

~~of Cotton Produced in Zones Affected by Stickiness' sponsored by the Common Fund for Commodities. The project is completed, but the overall gain is estimated at US\$95 million. Sudan is working to find economical and environmentally viable uses of cotton stalks. For the purposes of lowering the cost of production, short duration varieties have been developed that save on fertilizer and insecticide use. Minimum and~~

~~selective use of insecticides compared to broad spectrum insecticides is paving the way for stronger biological control systems.~~

~~The Committee on Cotton Production Research of the ICAC decided to hold the 2012 Technical Seminar on the topic "Intellectual Property Rights and the Role of Private Breeders".~~

Role of Biotechnology in Sustainable Development of Cotton

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Abstract

The prospects of biotechnology to provide cost-efficient sustainable cotton production under a safe environment for the 21st century are enormous. The role of plant biotechnology in the improvement of cotton is a rapidly evolving area and very broad. The objective of this paper is to provide a report on three specific areas including transgenic technology, marker assisted selection and cotton genome sequencing with reference to the role of biotechnology in cotton improvement. The transgenic technologies in cotton are specifically targeted to overcome two major problems in cotton production: 1) the high cost of weed management and 2) the severe yield reduction caused by fruit-feeding lepidopteran insects, especially bollworm. The success story of transgenic technologies using *Bt* and herbicide tolerant genes in the improvement of cotton remains an outstanding scientific accomplishment. To improve the efficiency and delay the development of insect resistance to the Cry1Ac gene in cotton lines a pyramiding method of at least two or more genes. Cry2Ab, Cry1F and Cry1Ac genes have been applied in the second generation of *Bt* genes after 2009. The transgenic insect and herbicide resistant cotton lines have demonstrated the great potential of biotechnology in sustainable cotton production. Recent studies showed that the new emerging tool of RNAi technology will also have great impact in improving seed quality as a food source and also in providing resistance genes against pests and diseases including nematodes. Marker assisted selection (MAS) will provide breeders an efficient selection tool to augment traditional phenotypic selection with a gel-based DNA marker in the laboratory. The Simple Sequence Repeat (SSR) and Single nucleotide polymorphic (SNP) markers will be used as potential markers in MAS to expedite cotton breeding programs. Recently, a team of scientists from both public and private sectors initiated a partnership to put forward a report on the problems and prospects of cotton genome sequencing. Scientists at the University of Georgia, USA and at the Texas Tech University, USA have led multidisciplinary international teams, which are nearing completion of the sequence of the ancestral D and A genome diploid species respectively of upland cotton. Knowledge gained from decoding the cotton ge-

nome will improve understanding of genes at the molecular levels and help to unlock the mystery of genetics for improving yield and fiber quality. Considering both environmental and economical impacts of the new technologies and cotton as a major cash crop in both developed and developing countries it is high time to consider establishing an independent international cotton research center. The center should recommend the complex, system-oriented solutions based on a knowledge-intensive plan using new emerging biotechnology tools for global food and fiber security.

Agricultural biotechnology will play a major role to provide cost-efficient sustainable cotton production in a safe environment for the 21st century. The world population is projected to be over nine billion people by 2050 (Wakelyn and Chaudhry, 2010) and the rapid increase in population will require a doubling of the current production levels for global food and fiber security within the next four decades. Farmers will have to fulfill this demand under conditions of rapidly declining agricultural resources including land and water. Biotechnology will be at the forefront of agricultural invention and innovation to solve many of these challenges. The power of new technologies to accelerate agricultural development is nowhere more visible than cotton because of the success story of biotech cotton.

Cotton, *Gossypium* spp., is the most important natural fiber source for the textile industry and is a major cash crop in 70 countries including USA, China and India (Smith and Coyle, 1997). It is a source of many renewable products including textiles for clothing, home insulation materials to save energy, protein- and energy-rich animal feeds, human food in the form of cooking oil and efficient use of energy through the use of the mulch and biomass (Cotton Incorporated, 2010). Scientists are also exploring the future potential of cottonseed as an important food source for people due to its high nutritional value (Sunilkumar *et al.*, 2006).

Cotton is one of the important crops that hold incredible promise to enjoy the benefits of plant biotechnology. It is one of the few crops to enjoy the benefits of genetic engineering since the introduction of *Bt* cotton in 1996. Currently, biotech cotton is grown on over 60% of world cotton production

areas (Wakelyn and Chaudhry, 2010). The discussion on the role of plant biotechnology in the improvement of cotton is a rapidly evolving area and very broad encompassing basic and strategic research and its application. This paper will focus on three specific areas on the role of biotechnology in cotton improvement considering its great impact: 1) use of transgenic technology in economically and environmentally sustainable cotton production, 2) marker assisted selection to expedite cotton breeding programs, and 3) the future of cotton genome sequencing to unlock the secrets of genetics for the improvement of cotton.

Transgenic Technologies

Transgenic technology uses exogenous DNA or RNA sequences by recombinant DNA technology to create transgenic organisms that express novel and agriculturally useful traits. The transgenic technologies in cotton are specifically targeted to overcome two major limitations in cotton production: 1) high cost of weed management in cultivation due to slow cotton seedlings' growth compared to weeds (herbicide tolerance) and 2) the reduction in fiber yield due to severe infestation from fruit-feeding lepidopteran insects, specially bollworm (Hake, 2010).

A recent report estimated that the value of plant protection chemicals used at the global level is about \$32 billion per year and 16% of all global insecticides are used to protect cotton (Kranthi and Kranthi, 2010). Cotton's share in pesticide consumption has declined by about 43% from 1986 to 2009 since the introduction of genetically engineered *Bt* cotton (Wakelyn and Chaudhry, 2010). It has also been reported recently in a special issue of *Nature* that the use of *Bt* cotton helps to improve yield by over 60% of that of conventional varieties and avoids at least 2.4 million cases of pesticide poisoning in Indian farmers each year, saving US\$14 million in annual health costs (Whitfield, 2003). The success story of *Bt* genes in the improvement of cotton remains one of the most shining accomplishments in agriculture in the history of mankind. ICAC published several reports on the use of transgenic technologies in cotton from the time of introduction to the present from experts (ICAC 2000, ICAC 2004, Hake 2010, Kranti and Kranti, 2010). Many of the thoughts in this paper with reference to transgenic cotton are collected from these papers and a recently published book *Cotton: Technology for the 21st Century* by ICAC (Wakelyn and Chaudhry, 2010). Readers are encouraged to review these for detail references.

As of 2010, twelve countries including Argentina, Australia, Brazil, Burkina Faso, China, Colombia, India, Indonesia, Mexico, Pakistan, South Africa and United States have officially approved biotech cotton planting since its inception in 1995 (Hake, 2010). Over the past two decades, demand for increased yield forced farmers to use more insecticides in pest management. This caused more insect species to develop resistance to insecticides and as a consequence high levels of pest resistance, especially bollworm resistance, caused a crisis in cotton pest management (Kranti and Kranti, 2010). The United States of America was among the first to commercially

release *Bt* cotton incorporating the *cry1 Ac* gene (derived from soil bacterium *Bacillus thuringiensis*) in 1996. The *Bt* toxin expressed in biotech cotton protects the fruit from lepidopteran insects but the toxin is safe to all other non-target organisms including beneficial insects, birds, fish, animals and humans (Hake, 2010). It has been estimated that most of the cotton area in Australia, China, India, Mexico, South Africa and USA is under transgenic cotton now covering over 15 million hectares worldwide (Kranti and Kranti, 2010). However, the single gene *Bt* technology (Bollgard 1TM) registered with the U.S. Environmental Protection Agency has been voluntarily withdrawn due to considerations on the development of resistance to the toxin by selected insects (Dodds and Bond, 2010). To improve the efficiency and delay the development of insect resistance to *Cry1Ac* gene in cotton a strategy of pyramiding at least two or more genes like *Cry2Ab*, *Cry1F* and *Cry1Ac* has been applied to the second generation of *Bt* genes after 2009.

High cost of weed management was always a major concern to cotton growers. The discovery of glyphosate-resistant cotton technology helped in the development of transgenic cotton lines with enhanced tolerance to glyphosate, a herbicide used to control weeds in cotton fields. The first herbicide tolerant gene to the herbicide bromoxynil in cotton varieties, sold under the trade name BXN, was developed using the *bxn* gene from the natural soil bacteria *Klebsiella pneumoniae* subspecies *ozaenae* (Dodds and Bond, 2010). The plant containing this gene produces an enzyme which detoxifies bromoxynil to its primary metabolite. The transgenic cotton lines resistant to glyphosate were developed by incorporating the glyphosate-insensitive EPSPS enzyme gene from *Agrobacterium* spp. strain CP4 (Green, 2009). Glyphosate-resistant and second generation glyphosate-resistant cotton (Roundup Ready FlexTM) covered about 35% of total cotton acreage in USA and 80% of total cotton area in Australia in 2006 (Holtzapffel *et al.*, 2008; USDA-AMS 2008; Wreth *et al.*, 2008). Since 2009, almost all commercial transgenic cotton varieties in U.S.A. contain second generation of glyphosate-resistant technology, in addition to the stacked insect resistant *Bt* genes (Dodds and Bond, 2010). The transgenic insect and herbicide resistant cotton varieties have raised our expectations for a continued flow of scientific accomplishments in sustainable cotton production for the 21st century.

RNAi Technology in Cotton Improvement

Scientists in cotton research will soon witness the legacy of biotechnology in another new emerging area of RNAi technology. Recently, the development of a new technology in which a double-stranded RNA (dsRNA) is introduced into an organism to induce sequence-specific RNA interference (RNAi) of a target transcript has become a powerful tool to discover gene function (Serenella *et al.*, 2007). RNAi is an emerging technique based on homology-dependent post transcriptional gene silencing, induced by double stranded RNA (dsRNA). Recently, several papers have been published describing the merits of this method in plant sciences (Nui *et*

al., 2010; Sindhu *et al.*, 2009; Kranti and Kranti, 2010). A source of dsRNA must be introduced through transformation techniques into a plant's DNA to create transgenic plants with RNAi-mediated traits, which can pass those traits on to the next generation. Selective inactivation of the genes using encoded DNA through RNAi technology will have great potential in future cotton improvement due to its high specificity, stability and efficacy especially in the area of plant resistance against pests, pathogens and nematodes and improvement of seed quality.

For every one kg of fiber, the cotton plant produces about 1.65 kg of seeds, which are an important source of high quality protein (23%) and oil (21%), and cotton is the third largest field crop in terms of edible oil seeds in the world (Sunilkumar *et al.*, 2006). The amount of protein present in cotton seed grown worldwide can provide enough protein to feed 500 million people per year (Star Tribune, 2009). However, the seeds also contain gossypol glands that provide resistance against insects, but gossypol lowers blood potassium to dangerous levels in humans and can harm the heart and liver in people and animals (Star Tribune, 2009). Cotton seed is used primarily as animal feed because the bovines' stomachs gradually digest the poisonous gossypol rendering it harmless to the animal. Recently Dr. Keerti S. Rathore's group at the Texas A&M University used the RNAi technology and made a breakthrough discovery to develop cotton plants eliminating gossypol production in the seed, leaving gossypol production to continue in stems, leaves, and flowers to protect the plant against insects (Sunilkumar *et al.*, 2006). This discovery provides a new tool for the use of cotton seeds as an important source of food products.

The RNAi technology also has great promise in controlling pests and pathogens in cotton. Researchers have discovered potential genes that are lethal for reproduction or fitness when silenced to free living nematodes. Scientists showed that silencing four genes from parasitic nematode through RNAi technology led to a reduction in the number of mature nematode females in transgenic *Arabidopsis thaliana* (Sindhu *et al.*, 2009). RNAi-mediated plant resistance has greater potential over conventional *Bt* resistant transgenic cotton plants (Niu *et al.*, 2010). For example, many pests and pathogens share distinct lineages and homologues of important genes, so silencing the appropriate target genes may provide resistance against a broad group of multiple pests or pathogen organisms. Also the resistance is more stable because it is based on RNA hybridization on few nucleotides rather than protein-protein interaction and the potential of mutation to impede RNA hybridization is less (Escobar *et al.*, 2001). So there will be less potential of pests overcoming the resistance. Theoretically all pests and pathogens carry genes with detrimental knockdown phenotypes, and identifying these target genes will provide a scope to use RNAi technology to make transgenic plants resistant against these pests and pathogens promoting eco-friendly crop protection methods (Niu *et al.*, 2010). One of the difficulties in RNAi technology is to identify genes that can be effective through a suitable delivery system. For ex-

ample, dsRNA rapidly breakdown in the digestive system of mammals and fail to uptake RNA into cells.

Although the transgenic technologies provide enormous benefits to produce higher agricultural yields with less resources and less environmental impact, sweeping adoption of these techniques without appropriate regulation concerns many on its potential hazardous environmental and health impacts. This is especially important considering that many countries have not developed appropriate safety and regulatory policies. As a consequence, many countries face new problems with pest management. For example, new sucking pests have emerged as major pests in India due to low use of insecticides causing significant economic losses to cotton production (Kranti and Kranti, 2010). It is important to practice integrated pest and weed management with transgenic cotton varieties using proper regulatory control for cost-effective sustainable cotton production. For example, the widespread use of glyphosate-resistant technology has led to a shift to some weed species that are tolerant to glyphosate in the USA (Holtzapffel *et al.*, 2008). Weeds can develop herbicide resistance due to selection pressure by excessive use of herbicides applied to the population. *Palmer amaranth* is one of the most important herbicide-resistant weeds found in the US cotton fields (Bennett, 2007). There is a possibility that cross contamination of pollen can also transmit the herbicide resistant trait to the wild relatives located in the same areas of cultivated cotton.

Marker Assisted Selection to Expedite Cotton Breeding Programs

The principle of plant breeding is based on the selection of desirable traits and assembling more desirable combinations of traits in a specific plant. Marker assisted selection (MAS), a tool in plant biotechnology, provides breeders an efficient selection system to replace traditional phenotypic-pedigree-based selection with a gel-based DNA marker in the laboratory. MAS is an indirect selection process where a trait of interest is selected not based on the trait itself but on a DNA marker linked to the trait of interest. Most of the economical important traits in cotton are controlled by complex quantitative trait loci (QTL) consisting of many genes affecting the phenotypes. DNA markers are 'landmarks' at the genome that can be selected for their close proximity to a QTL of interest. The selection of DNA markers linked to the QTL of interest increases the efficiency of breeding, decreasing costly, lengthy and subjective phenotypic selection and accordingly reducing significantly backcross generations.

MAS will have great potential in the following areas of cotton breeding program: 1) marker assisted pyramiding, 2) marker assisted backcrossing, 3) study of heterosis, 4) assessment of genetic diversity and parental selection, 5) cultivar identity and assessment of seed purity, and 6) marker assisted evaluation of breeding materials (Bertrand and Mackill, 2008). MAS will be very effective in cotton when breeders will use it in early generations because plants with undesirable gene combinations can be discarded and a lesser number of high-priority lines can be used in subsequent generations. Also if the

linkage between the marker and the selected QTL is not very tight, the greatest efficiency of MAS is in early generations due to the increasing probability of recombination between the marker and QTL. The major disadvantage of applying MAS at early generations is the cost of genotyping a larger number of plants in the population (Bertrand and Mackill, 2008).

Identification of informative DNA markers is the first critical step to develop a marker assisted breeding program and to expedite the variety development program. The major limiting factor in the use of DNA markers is the limited number of informative markers useful for MAS in cotton. The success of MAS is based on the information on the association of the markers with the traits of interest based on a molecular map. Most of the molecular maps in cotton are based on recombination map of a population developed from the crosses of specific parents of interest in a program. However, it has been reported that QTLs identified in a particular mapping population may not be effective in different genetic backgrounds (Liao *et al.*, 2001). Some collaborative studies to develop molecular methods of association mapping are reported by Abdurakhmonov *et al.* (2008 and 2009). This novel method of association mapping strategy provided a statistically more powerful tool in molecular map of cotton because it is based on the survey of large number of populations compared to the most commonly used recombination mapping method based on a few selected individual crosses. This is the first report on the use of association mapping strategy in cotton to the discoveries of: 1) genetic diversity in several hundreds cotton lines from Uzbekistan based on large number of molecular markers for fiber and agronomic traits, 2) the genome-wide linkage disequilibrium (LD) value in cotton genome, and 3) association of several DNA markers with important fiber and agronomic traits and their chromosomal locations. This research helped the geneticists to “mine” useful genes among large number of populations for germplasm enhancement. Currently, we are using MAS based on our association mapping study selecting the DNA marker from the donor parents associated with the improved trait of interest for improving fiber quality traits in some Uzbek cultivars. The study demonstrated that successful application of genetic association analysis using large number of populations will accelerate the discovery rate of gene/QTL and informative useful markers in cotton.

Selection of suitable markers is one of the key factors for the success of a MAS program and it must be based on a simple and efficient detection system, highly polymorphic and distributed across the genome. SSR and SNP markers are considered as the marker of choices for MAS in many crop species. Recently scientists have created the Cotton Microsatellite Database (CMD) [<http://www.cottonssr.org>] with the support from Cotton Incorporated (Blenda *et al.*, 2006). This is a curated and integrated web-based relational database providing centralized access to publicly available cotton microsatellites, an important resource for basic and applied research in cotton breeding. At present CMD contains publication, sequence, primer, mapping and homology data for nine major cotton microsatellite projects, collectively representing more

than 3,000 microsatellites. In addition, Monsanto also donated about 4,000 cotton SSR markers and associated information to Texas AgriLife Research, an agency of the Texas A&M System, in 2009 (Xiao *et al.*, 2009).

SNP marker discovery opens up a new paradigm in MAS especially considering many publicly available gene sequences now in GenBank. Single nucleotide polymorphic markers (SNPs) are normally associated with many candidate genes. Discovery of the SNP marker is very difficult in a polyploid species like cotton. It is like solving two jigsaw puzzles at the same time, because cotton has two duplicated sets of chromosomes. Many of the seed industries are now using SNP markers as a marker of choice in MAS in corn and other crops. However, there is almost no such information available in public cotton databases. Recently we discovered a strategy to identify SNP markers in tetraploid cotton species (An *et al.*, 2007, 2008; Buriev *et al.*, 2010, 2011). SNP markers are normally biallelic. However, detection of haplotype is essential to detect multiple alleles based on unique sets of SNP markers in a candidate gene. It is a difficult task to identify a haplotype that could distinguish allelic differences at a single locus in a polyploid species like cotton because of the presence of duplicated loci. Researchers used cluster analysis of the sequences from *G. hirsutum* and the diploid A and D genome ancestral species from a candidate gene of interest using Neighbor Joining clustering method and have grouped the tetraploid sequences into two subgenomes based on their association with the diploid ancestral species. The sequences of tetraploid genotypes from an individual clade of the phylogenetic tree were aligned and compared to detect the putative SNPs. The unique combination of SNPs in a sequence within a clade of the phylogram was considered as haplotype. Each clade in the dendrogram is considered as a putative locus. The hypothesis was based on the assumption that sequences at each locus will be more similar compared to the sequences between the loci. Sometime this strategy of identifying haplotype based on clustering analysis without prior genetic knowledge may separate significantly different alleles of a locus into two different clades, thus misrepresenting allelic differences as locus differences. Such a condition may fail to identify some of the true SNPs. Our strategy provided a very conservative estimate of putative SNPs in *MIC-3* gene family (Buriev *et al.*, 2010). The advantage of this method is the reduced number of false SNP in the analysis by avoiding the problem of comparison among the orthologue and paralogue sequences. This discovery of SNP markers was further confirmed using deletion lines to identify their chromosomal locations (Ann *et al.*, 2007, 2008; Buriev *et al.*, 2010, 2011).

SNP markers will provide a tool to associate candidate genes in MAS in cotton molecular breeding programs. Such discoveries providing the knowledge of candidate genes associated with complex traits will also have an indirect effect to explore the possibilities of genetic manipulation of the specific candidate genes to improve important traits. SNP markers discovery will have great impact in MAS of cotton breeding program.

Cotton Genome Sequence

Decoding the cotton genome is essential for efficient use of genomic technologies in the improvement of upland cotton. Recently, a multidisciplinary international team including scientists from both public and private sectors initiated a partnership as a community to put forward a report on the problems and prospects of cotton genome sequencing (Chen *et al.*, 2007). Readers are encouraged to study the report of Chen *et al.* (2007) for detail reference on cotton genome sequencing. This paper will summarize some of the key information from this report.

Cotton is enriched with many available genomic resources including bacterial artificial chromosomes (BACs), ESTs, linkage maps, and integrated genetic and physical maps for sequence analysis and assembly (Chen *et al.*, 2007). Sequencing cotton genomes will unveil the relationship of functional genome and agronomic performance, significance of polyploidy and genome size variation within the *Gossypium* genus.

The haploid cotton genome sizes are estimated to be approximately 880 Mb for *G. raimondii*, approximately 1.75 Gb for *G. arboreum*, and approximately 2.5 Gb for *G. hirsutum* (Chen *et al.*, 2007). It is essential to develop a comprehensive strategy for cotton genome sequencing based on economics, technology, and priorities. Due to continuing progress in high throughput sequencing technology and cost reduction, multiple and parallel approaches can be used to reveal complete genome information of *Gossypium* genomes. It is critical to have a comprehensive strategy for complete sequencing of one or more representatives of each A, B, C, D, E, F, G, K, and a tetraploid-derived AD ($n = 26$) *Gossypium* genome group to understand the complexity at the molecular level in the evolution of cotton genome.

Recently, scientists at the University of Georgia under the leadership of Dr. Andrew Patterson and at Texas Tech University under the leadership of Dr. Thea Wilkins have led the multidisciplinary international teams that are nearing completion of the sequence of the ancestral D and A genome diploid species respectively of upland cotton. Recently a report indicated that a joint venture of Monsanto Company (NYSE:MON) and San Diego-based Illumina Inc. (NASDAQ: ILMN) will help to unveil the cotton genome sequence using Illumina's next generation sequencing technology (Monsanto web page, 2010). The report also stated that the two companies have completed sequencing a wild Peruvian cotton species, *Gossypium raimondii*, and will donate their findings to the public. The U.S. Department of Energy's Joint Genome Institutes (<http://www.jgi.doe.gov/>) has taken a major step to support as a pilot study for shotgun sequencing of *G. raimondii* for a $0.5\times$ coverage (Chen *et al.*, 2007). USDA/ARS scientists are planning to sequence tetraploid cotton genome using a BAC-based sequencing approach in collaboration with scientists of the Anyang Cotton Research Institute in China (personal communication).

The AD genome sequence may offer superior opportunities to elucidate the types and frequencies of changes that distinguish

a polyploid cotton from a diploid cotton. Sequences from A and D genome diploid species will be very helpful in tetraploid AD genome sequence assembly and will provide valuable information in gene content and expression patterns and polyploid genome evolution. Sequencing representatives from each diploid clade will be important to understand molecular patterns and biological events associated in evolution including the genomic and morphological diversity within the genus to adapt to a wide range of ecosystems in warmer and arid regions of the world (Chen *et al.*, 2007). Knowledge gained from decoding the cotton genome will improve our understanding of gene function and ultimately benefit the growers with improved yield and fiber quality. Information from cotton genome sequencing will be very helpful to develop tools for making cotton plants resistance against biotic and abiotic stresses.

Due to the globalization of agriculture it is expected that commodity prices are likely to decline and efficient production will be the key factor in the competitive world market. Accordingly researchers may have to target manipulating the genetic system using biotechnology tools to adopt cotton plants in environmental diversity, making the most out of different natural resources including limited water supply - rather than using costly inputs to change the environment.

Cotton is also a major cash crop in many developing countries, which are the source of rapid population growth and environmental degradation and some farmers cannot afford to adopt the high-input packages of biotechnology. However, we live in an interconnected global village and failure to capture the benefits of biotechnology will have ripple effects all over the world in the future. But the challenges are not only biological - they are also institutional, financial, political and social. Considering both environmental and economical impacts of the new technologies, cotton as a major cash crop in both developed and developing countries, the role of private industries in new technologies and the complex agricultural problems in different countries perhaps it is high time to consider establishing an independent international cotton research center. It is essential to face these challenges for 21st century through a strong research partnership between public and private institutions. This international center will serve as a facilitator who can negotiate appropriate arrangements between the public and private sectors as catalysts in such partnerships. This center will make sure the benefits of tomorrow's breakthrough discoveries are shared properly in both developed and developing countries. This center should study the benefits of relative investments in favorable versus marginal environments in cotton because many cotton farmers in the world live in marginal areas cannot afford the high input packages of the biotechnology. Based on this study the center can develop ecologically friendly principles such as crop rotation, intercropping, and crop management systems to local conditions to maximize the benefits of biotechnology. This center will set up a regulatory system based upon sound science and agro-ecological factors of the specific country, develop cost-effective methods for quarantine or regulatory

purpose and assisting farmers in the detection of transgenic purity, an efficient mechanism of transgenic seed delivery. It is essential to develop a strategy to counteract the negative attitude towards the transgenic cotton. The center should recommend the complex, system-oriented solutions based on knowledge-intensive plan than just the simpler seed-centered technologies. The success story of biotechnology in cotton raised our expectations for a continued flow of scientific miracles to promote sustainable cotton production under a safe environment for the 21st century.

References

- Abdurakhmonov, I. Y., R. J. Kohel, J. Z. Yu, A. E. Pepper, A. Abdullaev, F. N. Kushanov, I. B. Salakhutdinov, Z. T. Buriev, S. Saha, B. Scheffler, J. N. Jenkins and A. Abdukarimov. 2008. Genome-wide linkage disequilibrium (LD) and LD-mapping of fiber quality traits in Upland cotton. *Genomics* 92:478-487.
- Abdurakhmonov, I. Y., S. Saha, J. N. Jenkins, Z. T. Buriev, S. E. Shermatov, B. E. Scheffler, A. E. Pepper, J. Z. Yu, R. J. Kohel and A. Abdukarimov. 2009. Linkage disequilibrium based association mapping of fiber quality traits in *G. hirsutum* L. variety germplasm. *Genetica* 136:401-417.
- An, C., S. Saha, J. N. Jenkins, B. Scheffler, T. A. Wilkins and D. M. Stelly. 2007. Transcriptome profiling, sequence characterization, and SNP marker based chromosomal assignment of the *EXPANSIN* genes in cotton. *Molecular Genetics Genomics* 278:539-553.
- An, C., S. Saha, J. N. Jenkins, D-P Ma, B. E. Scheffler, R. J. Kohel, J. Z. Yu and D. M. Stelly. 2008. Cotton (*Gossypium* spp.) R2R3-MYB Transcription factors, SNP identification, Phylo-genomic characterization, chromosome localization and linkage mapping. *Theoretical Applied Genetics* 116:1015-1026.
- Bennett, D. 2007. Ian Heap helps keep tabs on global weed resistance. In Delta Farm Press, January 17, 2007 at <http://deltafarmpress.com/ian-heap-helps-keep-tabs-global-weed-resistance> (verified on August 22, 2011).
- Bertrand, C., Y. Collard and David J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions Of The Royal Society Biological Sciences*. 363(1491): 557-572.
- Blenda, A., J. Scheffler, B. Scheffler, J. Lacape, J. Z. Yu, S. Jung, M. Staton, M. Palmer, C. Jesudurai, S. Muthukumar, P. Yellambalase, S. Ficklin, R. Eschelman, M. Ulloa, S. Saha, D. Feng, R. Cantrell and D Main. 2006. CMD: A cotton microsatellite data base resource for *Gossypium* genomics. *BMC Genomics* 7:132.
- Buriev, Z. T.*, S. Saha*, I. Y. Abdurakhmonov*, J. N. Jenkins, A. Abdukarimov, B. E. Scheffler, and D. M. Stelly. 2010. Clustering, haplotype diversity, and locations of *MIC-3*, a unique root-specific defense-related gene family in upland cotton (*Gossypium hirsutum* L.). *Theoretical Applied Genetics* 120:587-606. (*equally credited as first author)
- Buriev, Z. T.*. S. Saha* J. N. Jenkins, A. Abdukarimov, D. M. Stelly and I. Y. Abdurakhmonov*. 2010. Birth-and-death evolution of clustered *MIC-3* (*M*eloidogyne *I*nduced *C*otton - 3) supergene family of *Gossypium* species under purifying selection and gene amplification. *Molecular Biology and Evolution*. *Theoretical Applied Genetics* DOI 10.1007/s00122-011-1672-y. (online, *equally credited as first author)
- Chen, Z.J., B.E. Scheffler, E. Dennis, B.A Triplett, T. Zhang, W. Guo, X. Chen, D.M Stelly, P.D Rabinowicz, C.D Town, T. Arioli, C. Brubaker, R.G Cantrell, J.M. Lacape, M. Ulloa, P. Chee, A.R. Gingle, C.H. Haigler, R. Percy, S. Saha, T. Wilkins, R.J. Wright, A. Van Deynze, Y. Zhu, S. Yu, I. Abdurakhmonov, I. Katageri, P.A. Kumar, Mehboob-Ur-Rahman, Y. Zafar, J.Z. Yu, R.J. Kohel, J. Wendel and A.H. Paterson. 2007. Toward sequencing cotton (*Gossypium*) genomes. *Plant Physiol* 145:1303-1310.
- Cotton Incorporated. 2010. Cotton Today. At <http://cottontoday.cottoninc.com/sustainability-about/responsible-economic-development/> (verified Jul. 20, 2011).
- Cranston, J. J., J. D. Rivera, M. L. Galyean, M. M. Brashears, J. C. Brooks, C. E. Markham, L. J. McBeth and C. R. Krehbiel. 2006. Effects of feeding whole cottonseed and cottonseed products on performance and carcass characteristics of finishing beef cattle. *Journal of Animal Science* 84:2186-2199.
- Dodds, M. D. and J. A. Bond. 2010. Cotton weed pests and their control in the 21st century. Pp. 123-154. In: P. J. Wakelyn and M. R. Chaudhry (eds). *Cotton: Technology for the 21st Century*. Publ. International Cotton Advisory Committee, Washington, DC, USA.
- Escobar MA, E.L. Civerolo, K.R. Summerfelt, A.M. Dandekar AM. 2001. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. *Proc. Natl. Acad. Sci. USA*, 98: 13437-13442.
- Green, J. M. 2009. Evolution of glyphosate-resistant crop technology. *Weed Science* 57:108-117.
- Hake, K. D. 2010. Cotton biotechnology: Current and future applications. In: P. J. Wakelyn and M. R. Chaudhry (eds). *Cotton: Technology for the 21st Century*. Publ. International Cotton Advisory Committee, Washington, DC, USA.
- Holtzapffel, R., O. Mewett, V. Wesley and P. Hattersley. 2008. Genetically modified crops: tools for insect pest and weed control in cotton and canola. Australian Gov. Bureau of Rural Sciences, Canberra, http://www.daff.gov.au/_data/assets/pdf_file/0004/908266/GM-crops-insect-pest-weed-control-261108.pdf. (verified Mar. 17, 2009)
- International Cotton Advisory Committee (ICAC). 2000. Report of an expert panel on biotechnology in cotton. International Advisory Committee, Washington, DC, USA. <http://www.icac.org/> (verified Aug. 17, 2011)
- International Cotton Advisory Committee (ICAC). 2004. Report of second expert panel on biotechnology in cotton. International Advisory Committee, Washington, DC, USA. <http://www.icac.org/> (verified Aug. 17, 2011)
- Kranthi, K. R. and S. Kranthi. 2010. Cotton insects pests and their control in the 21st century. Pp. 99-122. In: P. J. Wakelyn and M. R. Chaudhry (eds.). *Cotton: Technology for the 21st Century*. Publ. International Cotton Advisory Committee, Washington, DC, USA.
- Liao C.Y, Wu P, Hu B, Yi K.K. Effects of genetic background and environment on QTLs and epistasis for rice (*Oryza sativa* L.) panicle number. *Theor. Appl. Genet.* 2001;103:104-111.
- Monsanto web page. 2010. Monsanto and Illumina Reach Key Milestone in Cotton Genome Sequencing. At <http://www.monsanto.com/newsviews/Pages/Monsanto-Illumina-Key-Milestone-Cotton-Genome-Sequencing.aspx>. (verified on August 22, 2011).

- Niu Jun-Hai, H. Jian, J. Xu, Y. Guo, and Q. Liu. 2010. RNAi technology extends its reach: Engineering plant resistance against harmful eukaryotes. *African Journal of Biotechnology*. 9(45): 7573-7582.
- Parakhi, V., V. Arkhi, V. Kumar, G. Sunilkumar, L. Campbell, M. Sing and K. S. Rathore. 2009. Expression of apoplastically secreted tobacco osmotin in cotton confers drought tolerance. *Molecular Breeding* 23:625-639.
- Reiser, R. and H. C. Fu. 1962. The mechanism of gossypol detoxification by ruminant animals. *Journal of Nutrition* 76:215.
- Sernella, A. S., J. McCuiston, Mui-Yun Wong, X. Wang, M.R. Thon, R. Hussey, T. Baum and E. Davis. 2007. Qualitative detection of double-stranded RNA-mediated gene silencing of parasitism genes in *Heterodera glycines*. *J. Nematology*. 39(2): 145-152.
- Sindhu, A.S., Tom R. Maier, Melissa G. Mitchum, Richard S. Hussey, Eric L. Davis and Thomas J. Baum. 2009. Effective and specific in planta RNAi in cyst nematodes: expression interference of four parasitism genes reduces parasitic success. *Journal of Experimental Botany*, Vol. 60, No. 1, pp. 315-324, 2009.
- Smith, C. W. and G. G. Coyle. 1997. Association of fiber quality parameters and within-boll yield components in upland cotton. *Crop Science* 37:1775-1779.
- Star Tribune. 2009. TEXAS: Genetic engineering turns 'Fabric of Our Lives' into edible cottonseed that may feed millions. In: *Bites*. Also available at <http://bites.ksu.edu/news/137208/09/12/03/texas-genetic-engineering-turns-fabric-our-lives-edible-cottonseed-may-feed-mil>. (verified Aug. 18, 2011)
- Sunilkumar G., L.M. Campbell, L. Puckhaber, R. D. Stipanovic†, and K. S. Rathore. 2006. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *PNAS*. 103(48):18054-18059.
- United States Department of Agriculture (USDA). 2008. Agricultural chemical usage-2007 field crops summary. <http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC/2000s/2008/AgriChemUsFC-05-21-2008.pdf>. (verified Mar 11, 2009)
- Wakelyn, P. J. and M. R. Chaudhry. 2010. Introduction. Pp. 1-2. In: P. J. Wakelyn and M. R. Chaudhry (eds.). *Cotton: Technology for the 21st Century*. Publ. International Cotton Advisory Committee, Washington, DC, USA.
- Werth, J. A., C. Preston, I. N. Taylor, G. W. Charles, G. N. Roberts and J. Baker. 2008. Managing the risk of glyphosate-resistance in Australian glyphosate-resistance cotton production system. *Pest Management Science* 64:417-421.
- Whitefield, J. 2003. Transgenic cotton a winner in India. *Nature* | doi:10.1038/news030203-12.
- Xiao, J., K. Wu, David D. Fang, David M. Stelly, John Yu, and Roy G. Cantrell. 2009. New SSR markers for use in cotton (*Gossypium* spp.) improvement. *J. Cotton Sci.* 13:75-157.

~~Technological Innovations for Sustainable Development of the Cotton Value Chain in Brazil~~

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~~Cotton production in Brazil had a change of regions in the last 25 years. From a production based in small areas in the Northeast, where perennial cotton with low use of inputs and lint yield of about 90 kg/ha was cultivated, and small areas in the South and Southeast, where upland cotton was planted and better technologies and inputs were used, today cotton is planted on large farms in the mid-west with high use of technology and inputs. The average lint yield is about 2,000 kg/ha in the main production regions of the Brazilian mid-west.~~

~~The Northeast region had a cotton area of about three millions hectares in 1983 planted under semi-arid conditions. All area was planted with perennial cotton and input use was almost none. The labor system was based on large farms distributed to small farmers who shared one-half or one-third of production with the owner of the farm. After the adoption of new labor laws, this system had to be abolished and the small producers had to work for themselves on their farms. Those producers who had no farms migrated to the cities in search of alternative work. Moreover, the arrival of the boll weevil in the region forced growers to spray large quantities of insecticides in a production system that did not use any inputs. These changes resulted in the end of cultivation of cotton on large farms in semi-arid region of Northeast Brazil.~~

~~The rebirth of cotton planting in another region of Brazil, and the technological advances of Brazilian cotton, are like a building held by many hands. The joint efforts were needed to overcome challenges of an unknown region for the majority of the producers. Since the first plantings in the early 1980s to the present day, numerous challenges have been overcome and others will be met. It was possible to overcome these challenges only through joint efforts of producers with business vision and enterprising spirit and state governments through direct or indirect incentives and research institutions. The private foundations like Mato Grosso Foundation, Goiás Foundation, Bahia Foundation and Chapadão Foundation and Federal Government agencies such as the Brazilian Agriculture Research Corporation - Embrapa played their role.~~

~~The first technological advances that resulted in what is now cotton in the Brazilian Cerrado were the selection of cultivars adapted to the region and discovery of the best time for planting. The first cultivar was developed from a partnership between Embrapa Cotton and the Itamaraty Group. The Itamaraty Group had a breeding program that resulted in the development of cultivars like CNPA ITA 90, responsible for raising cotton production in Mato Grosso, the main producing state until the present day.~~