut and plays a key role in the bollworms adaptation to gossypol. To down-regulate *CYP6AE14* expression, we developed a plant-mediated insect RNAi technology: engineering plants to express the dsRNA targeting the insect genes. When the transgenic plant tissues were used for feeding tests, the *CYP6AE14* expression in the midgut was substantially decreased. In a small scale assay, the transgenic cotton plants showed enhanced resistance to *H. armigera*. This provides a new strategy for pest management, and also suggests a new role of small RNAs in plant-insect interactions.

P7

cDNA microarray expression profiling for fibre elongation in *G. hirsutum* and *G. barbadense*.

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Cotton fibres are extensively elongated single epidermal cells that develop on the outer surface of ovules. To understand the molecular processes occurring during fibre development of *G. barbadense* and *G. hirsutum*, and to identify genes involved in these processes, we conducted cDNA microarray and qRT-PCR approaches to compare transcript levels in *G. barbadense* cv. Hai7124 and *G. hirsutum* acc. TM-1. 418 differential genes expression profiles during fibre cell elongation and in secondary wall deposition (5, 10, 15, 20 and 25 days post anthesis (DPA)) were identified in a cotton fibre cDNA library-based 12K microarray. Of these genes, 198 had greater expression in TM-1 and 116 had greater expression in Hai7124 during fibre elongation, respectively. Gene Ontology (GO) analyses of the differentially expressed genes indicated that biological processes primarily involved in carbohydrate metabolism, lipid metabolism and the response to phytohormone stimulus. In addition, during the fibre elongation stage sucrose and starch metabolism, flavonoid biosynthesis, phenylalanine metabolism, ascorbate and aldarate metabolism, pyrimidine metabolism and the phosphatidylinositol signalling system were involved by use of Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analyses. Moreover, we developed a set of functional markers with the differentially expressed genes and combined them with the previously reported cotton fibre quality QTL. These results will lead to a greater understanding of underlying mechanisms, and also help us understand how some expression alterations in *G. barbadense* and *G. hirsutum*, and enhancing the expression of this fibre QTL associated genes will be expected to alter fibre quality properties.

P8

Obtaining of new donors on the basis of phylogenetic relationships of intra-specific varieties of *G. hirsutum* L., *G. tricuspidatum* Lam. and *G. barbadense* L.

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Today due to ecological pollution increasing importance of stock material is becoming more obvious. In this connection it is quite necessary and urgent to study the genetic potential of a large group of intra-specific varieties of *G. hirsutum* L., *G. tricuspidatum* Lam. and *G. barbadense* L. Aim of the inquiry: an establishment the degree of phylogenetic types, specification of systematic status of wild, ruderal and cultural-tropical varieties of *G. hirsutum* L., *G. tricuspidatum* Lam. and *G. barbadense* L. and also revealing donors among them which are economically valuable attributes for using in practical selection. Methods of inquiry: Comparative morphology, intra- and inter-specific hybridization and genetic analysis. Statistical processing of material based on the methodology of B.A. Dospekhov (1985), determination of dominant coefficient significances using S. Wright formula (G.M. Beil and R.E. Atkins, 1965). On the basis of obtained data systematic status of some representatives are specified. Firstly on the basis of morphobiological differences (branching, ruggedness of a leaf, downiness, size and a structure of a flower and bolls, length and an output of fibre, degree of photoperiodicity), crossing, features of inheritance in F_1 and F_2, we consider it accurate to allocate ruderal form of var. el-salvador (ssp.*purpurascens)* in a rank of independent sub-species and to carry to wild forms of *G. tricuspidatum* Lam.. There has been identified isolation of var. *gambia* among the sub-species of ssp. *punctatum* (*G. hirsutum* L.). The opinion of A.A. Abdullaev and V.P. Klyat (2006) about removal of *palmerii* (var. *microcarpum palmerii*) and ssp. *darwinii (G. barbadense* L.) in a rank of an independent species was confirmed. There has been identified the phylogenetic kinship and distance of separate sub-species of *G. hirsutum* L., *G. tricuspidatum* Lam. and *G. barbadense* L.. Intra-specific and inter-specific hybrids with new germplasm to replenish potential cotton gene funds, and will be used as donors of valuable biological and economic attributes. The new scheme of phylogenetic relationship intra-and inter-specific versions of *G. hirsutum* L. and *G. tricuspidatum* Lam. and intra-specific varieties of *G. barbadense* L. will facilitate selection of an initial material and will provide efficiency of reception of new hybrid forms for genetically selection researches, in creation of competitive types of cotton in global market.

P9

Genetic effects of exotic alleles from Sea Island cotton (*Gossypium barbadense* L.) on fibre properties and other agronomic performances in upland cotton (*G. hirsutum* L.).

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Utilising the superior fibre quality introgression germplasm to enhance fibre quality of upland cotton (*Gossypium hirsutum* L.) has become increasingly important. Unfortunately, poor agronomic performance was usually transferred into the cultivar along with high fibre quality, known as linkage drag. F_2 and $F_{2:3}$ populations derived from a cross of an excellent fibre quality germplasm with introgressed genetic elements from *G. barbadense* L. and an elite upland cotton cultivar of high lint percentage were constructed to identify the QTLs for fibre quality and lint percentage and, consequently, investigate the association between fibre-related traits and exotic genetic components. A total of six putative introgressed chromosomal segments, which covered 132.7 cM, were identified and a total of 29 QTLs with 4.24-19.98% of the total phenotypic variance were detected. 5 among the 29 QTLs showed high stability when validated in an F_8 breeding population. As expected for fibre quality QTLs, a majority (70.6%) of favourable alleles were from the introgressed line (IL) parent and almost all of which were located in the introgressed *G. barbadense* components. For lint percentage QTLs, 50% of favourable alleles came from the upland parent. It is noted that the parent with inferior fibre quality or lint percentage also conferred favourable alleles. The co-localization of QTLs for different traits was also identified and it was the possible reason for the phenotypic correlations. The results presented here will help us better understand the molecular basis of linkage drag and enable further marker-assisted selection for synchronous improvement of lint yield and fibre quality in cotton breeding.

P10

***GhAGP31,* a cotton non-classical arabinogalactan protein, is preferentially expressed in roots and in response to cold stress.**

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Arabinogalactan proteins (AGPs), a superfamily of highly glycosylated of hydroxyproline-rich glycoproteins, are widely implicated in plant growth and development. A cDNA encoding a new class of non-classical AGP protein was isolated from cotton (*Gossypium hirsutum*) fibre cDNA libraries and designated as *GhAGP31*. The AGP31 protein shares features with several known and non-classical AGPs from other species: a putative signal peptide, N-terminal histidine-rich stretch, middle repetitive proline rich domain and a cysteine-containing PAC (for proline-rich protein and AGP) domain. Real-time quantitative RT-PCR results revealed that its transcripts were significantly accumulated in hypocotyls and roots, especially in root tips. As the roots developed, its mRNA gradually decreased to low levels. Meanwhile, its mRNA was affected by several abiotic stresses. It was induced at least three fold within 12h in response to cold treatment of 5-d-old roots. A *GhAGP31* promoter fragment isolated from cotton genome was fused with the β-glucuronidase reporter gene to demonstrate its specificity. Histochemical assays of the transgenic Arabidopsis plants demonstrated that the GhAGP31:GUS gene was specifically expressed in the tips of main roots and lateral roots. Cold treatment appropriately promoted the expression of the GUS gene in 14-d-old seedlings. Expression of an AGP31-eGFP (enhanced green fluorescent protein) fusion protein in transgenic cotton cells showed that the GhAGP31 protein was localized to the cell wall. These data suggested that *GhAGP31* may play an important role in root development and in response to cold stress during early seedling development of cotton.

P11

Differentially expressed genes in cotton plant genotypes and their evaluation under *Meloidogyne incognita* infection.

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Meloidogyne incognita is a nematode responsible for huge losses of economically important crops. The control of this pathogen is heavily centred on chemical nematicides, which are toxic to humans and the environment, besides being very expensive. Alternatively, resistant varieties of cotton generated from conventional breeding programs represent an attractive strategy for the control of *M. incognita*. In this context, the goal of the work reported here was to analyse the gene expression profile of one resistant and one susceptible cotton genotype infected with *M. incognita* aiming to understand the mechanisms involved in resistance. EST libraries of cotton in varieties both resistant and susceptible to infection by *M. incognita* were constructed and sequenced, generating 2261 sequences that were assembled into 233 contigs and 1593 singlets. Genes differentially expressed were observed in both resistant and susceptible cotton. Twenty genes were found to be expressed exclusively in the resistant cotton genotype, with functions related to pathogen recognition, signal transduction, defence mechanisms and protein synthesis transport and activation. The coordinated action of these genes suggests the existence of a complex defence pathway towards nematode attack in cotton. The candidate genes were evaluated, through real time PCR, aiming to analyse the expression pattern in resistant plants infected with *M. incognita*. In this assay, the infected cotton roots where collected 0h, 10h, 24h, 48h and 72h after inoculation with 1500 *M. incognita* j2. Total RNA extraction was made for each root, and these RNAs were used in Real Time PCR reactions. The results show the candidate genes expression patterns in a time-course experiment. Our data indicate some candidate genes for validation and use through transformation in other agronomically important plants.

P12

The gene *GbSTK* cloned from *Gossypium barbadense* might be involved in verticillium wilt resistance.

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Verticillium wilt caused by soil-borne pathogenic fungus is a vascular wilt disease, among the most destructive diseases in cotton and widespread throughout the world. In recent twenty years, verticillium wilt has caused severe yield and quality losses in three cotton-growing areas of China. It is particularly difficult to control the disease due to the long viability of the resting structures, the broad host range of the pathogens and the inability of fungicides to affect the pathogens once the pathogens enter the xylem. Because control of disease occurrence by reduction of inoculum density is rather difficult through solarisation, chemical soil fumigation or crop rotation, currently the best way to prevent verticillium wilt is the utilization of resistant cultivars. For cotton, tetraploid *G. barbadense* and some diploid species possess higher resistance to verticillium wilt than *G. hirsutum*. However, it is most difficult to breed a new variety by transferring disease resistance from tetraploid *G. barbadense* and some diploid species to *G. hirsutum* via wide-cross and conventional breeding method. Along with the rapid development of molecular techniques, several plant disease resistance genes have been cloned and assigned to five classes based on structural features. Among about 50 cloned R genes, four encoded serine threonine (S/T) protein kinases. In the present study, we cloned and analysed a *GbSTK* gene encoding serine/threonine(S/T) protein kinase from *G. barbadense* under *Verticillium dahliae* stress. The ORF of the *GbSTK* was 1314 bp length, which encoded a 437 amino acids polypeptide with a molecular weight of 38 kDa. Bioinformatic analysis indicated that *GbSTK* gene encoded a protein including serine/threonine protein kinase, protein tyrosine kinases activity domain and protein kinase ATP domain. Semi-quantitative RT-PCR showed that the expression of *GbSTK* remarkably increased after 48 h *Verticillium dahliae* stress in root in contrast to uninoculated control. A novel polypeptide with the molecular mass of about 74 kDa was expressed in *E. coli* BL21 by SDS-PAGE detection. The GbSTK-GFP fusion protein was located in the cytoplasmic membrane and the endoplasmic reticulum of onion epidermal cell by confocal microscopy. The eukaryotic expression vector of *GbSTK* gene was constructed and transformed to *Arabidopsis thaliana* (Columbia ecotype) through *A. tumefaciens* strain *GV3101* using the floral dip method. After three generation selection for transformants, we obtained seven T3 homozygous lines. Then wild control and four T3 homozygous lines were sown in eight 557-cm pots and four plants for each pot, and maintained at 251, 60% relative humidity and light intensity of 100 mol E m-2 sec-1 on a 16 h light/8 h dark cycle. After three weeks, they were inoculated with 1107 conidia per millilitre severe *Verticillium dahliae* strain using the root-inoculated method. The difference of leaf symptoms between wild and transgenic lines was visible eight days post-inoculation (dpi), subsequently became more and more obvious and intense. The transgenic lines presented higher resistance to verticillium wilt with less yellow leaves, slightly delayed flowering and higher flowering stems compared to Columbia ecotype. The above results indicated that the *GbSTK* might play an important role in the resistance to verticillium wilt in *Arabidopsis thaliana*.

P13

Development of cultivars with ultra-low gossypol cottonseed.

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Cotton (*Gossypium hirsutum*, L.) seed is a high-quality oil and protein source, but its utility is limited by gossypol which is a toxic compound to non-ruminant animals as well as humans. Gossypol is present throughout the plant and highly effective in providing host plant resistance to insect pests and other herbivores. Molecular biologist at Texas A&M University successfully developed transgenic plants with ultra-low gossypol (ULG) expression in seeds with a normal expression in the remainder of the plant. The current transgenic background is in Coker 312, which is a cultivar with moderate adaptability, yield potential and fibre quality. A program was recently initiated to integrate the ULG trait into improved germplasm as well as international germplasm. Six genotypes are being used as recurrent parents during the backcross procedure. F_1 plants indicate ULG is inherited with complete dominance and does not affect other morphological traits. PCR technology is needed to improve breeding efficiency and quality control. BC_3 plants are expected to be available in 2011. At the conclusion of this project, ULG germplasm with high-yield potential that are widely adapted will be created. As a result, a potentially new and abundant source of high quality protein and oil will be available for non-ruminant animal and human consumption.

P14

Cloning and functional characterization of the cotton basic helix-loop-helix transcription factor gene associated with cotton fibre elongation.

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As the longest cell type known in plants, cotton fibres are economically important seed trichomes and provide a unique single-celled model system for studying fundamental biological processes. A large number of genes related to cotton fibre differentiation and development have been cloned, but so far, the functions of few genes have been identified in transgenic cotton. Here, we isolated a cotton BHLH gene (temporary name: *GbBHLH*) from the normalized cDNA library of Sea-Island cotton (*Gossypium barbadense* L. cv. 3-79) fibre (-225 DPA, days post anthesis). Through the 5' RACE the complete 1366 cDNA sequence was cloned, and included a 1035 bp putative ORF encoding 344 amino acid. This protein contained a 60aa basic helix-loop-helix domain. Microarray and quantitative real-time PCR results showed that *GbBHLH* is preferentially expressed from 5 to 15 DPA and peaked at 10 DPA. Sub-cellular localization of GbBHLH in the nucleus of onion epidermal cells was demonstrated by biolistic transformation. We have been identifying the function of GbBHLH by over-expression and RNA interference strategies. A 2437 bp promoter of *GbBHLH* was isolated by genome walking method, and fused to the beta-glucuronidase (GUS) reporter gene. It has been transformed into cotton and Arabidopsis to elucidate the expression profile.

P15

GhHmgB3 deficiency deregulates proliferation and differentiation of cells during somatic embryogenesis in cotton.

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High mobility group box 3 protein (*HmgB3*) is a member of a chromatin-binding protein family that can alter chromatin and nucleoprotein structures to facilitate transcription. The gene encodes an Hmg-box domain in *Gossypium hirsutum* cv Coker 201 and was characterized as preferentially expressed among embryonic tissues. RNA interference (RNAi) was used to down-regulate the expression of *GhHmgB3* during cotton somatic embryogenesis (SE) by transforming both hypocotyl and embryogenic calli (ECs) via *Agrobacterium tumefaciens*. The GhHmgB3-deficient somatic cells of hypocotyls dedifferentiated more vigorously than the control cells, but they failed to differentiate to ECs. In another case, the proliferation and differentiation of *GhHmgB3*-deficient ECs were significantly improved, with many embryogenic structures, but failed to form plantlets. The differentially expressed genes between the control and *GhHmgB3*-deficient ECs were identified by Solexa sequencing technology. The Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis revealed an abnormal Wnt/-catenin signalling. The high expression of -catenin in the *GhHmgB3*-deficient ECs suggested a high level of Wnt/-catenin signalling that promoted cell self-renewal. The induced mitogen-activated protein kinase (MAPK)/p38 signalling repressed the canonical ternary complex factor (TCF)/-catenin pathway and then led to a lack of growth of *GhHmgB3*-deficient EC cells. In contrast, the special expression pattern of calcium-related genes and high expression of actin 10 in *GhHmgB3*-deficient ECs reflected an induced cadherin/-catenin pathway that promoted the differentiation of ECs. These results in response to the down-regulation of *GhHmgB3* revealed a series of –catenin Ca dependent mechanisms that regulated the proliferation and differentiation of cotton SE.

P16

Deep sequencing analysis of transcriptomes from different cotton tissues.

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As the most important resource of natural fibres used in textile industry, upland cotton (*Gossypium hirsutum*) has not been sequenced yet mostly because of its very complex allotetraploid (AADD) genome (2n = 4 = 52) of approximately 2.15G base pairs. Here we performed Illumina 1G deep sequencing analysis of cotton transcriptomes by using 4 different cotton tissues: leaf, flower, 0 days-post-anthesis (dpa) ovules (O-0) and 5 dpa ovules (O-5). Over 208M clean reads (15.6G base pairs) were obtained and assembled into 147,461 unigenes with average length of 775bp, among which 20,038 were longer than 1000bp. All unigene sequences with its raw data and annotation information were built into a database at the CBI website. (Center for Bioinformatics, Peking University, http://cbi.pku.edu.cn). Statistically, there are 90,037 unigenes in leaf tissue, 78,100 in flowers, 90,415 in 0 dpa ovules and 78,208 in 5 dpa ovules. After in-depth analysis of unigenes from different cotton tissues, we obtained 11,980 fibre-specific transcripts in 5 dpa ovule samples. Annotation, conserved domain and gene ontology analysis of these unigenes showed that, in cotton fibres, GO biological processes ontology of protein amino acid phosphorylation, regulation of transcription and glycolysis are mostly enriched, which indicates that phosphorylation of transcription factors may be important in cotton fibre development. Ahead of the upland cotton genome sequencing data, the current research provides an insight of cotton transcriptome and gene expression profiles in different cotton tissues. Also, novel fibre enriched/specific genes may be fished out by digging into these data.

P17

Exploring the genetic potential of herbicide resistant transgenic cotton cultivar against the direct treatment of cadmium.

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Here we report the successful integration of the glufosinate resistance (Bar) gene into the cotton genome and an investigation of the genetic potential of our own developed glufosinate resistant transgenic cotton cultivar (BR001) against cadmium stress. First of all, the Bar gene was transferred in Coker 312 using the shoot apex and pollen tube pathway via ovarian injection approaches. Both the PCR and southern blot results revealed the successful integration of the foreign gene. Both the Mendelian inheritance and segregation analyses confirmed that a single nuclear dominant gene governed the herbicide resistance characters in BR001. In the second phase of our study, BR001 and its parent line (Coker 312) were put under cadmium (Cd) stress at increasing concentrations (i.e. 0, 10, 100, 1000 µM). As against their respective controls, low concentrations (10 and 100 µM) of Cd stimulated the seed germination in BR001, while the highest Cd (1000 µM) concentration inhibited its germination. A linear decrease was observed in the mean lengths of root stem and leaf and leaf width as well as their fresh and dry biomasses in BR001. Moreover, a progressive stimulation in the water absorption capacities of root, stem and leaf was noticed, which were more in leaf followed by root and stem. Also the accumulation of Cd was more in BR001 as compared to Coker 312. An increase in plasmolysis of the plasma membrane, number of nucleoli as well as vacuoles in BR001 was found in the root tip cells. However, these ultrastructures were well-developed in Coker 312 except enlarged vacuoles and greater number of mitochondria. With the elevation of Cd stress levels, the ultrastructural modifications in leaf mesophyll cells also became prominent, which were more obvious in BR001 than Coker 312. Modifications in morphology of chloroplast, increase in number and size of the starch grains as well as increase in the number of plastoglobuli reveal the toxic effect of Cd on photosynthetic organs of the leaf in the case of both experimental cotton cultivars. Moreover, TEM studies revealed the accumulation of Cd in the form of electron dense granules and crystals both in vacuoles and attached to cell walls in both BR001 and Coker 312. These results suggest that the transgenic cotton cultivar and it parent line showed positive responses towards Cd stress at the seedling stage as well as that the internal Cd-detoxification in these studied cultivars might be through apoplastic and symplastic binding.

P18

Development of mapping population RILs in diploid cotton (Asiatic cotton).

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A long-term challenge facing a cotton breeder is the simultaneous improvement of yield and fibre quality to meet the demands of the cotton producer as well as the textile industry. A negative association between lint yield and fibre quality is still present after many years of exhaustive breeding for improved fibre properties. Conventional breeding procedures are difficult for further improving fibre quality because of high costs, long duration and low selective efficiency. Studies in other crop plants have demonstrated that biotechnology promises to provide powerful tools for enhanced genetic improvement of qualitative traits. Advances in the identification of DNA markers for fibre quality QTLs have been reported. Many QTLs for fibre traits were identified from four inter-specific populations of *Gossypium hirsutum* and *barbadense* (Jiang et al. 19989; Kohel et al. 2001; Mei et al., 2004; Paterson et al., 2003). Development of a mapping population (RILs) has been initiated in case of diploid cotton using two extreme parents for fibre traits RG 8 (having very short fibre, 15mm and least bundle strength, 14g/tex) and Arbha 35 (having long fibre, 28mm and high bundle strength, 25g/tex). In F2, 268 single plants obtained were advanced to develop RILs through repeated selfing or sib-mating to develop a set of inbred lines. The 268 inbred lines are presently in F4 generation and will be screened using SSR markers for fibre quality traits and lint yield after fixing in F6 or later generation. The range of variation for fibre length was from 13mm to 29mm, for ginning outturn (%) from 22 to 48. The RILs being developed will be screened to identify molecular markers or QTLs for fibre traits and such markers or QTLs would be used to accelerate the selection of elite cotton lines in early segregating breeding generations.

P19

Assessing Genetic Diversity in *Gossypium arboreum* L. cultivars using genomic and EST-derived microsatellites.

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The cultivated diploid, *Gossypium arboreum* L., (A genome) is grown throughout India under irrigated as well as under rainfed situations. The level of lint yield as well as fibre quality traits also varies from low to medium or even high. In the present study the cultivars representing various states of India, Andhra Pradesh (Mudhol), Jalgaon, Akola, Nagpur (Maharashtra), Surat (Gujarat), Haryana (Hisar), Sriganganagar (Rajasthan) and Ludhiana (Punjab) were taken to assess the genetic diversity using genomic and EST-derived microsatellites (SSRs). A total of 61 polymorphic bands were generated using genomic and EST derived-SSR markers. Maximum similarity up to 0.73 was observed between the cultivars RAC 024 and AH-1 and minimum between GBav 110 and RG 359 representing different centres in north and central India. The cultivars (RAC 024, AH-1, CINA 343) which showed maximum similarity from 0.70 to 0.73, were from the same zone or state except HD 464. The dendrogram analysis exhibited the range of similarity coefficient from 0.47 to 0.88. The cultivars grouped in two main clusters. The cluster 1A had only one cultivar, GBav 110 while the cluster 1B had all other cultivars. The cultivar RG 359 and HD 464 were clustered together representing the nearby states whereas LD 955 also from nearby state was grouped in another cluster having genotypes GBav 108 and GBav 109 representing Gujarat state. A total of six clusters were formed which were representative of various states/centres.

P20

Molecular mapping of photoperiodic flowering in cotton.

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Wild cotton germplasm resources are largely under-utilized because of photoperiodic-dependent flowering of exotic cottons. Cottonseeds of several photoperiodic wild and primitive cottons treated with gamma-radiation using 60Co, and beta-radiation using 32P that converted non-flowering wild cottons directly into day-neutral plants. These mutant cotton germplasm had been self-pollinated and selected for early flowering phenotype for M_{8-10} generations that led to development of more than 200-photoperiod converted mutant cotton germplasm. In this work, we created a $F_{2:3}$ segregating populations derived from the cross of mutant (day-neutral) and original (photoperiodic) genotypes for two species via genetic hybridization between *G. hirsutum* ssp. var. el-salvador (mutant) x *G. hirsutum* ssp. var. el-salvador (original) as well as between *G. darwinii* (mutant) and *G. darwinii* (original). These $F_{2:3}$ segregating populations for both crosses were evaluated in long-summer day field condition of Uzbekistan in two consequent years of 2008 and 2009, and a genetic segregation pattern for flowering phenotype was recorded. The genomic DNAs of individuals from each $F_{2:3}$ segregating populations were isolated and genotyped with more than 400 SSR markers from different SSR collections as well as three candidate gene markers developed from cotton phytochrome gene family. Linkage analysis and QTL-mapping of photoperiodic flowering will be presented.

P21

Quantitative cDNA-AFLP reveals the extent of transcriptional polymorphism in developing cotton fibres.

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Genetic variability in fibre quality among the two major cultivated cotton species, *Gossypium hirsutum* (Gh) and *G. barbadense* (Gb), shows a complex multigenic inheritance. We developed a quantitative 3 targeting cDNA-AFLP analysis strategy in order to dissect transcriptional regulation differences between the developing fibres of these 2 species. Two studies were undertaken. In the first study the expression profiles of over 3000 transcripts from the 2 parental species were analysed by quantitative cDNA-AFLP during the time-course of fibre development, between 6 and 28 days post anthesis (dpa). The 2nd study (4400 transcripts profiled) focused on two key developmental stages of fibre development (10 and 22 dpa, respectively) at a population-wide level using an inter-specific RIL population. Major achievements include: (1) the partitioning of genes among significant expression profiles and its comparison between Gh and Gb; and (2) the mapping of a large (>5000) number of expression QTLs on the RIL genetic map and a comparison of their distributions with QTL for fibre phenotypic traits. This research is part of a larger project aimed at the genetic and genomic dissection of cotton fibre quality.

P22

Functions of three conservative cysteines of GhABP1.

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As an essential plant hormone, auxin orchestrates a multitude of developmental and morphological phenomena in plants, most notably those involved in apical dominance, root initiation and a variety of tropisms. Nowadays, Auxin Binding Protein 1 (ABP1), that binds auxin at physiologically relevant levels, has been characterized as a potentially important mediator of auxin action in plants. However, the role of ABP1 in auxin signalling still remains to be understood. Therefore, we took a transgenic approach to examine the functions of three cysteines and C-terminal of the GhABP1 *in vivo* to elucidate the structure-function relationship of ABP1. The cDNA sequence of *GhABP1* encoded a polypeptide of 191 amino acid residues with significant homology to the ABP1 in other plants. After removed the 24 amino-acid residues signal peptide at N- terminal, the three conservative cysteine residues located on the site of 3, 63, 157 in sequence respectively. Interval strips excised from growing transgenic tobacco leaves, in which *GhABP1* was driven by the CaMV35S promoter, have stronger auxin-specific, epinastic growth response to NAA than the wild-type. This suggested that the GhABP1 has the same function as AtABP1 in transgenic tobacco. To investigate the function of the alpha-helix in the C-terminus of GhABP1, the GhABP1-NC without a C-terminus were introduced into tobacco driven by CaMV35S promoter. The same curvature of transgenic and wild-type strips treated with NAA was observed and the transgenic tobacco showed normal morphology. This implied that removing the alpha-helix in the C-terminal end of GhABP1 resulted in the loss of its function. Furthermore, GhABP1-NC did not show the competing reaction to the docking site with wildtype ABP1. To confirm the functions of three conservative cysteines in GhABP1, the GhABP1-C3S, GhABP1-C63S and GhABP1-C157S mutant were obtained via converting cysteine into serine, respectively. The leaves from the transgenic tobacco over-expressing GhABP1-C3S and GhABP1-c63s showed the same auxin-specific response to NAA as the transgenic tobacco over-expressing GhABP1. However, the reaction of epinastic growth in plants over-expressing GhABP1-c157s was not observed. It demonstrated that the GhABP1 would lose its function after being mutated at Cys157 to Ser157. By contrast, mutating the Cys3 to Ser3 or the Cys63 to Ser63 caused the GhABP1 to keep its epinastic growth. It is interesting that there are different observation between the GhABP1-C3S and GhABP1-C63S over-expressing plants. The epinastic growth test showed that the curvature of ABP1-C63S over-expressing plant is much larger than those of wildtype and plants over-expressing GhABP1-C3S and GhABP1-C157S without auxin treatment. The curvature of the leaf strips from GhABP1-C3S over-expressing plants is the same as that of wildtype. It is concluded that the Cys3 is not essential for the function of GhABP1, while the Cys63 mutant will make GhABP1-C63S be easy in active phase, even in low concentration of NAA. Plants over-expressing GhABP1-C63S show high activity with low levels of NAA. The transgenic plants are smaller in size and slower in development than the wild-type.

P23

Cloning and expression characteristics of a novel GhMATE1 gene in a brown pigment synthesis of brown fibre cotton.

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The brown fibre comes from a type of natural brown cotton, which keeps the characteristics of nature, environmental protection non-pollution in the processing of garment dying. In order to understand the formation of brown pigment of cotton fibre and cloning of brown pigment synthesis associated with important genes, brown cotton "Zhecaimian 2" and white cotton "Zhemian 102" were selected in this study. Cotton fibre total RNA was extracted form the cotton fibre development period of 10 DPA, 15 DPA, 20 DPA, 25 DPA and 30 DPA. Ultimately, only one expressed fragment of 420 bp was obtained from the cDNA of brown cotton fibre using Gene Fishing Screening ACP primers. After sequencing, bioinformatics analysis, screening and target integrated cotton EST database, one gene was cloned using the RT-PCR method. The sequence of the gene showed 75% homologous with brown pigment synthesis *TT12* gene of Arabidopsis in amino acid sequence. The gene was named *GhMATE1*, which encodes 497 amino acid residues with the molecular weight of 53.6KD, containing conserved domain MATE and belonging to a member of MATE super-gene family. The expression analysis of the gene showed that: the gene presents super-dominant expression during brown cotton fibre development by the Real-time quantitative PCR method. In 20DPA, the expression of the *GhMATE1* was nearly 30-fold in brown cotton fibre compared with the white cotton fibres. The cloning of the gene gives a clue for further understanding its function participating in the formation of brown pigment of cotton, the relationship of brown pigment of cotton fibre and brown pigment of seed coat.

P24

Research on the regeneration capacity of different genotypes in upland cotton.

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The regeneration system of upland cotton (*Gossypium hirsutum* L.) is required for genetic transformation as well as the study of Genomics. However, there are few cultivates of upland cotton that can be regenerated efficiently. Different genotypes show different results in the process of cotton somatic culture and plant regeneration and some show inducible because cotton is one of the most reliant genotype plants. In this study, totally 20 varieties of upland cotton, which were verified with good agronomic trait before, were used to compare the regeneration capacity so as to screen out varieties which could be regenerated efficiency.

The best medium for the solid callus initiation, MS + 0.1mgL^{-1} IAA + 0.1mgL^{-1} KT + 0.1mgL^{-1} 2, 4-D + 30gL^{-1} glucose, was selected for callus induction from hypocotyls. Notably, the first time of the callus to emerge, the colour and the texture of the callus displayed no difference among these varieties in the process of callus induce, while the proliferated weight showed obviously discrepancy between 0.2g and 0.6g after 30 days of callus inducing. After the subculture and the embryogenic callus induced in a halved NH4NO3 solid medium, MS + 2mgL^{-1} glycine + 0.3mgL^{-1} IBA + 0.1mgL^{-1} KT + 30gL^{-1} glucose, the phenotype of the callus turned to be quite dissimilar and could be classified into three types: 15% of the materials were induced to type I callus, the typically embryogenic callus that showed in light yellow or gray colour, friable or alveolate structure and a little moist characteristics; 75% of the varieties were the type II callus in brown or green colour with the compact surface; and the others were type III callus proliferated in "mad".

An excellent genotype, upland cotton variety JW204, has been screen out. It could form somatic embryogenesis and plant regeneration in no more than 130 days in the most proper somatic embryogenesis and plant regeneration medium, which halved NH4NO3 MS + 2mgL^{-1} glycine + 0.5mgL^{-1} IBA + 0.2mgL^{-1} KT + 0.5gL^{-1} asparagine + 0.5gL^{-1} glutamine + 30gL^{-1} glucose. And the embryos per gram on somatic embryogenesis medium for JW204 were higher than those for Coker 312 reported previously. Then the regeneration plantlets were cultivated on MS medium with no hormones for rooting and were acclimatized before transfer to the greenhouse. Abnormal embryos both in structure and in physiology were observed in the process of somatic embryogenesis of JW204 too. So our study provides a useful protocol and a new genotype for the regeneration system of cotton.

P25

Transformation of SLL705 cotton cultivar by biolistics aiming for resistance to cotton boll weevil.

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Among the crops of agronomic importance, cotton is highlighted by many factors. For example, the cotton fibres, which are of great value to the textile industry, and the seeds, which are used to produce oils for human consumption and animal feed. However, cotton production has been strongly affected by attack of insect pests, which causes crop losses and increased production costs. Consequently, the use of pesticides is mandatory, causing contamination of the environment, animals and human beings. Since the introduction of cotton boll weevil (*Anthonomus grandis*) into Brazilian fields, huge agronomic losses have been recorded. The boll weevil attacks floral buds internally, so is inaccessible to pesticides. The incorporation of genes coding entomotoxic proteins by genetic engineering has been used as an alternative method of pest control. In this context, this study aimed to transform cotton plants (cultivar SLL705) via biolistics to introduce the *cry8ka5* gene and to induce resistance to the cotton boll weevil. In this work, 640 apical meristems from embryos were bombarded, resulting in 221 acclimatized plants in the greenhouse. These plants were tested for kanamycin resistance, by spreading kanamycin (100 mg/ml) at halo-shaped leaves of potentially transformed plants. Chlorotic spots were observed in 181 susceptible plants. Positive plants (40) were evaluated by PCR using primers for *cry8ka5* and by ELISA (neomycin phosphotransferase expression). Until now, only six plants showed consistently positive results for all three replicates for all three preliminary tests that could mean 0.93% transformation efficiency. After the complete molecular characterization (Southern blot, qPCR, Western blot), these plants will be submitted to bioassays against *A. grandis* to verify the resistance.

P26

Arabinogalactan proteins specialized for secondary cell-walls of arabidopsis and eucalypts.

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Our studies in Arabidopsis and eucalypts indicate that closely related arabinogalactan proteins (AGPs) are candidates for impacting on secondary cell wall formation. We observed that in eucalypts the expression of these genes is correlated with the development of wood with different cellulose microfibril deposition. These eucalypt AGPs are phylogenetically related to a subset of Arabidopsis AGPs - the fasciclin-like AGPs (FLAs). The eucalypt and Arabidopsis FLA genes are specifically expressed in stems, with little expression in other tissues. We have used Arabidopsis as a model to investigate the role/s these FLAs might play in cell-wall architecture and properties. Phenotypic analyses of knock-out mutant Arabidopsis lines that lacked expression of these Arabidopsis FLAs yielded some fascinating results. We present evidence implicating expression of these FLAs with the stiffness, strength and cellulose deposition of secondary-cell walled cells such as those found in wood, and discuss how these genes are likely to be involved in cell wall matrix properties.

P27

Cloning and functional analysis of cotton casein kinase I.

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Genes of Casein kinase I (*CKI*) family are Ser/Thr protein kinases that are involved in various cellular, physiological, and developmental processes in yeasts and animals, but the biological roles of CKI members in plants are not well understood. Great difference was found in gene expression profile of a transcript with homology to CKI in Arabidopsis between Xu142 and its fuzzless-lintless mutant at the stage of fibre differentiation through real-time quantitative RT-PCR. A 1392-bp length of cDNA sequence was obtained by 5'/3' terminal extension, named *GhCKI* which encoded a protein of 463 amino acids. *GhCKI* was predicted to have seven conserved domains and a putative nuclear localization site with high homology to *AtCKI* except for the C-terminal, which may contain a substrate binding domain. Construct of ProGhCKI:GUS was constructed and transformed into Arabidopsis, the result revealed *GhCKI* was expressed highly in cotyledons, young roots and pollen. Furthermore, ectopically over-expressing *GhCKI* resulted transgenic Arabidopsis exhibiting pleiotropic phenotypes, including delayed senescence, long inflorescence, reduced fertility, more secondary branches, large seed size and short siliques, compared with wild-type. The study on function of *GhCKI* in plants is still on-going.

P28

Transcriptome analysis of cotton (*G. hirsutum L.*) during boll development under drought stress.

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Cotton is the most important fibre crop in India occupying 9 mha area which represents world highest acreage of 34 mha under cotton cultivation. Drought is the most important environmental factor limiting plant development. Transcriptome analysis was demonstrated to be an efficient approach to reveal the components responsible for abiotic stress tolerance. To identify candidate genes for drought resistance expression profiling was performed on cotton (*G. hirsutum* L. cv. Bikaneri Nerma) plants subjected to water stress under field condition. RNA samples were collected separately from leaf and different boll developmental stages viz. -5 dpa, 5 dpa, 10 dpa and 20 dpa. Gene expression profiling was conducted using Affymetrix Cotton GeneChip with probe sets of ~23,500 genes. Microarray data were processed with Genespring GX 11.0.1 (Agilent) software. Genes with corrected p value <0.01 and a log fold change >2 were identified as differentially expressed genes. Genes with more than 3 fold change were taken for further analysis. The results showed that under water stress condition 2035 genes (638 up and 1397 down) in leaf, 298 genes (183 up and 114 down) in -5 dpa, 1182 genes (908 up and 274 down) in 5 dpa, 2331 genes (1177 up and 1154 down) in 10 dpa and 1223 genes (731 up and 492 down) in 20 dpa were differentially expressed. Among the boll development stages (5 dpa, 10 dpa and 20 dpa) 146 genes were commonly regulated. In each stage 40 differentially expressed genes were selected for qRT-PCR to validate the microarray data. Differentially expressed genes were assigned to Arabidopsis locus identifiers by BLASTx to Arabidopsis gene models in TAIR database and grouped into various functional categories based on MIPS Functional Catalogue. In all the stages 50% of the differentially expressed genes were involved in metabolism, protein function related (protein synthesis, protein fate and protein with binding function or cofactor requirement) and sub-cellular localization categories followed by genes involved in defence, cellular communication, signal transduction, transcription and biogenesis of cellular components. Except in leaf stage in all other boll developmental stages more up regulated genes were involved in each functional category compared to down regulated genes. The differentially expressed genes were further analysed according to pathways and transcription factor families in which they were involved. Statistically enriched metabolic pathways were identified using the KOBAS database. During boll developmental stages genes involved in alpha-linolenic acid metabolism, amino sugar and nucleotide sugar metabolism, cytochrome P450, fatty acid metabolism, flavonoid biosynthesis, gamma-Hexachlorocyclohexane degradation, indole alkaloid biosynthesis, receptors and channels, phenylalanine metabolism, phenylpropanoid biosynthesis, riboflavin metabolism and starch and sucrose metabolism were enriched. The significantly overrepresented transcription factor families included *AP2/EREBP*, *bZIP*, *bHLH*, *HB*, *MYB*, *NAC* and *WRKY* in differentially expressed genes during boll development stages. Data from these studies provide a robust reference data set for improvement of drought resistance in upland cotton and other plant species.

P29

Selection of germplasm for development of heterotic groups in hirsutum cotton.

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The selection of appropriate germplasm in a breeding programme strongly depends on the type of cultivar to be developed. In G. hirsutum cotton, broadening the genetic base is a key to ensure continued genetic gains in hybrid breeding. There are no recognized heterotic groups in cotton. Therefore, the development of a method for choosing potential parents/germplasm before making all possible crosses and their field evaluation could improve the efficiency of hybrid breeding. In the present study, for identifying heterotic groups, parents/germplasm were selected based on adaptability in diverse agro-ecological zones of cotton cultivation. These diverse agro-ecological zones are Central, South and North which represent cotton cultivation in India. Parents G.Cot.16, Daet-S-SL, P 56-4, Reba B 56 and JK-4 were identified as best combiners for seed cotton yield. Based on specific combining ability, crosses G. Cot 16 Daet-S-SL, JK 4 Pusa 56-4, JK 4 G 67 and Nh 615 P 56-4 were identified as potential for developing hybrids.

P30

Enhanced drought tolerance in *Gossypium hirsutum*

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Gossypium arboreum harbours built-in drought tolerance, therefore, it is highly suited to dry land and low input farming. On the contrary, American (*G. hirsutum*) varieties are high yielding, but lack such important characteristics. We have studied several local varieties of *G. arboreum* for tolerance against water shortage. Searches for the identification and expression of wax genes, by RACE-PCR, led to the identification of two genes that showed homology with 3-ketoacyl CoA synthase and Cer3 genes of *Arabidopsis thaliana*. The differential display technique was used to isolate and clone 7 up-regulated fragments. From these fragments two genes, alpha-crystalline small heat shock protein gene (GHSP26) and the universal stress protein gene (GUSP1) were further isolated and characterized. GHSP26 and GUSP genes were cloned in the plant expression vector, pCambia-1301 (CaMV 35S promoter, neomycin phosphotransferase and GUS, as a reporter gene). These genes were transformed in *G. hirsutum* variety CIM 496. Expression studies of both genes in different tissues revealed that the GHSP26 is expressed mainly in leaf tissues while the GUSP1 is ubiquitous in its expression. mRNA expression was found to be 6 fold over expression of GUSP1 and 60 fold over-expression of GHSP26. Heat tolerance capacity of the plants showed that transgenic plants were more tolerant at 50^{o}C for 3hrs than non-transgenic plants. The inheritance pattern of drought tolerant genes have been studied in the T_1 progeny of transgenic plants and the over expression of drought tolerance was found in the progeny. The promoters of these two genes were isolated through genome walking. The putative cis-acting elements involved in stress responses with special emphasis on water stress were found in both promoter regions. Promoters were analysed for their stress tolerance activity through agro-infiltration and transient GUS expression assays. Both promoters showed enhanced GUS expression when treated with dehydration, ABA, Salt and heavy metals stresses. On the basis of comparative studies of morphological and physiological characteristics of *G. arboreum*, the most drought tolerant variety was selected and cDNA libraries were constructed in order to carry out high throughput analysis of drought tolerant genes. Twelve thousand clones were picked, cultured and 1401 clones were sequenced and submitted to NCBI Genbank. Of these clones, 73 % did not show any homology with other genes in the NCBI data bank. Nine thousand four hundred and eight clones were spotted onto microarray slides and hybridized. Thirty seven (37%) ESTs were obtained that were up-regulated under drought stress. Moreover, wax mutants were developed by physical and chemical mutagens and confirmed by SEM and GCMS. cDNA libraries were constructed. Ten thousand clones were picked, cultured, 778 clones were sequenced and submitted to NCBI Genbank. Among those clones, 73 % did not show any homology with other genes in the NCBI data bank. Four Thousand seven hundred and two clones were spotted onto microarray slides and hybridized and 40 ESTs responsible for wax biosynthesis were identified. These studies will provide the number and nature of all genes that are implicated in stress tolerance and their response to alternation of stresses.

P31

Efforts on identification of new monosomic stocks of cotton, *G. hirsutum* L..

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We developed a new sets of cotton monosomics from common genetic background of highly inbred line L-458 (*G. hirsutum*) after irradiation of seeds or pollens in M_1, M_2 and M_3 generations. We studied cytological and morphological features of the cotton our monosomic lines and identified them by means of translocation tests using our translocation lines. We reported reduced stigma as a new phenotypic marker for cotton monosomics, which makes it possible to distinguish cytotypes without cytological analyses. In past, we identified eleven cotton monosomes by translocation tests using our 28 translocation cotton lines. In this study, we begin a new effort to identify our cotton monosomic lines using a well-defined tester-set of translocation lines from Cytogenetic collection of the USA, kindly provided by Dr. D.M. Stelly through USDA-Uzbekistan cotton germplasm exchange program. We observed specific features of tester-set translocation lines of USA collection in the growing condition of Uzbekistan such as existence of specific morphological characteristics of each line, low level of seed germination, sterility during self-pollination, dwarfism and absence of flowering organs. We crossed our monosomic stocks with tester-translocation lines to analyse monosomic-translocation hybrids. Currently, we are studying the chromosome pairing patterns to identify the monosomes. Translocation tests involving 12 monosomic lines of our collection have not yet revealed any homology with monosomes and the chromosomes involved in interchanges of the translocation tester-set. They showed detections of chromosome pairing with 23 bivalents plus one univalent plus one quadrivalent. Moreover, our analysis detected differences in frequency of the formation of the quadrivalents in hybrid monosomic plants derived from crosses of different monosomic and translocation lines. Furthermore, we created chromosome substitution lines through hybridization of our monosomic stocks with *G. barbadense* genotype (Pima 3-79) and developed 35 F_1 hybrids with different monosomic stocks. The subsequent generation hybrids are being analysed with a set of SSR markers, a priory associated with each specific chromosome of cotton. Our efforts toward this direction are discussed.

P32

Use of the cotton leaf curl satellite-like component as a silencing/expression vector.

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Alphasatellite formerly known as (DNA 1) is a satellite-like component associated with cotton leaf curl disease (CLCuD) that require betasatellite for symptom induction but it depends on DNA A for systemic movement. We converted alphasatellite into a gene-silencing vector (modified alphasatellite (ΔDNA 1)) by deleting its a-rich region and this does not affect the replication and movement of the component with the helper virus. This allowed the effect of the foreign gene insert to be easily recognized. Insertion into ΔDNA 1 of a transgene green florescence protein (GFP) resulted in the silencing of the cognate gene in *Nicotiana benthamiana*. The silencing persisted for more than one and half months and was associated with the decreased levels of mRNA of the targeted gene. This satellite-like DNA vector base form of virus induced gene silencing (VIGS) promises to be applicable to other begomovirus/alphasatellite systems, thereby providing the powerful approach to gene discovery and the analysis of gene function in malvaceous crops.

P33

Enrichment of cotton breeding with the development of marker-assisted selection (MAS) tools in Uzbekistan.

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Most agriculturally important elite cultivars have been produced using the conventional breeding methods: hybridization and backcross introgression of a trait of interest into breeding pool. After the genetically variable populations or lines have been developed through conventional approaches, they usually must undergo extensive selection cycles of plant breeding to test and select the variety for the trait of interest. However, the process of conventional crop breeding is costly and takes much efforts and time. The introduction marker assisted breeding or so called marker-assisted selection (MAS) is of critical importance in reducing the time for crop introduction and commercialization because development time of new crops have a large impact on the profitability of a new agriculturally important trait. In Uzbekistan, with the long-term objective of introducing and enriching the currently-applied traditional breeding approaches with more efficient modern MAS tools, we began marker assisted-selection efforts using molecular markers associated with important fibre traits. For this purpose, we selected 1) candidate SSR and gene-specific markers and 2) donor genotypes that were identified in our previous studies on QTL-mapping and LD-based association mapping through the evaluation of large number of diverse cotton germplasm resources. Briefly, we selected 23 major (micronaire, fibre strength and length, and elongation) fibre trait-associated SSR markers and several phytochrome specific candidate gene markers as a tool to control the transferring of QTL loci during a genetic hybridization. We also have selected 37 (11 wild race stocks and 26 variety accessions from diverse ecotypes) donor cotton genotypes that bear important QTLs for fibre traits. These donor genotypes were crossed with 9 commercial cultivars of Uzbekistan in various combinations with the objective of improving one or more of fibre characteristics of recipients. These 9 parental recipient genomes preliminarily were screened with our DNA-marker panel to compare with 37 donor genotypes. The polymorphic states of marker bands between donor and recipient genotypes were recorded. $F_{1\text{-}2}$ generation of hybrid plants from each crossing combination were tested using DNA-markers at the seedling stage and hybrids bearing DNA-marker bands from donor plants were selected for further breeding. The efforts 1) should assess the effectiveness of fibre trait-associated DNA-markers in a real breeding process and 2) should develop the MAS tools for our cotton breeders. Furthermore, we also are creating Nested Association Mapping populations (NAM) from the hybridization efforts in our diverse donor and narrow recipient genotypes that will be useful for fine mapping of the loci conditioning the major fibre traits in the future.

P34

Expression of critical genes during cell wall biogenesis.

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Cotton is one of the most important crops in the world, and over ninety percent of the value of cotton comes from its fibre; however, the genetic mechanism governing fibre development is poorly understood. Cotton fibre mutants have been useful in examining fibre development due to their biochemical and morphological diversity in fibre cells; therefore, using the Ligon Lintless (*Li-1*) mutant, a monogenic dominant cotton mutant characterized as having very short fibres, we employed the high throughput approaches of microarray technology and real time PCR to gain insights into what genes were critical during the secondary cell wall synthesis stage of cotton fibre development. Comparative transcriptome analysis of the normal TM-1 genotype and the near isogenic *Li-1* revealed that over 100 transcripts were differentially expressed at least 2-fold during secondary wall biogenesis, though the genetic profile of the expansion phase showed no significant differences in the isolines. Of particular note, we identified at least three candidate gene families; expansin, sucrose synthase, and tubulin whose expression in *Li-1* deviates from normal expression patterns of its parent, TM-1. These genes may contribute to retarded growth of fibres in *Li-1* since they are fibre-expressed structural and metabolic genes. Real time-PCR was used to validate selected genes from the microarray data. This work provides more details into the mechanisms of fibre development, and suggests the *Li* gene is active during the latter stages of fibre development.

P35

The genes related to cotton fibre development enhance resistance to abiotic and biotic stress in cotton and tobacco.

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By microarray analysis and mRNA sequencing, we found that many genes related to cotton fibre development were similar to the genes involved in the plants abiotic and biotic stress response. It may be that cotton fibre development has similar biological processes to stress responses. After producing some transgenic plants, we found that some cotton fibre related genes were actually involved in abiotic and biotic stress responses and the resistance of some transgenic cotton or tobacco to stress was enhanced. GbCaM1 was preferentially expressed in the elongating fibre. After treatment of the plants with the addition of 200 mM NaCl to the medium for 3 days, obvious differences could be observed between transgenic and non-transgenic plants. Lower leaves of RNAi transgenic cotton and the wild type became yellow and gradually fell, with the RNAi plants being more affected. But the over-expression (35S) plants had no obvious phenotype. GbTLP1 (thaumatin-like protein) is a gene related to the secondary cell wall synthesis stage in fibre. Transgenic tobacco constitutively over-expressing GbTLP1 showed enhanced resistance against different stress agents, particularly in its performance against *Verticillium dahliae* which was exceptional. Transgenic tobacco plants also exhibited considerable resistance against *Fusarium oxysporum* and some abiotic stresses including salinity and drought. Our results support the hypothesis that the genes related to fibre development may also enhance biotic and abiotic stress tolerance in transgenic plants.

P36

Structure and size variations between 12A and 12D homoeologous chromosomes based on high-resolution cytogenetic map in allotetraploid cotton.

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Cotton is a model system for studying polyploidisation, genomic organization, and genome size variation because the allotetraploid was formed 1-2 million years ago, which is old enough for sequence divergence but relatively recent to maintain genome stability. In spite of characterizing random genomic sequences in many polyploidy plants, the cytogenetic and sequence data that decipher homoeologous chromosomes are very limited in allopolyploid species. Here we reported comprehensive analyses of integrated cytogenetic and linkage maps of homoeologous chromosomes 12A and 12D in allotetraploid cotton using fluorescence in situ hybridization (FISH) and a large number of bacterial artificial chromosomes (BACs) that were anchored by SSR markers in the corresponding linage maps. Integration of genetic loci into physical localizations showed considerable variation of genome organization, structure, and size between 12A and 12D homoeologous chromosomes. The distal regions of the chromosomes displayed relatively lower levels of structural and size variation than other regions of the chromosomes. The highest level of variation was found in the pericentric regions in the long arms of the two homoeologous chromosomes. The genome size differences between A and D sub-genomes in allotetraploid cotton were mainly associated with uneven expansion or contraction between different regions of homoeologous chromosomes. As a novel approach and attempt for studying on the polyploidy homoeologous chromosomes, we showed macro-view (chromosomal) of genome organization, structure, and size variation between the homoeologous chromosomes in tetraploid cotton. These results are of general interest to the understanding and future sequencing of complex genomes in plant species.

P37

Anti-abiotic genes cloning and their identification in Chinese cotton germplasm.

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Soil salinisation has become a serious global problem affecting the agricultural development and the ecological environment. Cotton, the major cash crop in China, is playing a crucial role in national economic development. China, with less cultivated lands and more people, faces the contradiction between food and cotton, which seriously affects the cultivation and production of cotton. Therefore, an effective way to farm saline land and to enhance sustainable agricultural development is by developing salinity-tolerant varieties of cotton. Identification of salinity-tolerance also plays a vital role in cotton breeding. The abiotic-tolerant identification methods used previously, mainly based on morphological characters, were usually restricted for time-wasting and labour-costing, environmental influences, and seasonal restrictions. A new set of preliminary methods, called the SSR multi-marker salinity-identification method, was initially established to identify salinity tolerance of cotton by the standardisation of the whole process of seedling nurseries, DNA extraction, PCR amplification, amplification product detection, and marker-combination. Another 11 materials were used to validate this method, which showed a coincidence of 90.91% in consistency with the identification results based on a 0.4%NaCl identification method. This study proved that the multi-marker identification method could be used to assist identification of salinity tolerance in cotton germplasm. Two salt-tolerance related genes, H+-pyrophosphatase gene and S-adenosylmethionine synthetase gene, were cloned from the salt-tolerance material of *Gossypium hirsutum*, and were named *GhVP* and *GhSAMS*, respectively. Bioinformatic analysis was conducted. At the same time, Real-time PCR analysis, prokaryote expression vectors for expression of SAMS in *E. coli* were performed and constructed.

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Cloning and characterization of the phosphatidylinositol 4-kinase gene (*GbPI4K*) from *Gossypium barbadense*.

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Phosphatidylinositol (PtdIns) constitutes about 10% of total cellular phospholipids in all eukaryotic cells. In different combinations, three of the five free hydroxyl groups of PtdIns can be phosphorylated by several kinds of PI3K/PI4K-domain-containing proteins, including PI3Ks (phosphoinositide 3-kinases), PI4Ks (phosphoinositide 4-kinases), PI5Ks (phosphoinositide 5-kinases) and PLC (phospholipase C), and as a result, two important second messenger molecules of Ins(1,4,5)P3 and DAG (diacylglycerol) were generated. Recent studies revealed the important roles of phospholipid signalling pathways in multiple processes of higher plants, including root and pollen growth, vascular development and plant morphogenesis. Evidence in *Arabidopsis thaliana* root, soybean cell and *G. hirsutum* fibre revealed that PtdIns-dependent signalling pathway was regulated by a small GTPases of RAC/ROP, and influence was detected in the secondary cell wall thickening process. In metabolism pathway of PtdIns, the major precursor of PtdIns(4,5)P2 is PtdIns4P which was phosphorylated by PI4Ks from PtdIns. Therefore, PI4Ks were considered as gatekeepers for the production of most phosphoinositides in the PtdIns-dependent signalling pathway. Two major types of PtdIns4-kinase, II and III, had been detected in different plant tissues. The isolation and functional expression of a full-length plant PtdIns4-kinase cDNA was first reported in 1999, but up to present the majority of information of type II PI4Ks was derived from yeast and mammals, the minority from *Arabidopsis thaliana*, little is known about other plants. In *Arabidopsis thaliana*, PI4Ks were found participating polarized secretion of cell wall components in tip-growing root hair cells. And the double mutant PI4K1/PI4K2 displayed aberrant root hair morphologies. Identification of further proteins regulated by PtdIns4P is one of the points to a better understanding of the function of PtdIns 4-kinase. *Gossypium barbadense* has been highly valued for its high fibre quality, especially fibre strength. Fibre strength is mainly determined in secondary cell wall thickening stage. Based on a transcript-derived-fragment originated from QTLs mapping, we firstly cloned fibre strength related candidate gene of phosphoinositide 4-kinase cDNA, designated *GbPI4K*, and characterized its expression in the secondary cell wall thickening stage of *G. barbadense* fibres. The ORF of *GbPI4K* was 1926 bp in length and encoded a predicted protein of 641 amino acid residues. The putative protein contained a clear PI3_PI4_kinase catalytic domain and fell into the plant type II PI4Ks cluster in phylogenetic analysis. The expression of cotton PI4K protein was firstly induced in *E. coli* BL21 (DE3). Semi-quantitative RT-PCR analysis found that the gene expressed in cotton root, hypocotyl and leaf tissues. Real-time RT-PCR indicated that the gene in Sea Island cotton fibres had ten days longer of expression duration than that in Upland cotton fibres, and the main expression difference between *GbPI4K* and *GhPI4K* was located in the stage of the fibre secondary cell wall deposition. Further analysis suggested that PI4k could be a crucial factor in Rac-proteins function of regulating phospholipid signalling pathway.

P39

Transgenic cotton plants expressing a fungal phytase gene.

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Phosphorus (P) is one of the least available macronutrients restricting crop production in many ecosystems. Although multiple forms of P exist in soil, only the inorganic form (Pi) in soil solution is readily available for plant root uptake. However, 50-80% of the total P in agricultural soils exists in the form of organic phosphate, in which, about 60-80% is myo-inositol hexakisphosphate (phytate). Since plant can not directly absorb phytate-P directly, low-P availability becomes one of the limiting factors to plant growth. Cotton is one of the most economically important crops in the world. Low efficiency and imbalanced use of phosphate fertilizers are important factors that are responsible for low yield. Phosphorous deficiency in cotton plants is often expressed as dark-green leaves, abscission of buds and bolls, deep-purple markings on the lower leaves and low cotton fibre quality. Although previous studies have indicated that genotypes showed different behaviour at various P levels for a number of parameters and cotton root growth was affected by P fertilizer, improved P uptake by molecular genetic approaches has not been reported. Plant genetic transformation is a process whereby agronomically useful genes are directly introduced into important crops. It is an important tool to produce novel and genetically diverse plant materials. In the last few years, many transgenic cotton plants have been obtained by direct gene transfer, such as insect-resistant transgenic cotton and herbicide-resistant transgenic cotton. To increase phosphorus (P) acquisition efficiency, a phytase gene (*phyA*), isolated from the *Aspergillus ficuum* (patent number: 00102974.6), was introduced into cotton (*Gossypium hirsutum* L.) by *Agrobacterium*-mediated transformation in our laboratory. The transgene was driven by the root-specific promoter of the myrosinase (*pyk10*) gene. To ensure secretion of the expressed phytase into the extracellular space, an expression cassette was designed (*pyk10*-SP-*phyA*) where a carrot extracellular targeting peptide sequence was inserted between the promoter and the phytase coding region. Southern and Northern blot analyses showed that the *phyA* gene was successfully incorporated into the cotton genome and expressed in eleven cotton lines. After growing for 45 days in pots supplied with organic phosphorus (Po) as the only P source, the shoot- and root-dry-weight of transgenic plants all increased by nearly 2.0-fold than those of wild-type plants, and were similar to those of counterparts supplied with inorganic phosphorus (Pi). Phytase activity in root of the transgenic plants increased from 3.6- to 2.5-fold compared to the wild-type plants. Shoot P concentrations of the transgenic plants were significantly increased compared to the wild-type plants as well. Transgenic plants accumulated much higher concentrations of total P (up to 2.1-fold after 30 days of growth) than the wild-type plants with Po treatment. These findings clearly showed that the transgenic plant roots do secrete an extracellular phytase, and their ability to utilize P from phytate is markedly improved. And it also indicated that the phytase expression lines could be used for development of new cotton cultivars with improved phosphorus accessibility.

P40

Mapping of Sub-red mutant gene (Rs) in *Gossypium hirsutum* L..

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Sub-red mutant, used as a female parent, was crossed with GK19 to generate F_1 plants, which surpassed their parents with its excellent photosynthesis efficiency. In this sense, sub-red mutant would be regarded as a potentially excellent germplasm. Molecular markers linked to sub-red mutant gene are of great importance for fine mapping of the sub-red gene and its eventual cloning. To screen for SSR markers closely linked to sub-red gene in upland cotton, F_1 and BC_1 from intra-specific crosses were developed. Screening of 419 SSR markers, covering all the identified chromosomes and most linkage groups of cotton, was performed by bulked segregant analysis, revealing informative SSRs, which were afterwards mapped on the above populations. One co-dominant SSR marker BNL2634 was identified closely linked to the sub-red gene (abbreviated as *Rs*), with an estimated distance of 10.3 cM, depending on the population used. The other 4 molecular markers are CIR393, CIR335, BNL1597 and NAU1362, all allowing the *Rs* gene to be located on cotton chromosome 7. Furthermore, we built an F_2 generation containing 1214 individual plants from the sub-red mutant and Hai 7124 parent cross, and screened with 55 pairs of SSR and SRAP primers on chromosome 7. We found *Rs* is located between marker NAU3735 and NAU1048. The genetic distances between *Rs* and these two markers are 0.1 and 0.2 cM, respectively.

P41

Identification of differentially expressed miRNAs and their target genes between salt-tolerant and salt-sensitive cotton cultivars under salt stress.

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MicroRNAs (miRNAs) are a highly conserved class of small non-coding RNAs that regulate gene expression at the post-transcriptional level. In plants, miRNAs play important roles not only in multiple developmental processes, but also in the response to environmental stimuli. Salt stress is one of the important elements for reduced crop production. In order to adapt to high soil salinity, plants express a variety of genes to enhance their tolerance. However, the regulatory networks governing these genes are poorly understood. Cotton (*G. hirsutum*) is considered a relatively salt-tolerant non-halophytic species. Variations in salt tolerance have been observed among different cultivars. In this study, the salt-tolerant cotton cultivar (TOL cv.) SN-011 and the salt-sensitive cotton cultivar (SEN cv.) LM-6 were used to detect and annotate differentially expressed miRNAs involved in salt tolerance. We used a microarray to compare miRNA expression profiles between the two cotton cultivars. 15 miRNA homologs exhibited significantly different expression. Using their sequences as query in a homology search approach, 12 cotton miRNAs were identified in a cotton genome database. Among them, ghr-miR156d, ghr-miR159, ghr-miR167a, ghr-miR167b, and ghr-miR399b were newly identified. Using quantitative real-time RT-PCR, seven miRNAs were confirmed with a genotype-specific expression model in response to high salinity. They were grouped into three categories: (1) miR156a, miR156d, miR156e, and miR169 were dramatically down-regulated by salt stress in TOL cv., but were not affected in SEN cv.; (2) miR159 was more sustained in TOL cv., but was down-regulated in SEN cv.; (3) miR167a and miR399a were dramatically up-regulated in TOL cv., but were not affected in SEN cv.. These differentially expressed miRNAs could be of interest in determining the contrasting responses to salt stress of the two cotton genotypes. To gain insight into their functional significance, 23 target genes were further predicted from an mRNA database. Unigene entries were selected to examine their functional similarity. Ghi.13781, Ghi.16537, Ghi.18144, and Ghi.10300 were targeted by miR156. They encoded squamosa-promoter binding-like proteins (SPL) which are involved in early flower development and vegetative phase changes. Ghi.14555, Ghi.20503, Ghi.21682, and Ghi.2038 were targeted by miR167. They encoded Auxin response factors (ARF6/8) which can bind to auxin response promoter elements and mediate gene expression responses to auxin. Two miR169 targets, Ghi.713 and Ghi.9444, encoded heme activator protein (HAP2). Family members of SPL, ARF and HAP2 proteins have previously been verified as targets of miR156, miR167 and miR169, respectively. Quantification of the target transcripts revealed that these target genes showed significant inverse correlation with corresponding miRNAs in expression. Our results suggested that to enhance the salt tolerance of cotton, the down-regulated miR156 and miR169 enhance the amount of SPL and HAP2 transcription factors respectively, further with an ultimate operation on the physiological and morphological adaptation to salt stress. The salt-induced miR167 leads to the down-regulation of ARF and acts as a responsive component of auxin signalling pathways to enhance salt tolerance.

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CottonDB: An integrated repository resource for cotton

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CottonDB (http://cottondb.org), the first and the most comprehensive cotton genome database established by USDA-ARS, has been updated and extensively redesigned since 2006. The CottonDB project includes both a website and database creating a repository of information for germplasm, reference, personnel, pathology, and other accessions, over 480,000 gene, EST and contig sequences, many with genetic and physical maps, and DNA primer or probes. Over 1,100 Chromosome or Linkage Maps of AD, A, D, and G genomes (contains over 20,000 loci and over 1,000 QTLs) from over 40 map studies can now be viewed, compared, and downloaded via CMap Viewer. A BLAST server offers custom databases such as cotton ESTs, EST-SSRs, contigs, repeat sequences, and proteins. Through CottonDB Genome Browser Viewer, the comparisons among genetic and physical maps or between cotton and Arabidopsis genomes can be viewed and downloaded at nucleotide or peptide levels. The Quick Queries provides the query results dynamically for the most frequently asked queries. Other improvements include Cotton Breeder's Tool Box, a separate page that selected existing query results from Quick Queries for those most frequently asked queries by cotton breeders. CottonDB has been guided by comments and suggestions from our users, and we welcome further feedback as we continue to enhance its value as a resource for the cotton research community. CottonDB is a product of the USDA, Agricultural Research Service (ARS).

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Cotton genetic resources: their molecular diversity and association mapping in China.

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To enrich cotton genetic resources in China, a total of 8193 accessions were collected over the last 100 years, which made China ranked 4th in the world. Accessions of four cultivated species were kept in a seed bank, and the wild species grown in the Cotton Garden at Hainan Island. A cotton germplasm digital database was also established. 402 Simple sequence repeat (SSR) primers were used to evaluate the genetic diversity of 81 varieties. 197 SSR markers of the 402 were polymorphic and a total of 522 alleles were detected. The average number of alleles per locus was 2.69, ranging from 2 to 7. 112 primers amplified 2 alleles, which are more than half of primers with polymorphic. The average genetic diversity index was 0.3120 with a range of 0.0244 to 0.7724. In addition, the average Polymorphism information content (PIC) value was 0.2664 with a range of 0.0241 to 0.7385. These results indicated that the variation between cotton varieties was rich at the genome level but narrow in genetic basis. Association mapping performed using the general linear model of the software TASSEL indicated that many SSR markers were associated with traits ($P < 0.05$), which were consistent with the QTL mapping results using traditional family-based linkage method. This supports LD-based association mapping using diverse sets of cultivated cotton germplasm. Association analysis with combined data of four environments identified marker-trait associations ($P < 0.01$) for all the traits evaluated. A total of 61 marker-trait associations were identified with 43 different SSR markers.

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A comprehensive method to isolate genes responsive to Salt/Cold stresses from nucleotide resources in *Gossypium hirsutum*.

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Transcriptome analysis based on microarrays is a powerful tool for discovery and comprehensive evaluation of genes and has been widely applied in model organisms research. Though there are more than 20,000 Unigenes clustered from *Gossypium hirsutum* in GenBank and most of them isolated from fibre, few are well elucidated. For the purpose of making better use of these resources of nucleotide sequences, a data-mining method combined with verification by real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to identify putative genes responsive to salt and cold stresses from cotton. Totally, 826 genes up-regulated or down-regulated significantly in roots or leaves during salt/cold treatment in Arabidopsis were identified based on the compiled microarray database. In comparing to these 826 Arabidopsis genes annotated, 38 homologous expressed sequence tags (ESTs) from *G. hirsutum* were selected and their expression profiles were confirmed using real-time qRT-PCR. Among these 38 ESTs, 37 were responsive in abscisic acid treatment or salt/cold stresses responses in cotton. Totally, 14 genes and corresponding homologous ESTs were found with identical expression patterns in seedlings (combined with data from roots and leaves) between Arabidopsis and cotton. The expression pattern of about 55% of the genes (21 of 38 genes) was different in response to ABA between cotton and Arabidopsis; whereas >70% of genes had similar responses to cold and salt treatment. Among the 37 ESTs responsive to ABA or abiotic stress, the functions of 15 corresponding Arabidopsis genes are not characterized. This means novel genes involved in abiotic stress in cotton could be isolated through this method. According to these results, this approach appears effective in large-scale identification of cotton genes involved in abiotic stress and could be adopted to determine gene functions in various biologic processes in cotton and other organisms.

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