



Highlights of the World Cotton Research Conference-6

**Goiânia - Goiás, Brazil,
May 2 - 6, 2016**

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The World Cotton Research Conference-6 (WCRC-6) and 2016 Biennial Conference of the International Cotton Genome Initiative were held in conjunction in the city of Goiânia, Brazil, from May 2-6, 2016. It was a great opportunity for researchers to present their research, expand and strengthen their networking and learn about the most important research work being done in the world. The Cotton Growers Association of Goiás-AGOPA (Associação Goiana dos Produtores de Algodão-AGOPA) served as the primary host. AGOPA is affiliated to the Brazilian Association of Cotton Producers-ABRAPA (Associação Brasileira dos Produtores de Algodão) and is one of the nine cotton producer associations in the country. The associations work to promote the profitability of the cotton sector, through unification and organization, in order to foster the sustainable production of cotton. The cotton research program 'EMBRAPA Cotton' of the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária-EMBRAPA) played a crucial role in designing the technical program and executing it during the Conference.

The ICAC takes pride in organizing the world cotton research conferences. The ICAC Secretariat coordinated international sponsorships and worked with the Organizing Committee, Program Committee, International Cotton Researchers Association and leadership of the International Cotton Genome Initiative (ICGI) to shape a successful conference and to ensure a clear understanding among various organizers of the WCRC-6. For the first time the Conference was held under the auspices of the International Cotton Researchers Association (ICRA), whose chairman headed the International Committee that selected keynote and plenary speakers. The International Committee advised the Organizing Committee and Program Committee when needed. The roles of various institutions were well defined, but the active involvement of the ICAC as a neutral and umbrella organization and to provide guidance based on experiences with the previous world cotton research conferences was still required.

The program included two keynote speakers and eight plenary speakers with 28 specialized concurrent sessions. A number of sessions were devoted to genomic research in order to follow the biennial conference format of the ICGI meetings.

Sponsorship of WCRC-6

The ICAC has sponsored the world cotton research conferences since their inception in the early 1990s. The Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), of France, has sponsored the

conferences since 1998 and continued its support for the WCRC-6. The Food and Agriculture Organization of the United Nations (FAO) has also provided support to world cotton research conferences, including the WCRC-6. For the first time, CABI also provided sponsorship to the Conference. Support from the private sector in each host country was a tremendous asset in the success of the WCRC-6, as was the case in previous Conferences. The ICAC has provided major sponsorship to all WCRCs, in addition to staff time and services. For the first time, the international sponsorship was channeled through the International Cotton Researchers Association (ICRA). Sponsorships were pooled and formally advertised through the ICAC and ICRA web pages and mailing lists. Four kinds of sponsorship were made available.

- Air ticket;
- Hotel and registration fee;
- Cash support (US\$1,000); and
- Registration fee.

Applications were received at the ICAC, on behalf of ICRA, up to November 15, 2015. The Executive Committee of ICRA met in Mumbai, India, on December 6, 2015, and finalized the names to be sponsored. In total, 36 researchers received four kinds of sponsorship. The Organizing Committee also decided to sponsor 10 researchers for registration, accommodation and technical tour. These 10 names were also taken from the ICRA applicants. Eighty-two researchers applied to ICRA for sponsorship, of which 46 were sponsored. In addition, ICAC also sponsored researchers through the ICAC Research Associate Program and the Southern and Eastern African Cotton Forum (SEACF). For the Research Associate Program, which ICAC conducts every year, researchers usually come to the ICAC headquarters for 10 days. However, during the years of WCRCs, funds are used to partially sponsor researchers to attend the conference. ICAC selected 16 researchers from 10 countries to attend WCRC-6 and receive sponsorship under the Research Associate Program. However, only 10 of the 16 were able to make use of the sponsorship. Six others could not attend due to various reasons, including inability to arrange remaining funds. The Southern and Eastern African Cotton Forum organized their meeting during the WCRC-6 and the Forum used ICAC sponsorship to bring researchers to the WCRC-6.

This issue of the *ICAC RECORDER* is devoted to WCRC-6 with a primary focus on abstracts of the presentations made by the keynote and plenary speakers. Abstracts of all papers submitted to WCRC-6 were published in a handbook. The

book of abstracts will be made available on the WCRC-6 web page as soon as it is updated based on actual presentations. Abstracts of the keynote and plenary speakers were not included in the publication, so they are produced here for the benefit of *ICAC RECORDER* readers in English, French and Spanish languages.

Keynote Paper Abstracts

1. Innovative Research Solutions to Enhance Cotton Production; How Close We Are?

Yusuf Zafar, ICAC Cotton Researcher for the Year 2012, Vienna International Centre, International Atomic Energy Agency, Austria

Cotton though covers only 2% of arable land of this planet and has 6% share of global trade, but this natural fiber is responsible of sustaining a textile and fashion industry that is worth trillions of dollars. Moreover, it is the backbone of several developing countries whose economies are heavily dependent on cotton. The cotton research community has made commendable efforts to arrest the declining trend of its share in the wake of ever-rising use of man-made fibers (polyester, rayon, viscose, etc.). Still, global cotton production and demand have remained stagnant for several decades. Recent very low prices of oil, low demand for garments and natural disasters in areas of large cotton producing countries due to climate change are putting an extra burden on cotton production systems, which is already delicate and vulnerable to multiple stresses.

The genetic make-up of any crop plant is embedded in its seed. According to the FAO, the seed contributes nearly half towards crop production. Despite some regional efforts, there is no global platform to exchange cotton seeds (germplasm) among cotton-producing countries. In the more recent past, many countries have increased restrictions on the sharing of germplasm, resulting in a lack of exchange of germplasm even for classical breeding programs. All cotton-breeding programs in the world are suffering due to a narrow genetic base.

Cotton is one of the top three crops (with corn and soybeans) modified through genetic engineering (biotech cotton). Biotech cotton has adapted very well in top cotton-producing countries (Australia, Brazil, China, India, Pakistan and the USA). However, only two traits (insect resistance and herbicide tolerance) are available in biotech cotton. The spread of biotech cotton to other cotton-producing countries is slow and faces extensive resistance. Recent news about patent issues and the price of biotech seed in India clearly demonstrate the need for a more equitable arrangement of exchange of technologies and germplasm.

The advent of the WTO in 1995, along with emergence of private seed sector conducting business in biotech crops (inclusive of cotton), raised the issue of monopolies of multinational companies. The long-term issues of patents,

plant breeder rights (PBR) and the International Union for the Protection of New Varieties of Plants (UPOV), complex biosafety protocols, diminishing role of public sector research and, above all, the steep rise in input costs, in conjunction with global volatility of raw cotton prices (dependent on import/export of cotton of a small number of countries) impacted research and development work on cotton and thus its production.

The present challenges to cotton production could be met easily with recent developments in genomics. As a result, fully sequenced data of both diploid and tetraploid cottons are available. Progress in bioinformatics and the clustered regularly interspaced short palindromic repeats (CRISPR) system raises the hope of new era in cotton research. However, this progress will be possible only with a paradigm shift of policies and improvements in the regulatory process.

One major factor that requires immediate attention is strengthening of public sector research with more funds, sustainability and enabling environment. The equitable and easy access of modern research to all the cotton research community, especially LDCs and developing countries, is another important component of bringing revolutionary change to the cotton research sector.

The long cherished goal of having an International Cotton Research Institute (ICRI), like that of International Rice Research Institute (IRRI), remains valid. The emergence of the BRICS group and the New Development Bank, led by China, raises the hope that the emerging economies of Brazil, China and India will seriously consider the establishment of this entity. This will revolutionize the present cotton research system and would be a game changer in cotton R&D. After the establishment of ICRA, the next major step forward could be the development of an ICRI by the global cotton research community.

2. Connecting Cotton Growers with Research

Adam Kay and Nicola Cottee, Cotton Australia, Australia (Presented by Adam Kay)

The success of the Australian cotton industry can be largely attributed not only to significant research investment, but also to the rapid adoption of emerging science by cotton growers to enhance yield, quality, sustainability and profitability. A solid extension framework that relies on a strong partnership among industry groups, researchers, growers and extension officers enabled the success of technology transfer in Australian cotton. The industry's best management practice program (myBMP) is relied upon, in conjunction with traditional extension initiatives, to deliver the latest scientific understanding to growers. Despite this success, transforming the latest research into changes in practice on the farm continues to present a challenge to the Australia cotton industry.

A key challenge for research-extension is identifying the overall key limitations of the crop production system, to

ensure that the extension effort delivers a net benefit to overall productivity. For example, selection of Australian germplasm with proven desirable yield and fiber quality attributes may be considered by overseas growers as a simple solution to improve yields in their cotton system. However, this is not always the case when local constraints are taken into account; imported germplasm may fail to outperform locally bred varieties. In addition to genetic constraints, local solutions and limiting factors need to be considered in order to maximize yield. For a water-limited environment, an extension effort focused on soil water storage and management strategies would probably deliver greater yield benefits.

Over the last 30 years, the Australian cotton industry has developed a diverse range of initiatives and tools to connect cotton growers with research in pest management. Early field trials showing that square loss did not significantly affect yield were used to challenge the thinking around early season insecticide use in cotton. Subsequent development of printed materials and decision management tools allowed growers to make informed decisions about integrated pest management, particularly in relation to pest thresholds. This philosophy around responsible pesticide management ensured that the industry was well placed for the introduction of genetically modified insecticidal cotton. This technology was accompanied by a number of industry-endorsed tactics for resistance management, deployed through a strong network of technology providers, researchers, extension officers and cotton growers. This approach remains relevant to this day, where currently a full array of techniques including fact sheets, field days, meetings, conferences, and online media continue to convey the importance of pest management with the impending release of triple-stack Bt cotton.

The Australian cotton industry has been able to deliver innovative practices and technologies to on-farm practice change through a strong collaborative approach between research teams, extension teams and grower networks that are underpinned by a best management practices program. As an industry on the cusp of a digital agricultural revolution, the Australian cotton industry continues to review and refine its extension framework and network to enable growers to make informed decisions across the whole farming system. An ability to connect growers with research, through an extension framework, continues to be of extremely high priority to ensure the longevity, productivity and competitiveness of the industry.

Plenary Paper Abstracts

1. 'Cotton Physiology' the Cornerstone of Future Cotton Science

Michael Bange, Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia

Cotton production worldwide will be influenced by changes in climate as well as by indirect effects, such as the regulation of water resources. Combating these changes as well as dealing

with increasing costs will mean that sustainable production will need to adopt practices in combination that will: increase and/or maintain high yields and quality; improve a range of production efficiencies (water, nitrogen, energy, emissions etc.); seek to improve a better return for products; or consider other cropping options as alternatives. The presentation covers present impacts of these changes on production systems and highlights some options for adaptation with an emphasis on the role of plant and crop physiology to support these. Crop management and plant breeding options include: high yielding/high quality stress tolerant varieties; optimizing water and nutrition; manipulating crop maturity; varying planting time; optimizing soil and health for crop nutrition; and maintaining diligent monitoring practices for weeds, pests and diseases to enable responsive management.

2. Evolution of Cotton Fiber Quality: An Imperative for Future Market Needs

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³Electrical and Computer Engineering, Texas Tech University, ⁴CSIRO Australia, (Presented by Eric F. Hequet)

Upland cotton, *Gossypium hirsutum* L., ranks fourth in planted acreage in the United States, behind corn, wheat, and soybeans. In response to the demand for cotton fabric, worldwide consumption of cotton fiber more than doubled from 1960 to 2011. Though cotton fiber consumption has increased, cotton has lost half its market share to competition from synthetic fibers.

While consumers demand cotton yarns and fabrics, variability in cotton fiber quality makes it a challenging natural raw material to transform into a consistent industrial product. Natural variability in cotton fiber quality can translate into imperfections in spun yarns. Imperfections in the yarns, in turn, result in imperfections in the finished textiles. In addition to impacting the value of finished yarns and textiles, variability in cotton fiber negatively impacts processing ability. Indeed, yarn imperfections translate into weak points that increase yarn breakages and lower productivity at the mill.

Developing cotton varieties with improved spinning performance and yarn quality poses a formidable challenge. As we know, cotton breeders face the task of developing cultivars that will perform well in the field, at the gin, and in textile processing. One of the most challenging tasks is predicting the processing performance of the raw material. Indeed, producing yarn from each entry in a breeding program is not possible because of both the limited quantity of lint available and the prohibitive cost of spinning tests. Hence, how could we predict the industrial yarn quality of a breeding line without spinning the lint into yarn? The logical answer to this question would be: by carefully evaluating fiber quality. Unfortunately, most of the breeding programs use HVI (High

Volume Instrument) only to assess fiber properties. Is this sufficient?

Selections based on fiber quality parameters should be done with the aim of improving yarn quality. It is important to ask if the fiber quality parameters provided by HVI testing are adequate for selecting elite cotton lines for improved spinning performance. While HVI measurements are fast, they cannot characterize variations in cotton fiber quality among fibers (within a sample). We demonstrated that capturing within sample variability is critical for predicting spinning performance. The main tool for measuring within sample fiber-to-fiber variability is the Advanced Fiber Information System (AFIS). It is now well understood that, in order to improve its competitiveness as compared with man-made fibers, cotton fiber must exhibit reduced variability so that it may perform more predictably at the mill. This can be achieved by breeding for an improved distribution in fiber quality using non-HVI fiber properties (AFIS).

3. Development of GM Cotton Varieties - Challenges for a Tropical Environment

Camilo de Lelis Morello, Cotton Researcher, EMBRAPA, Brazil

Increases in productivity in Brazil were achieved during different periods in various regions where cotton was historically produced. The environments (E) in which cotton was grown along with management practices (M) and genetics (G) plus G x E x M interactions all explain increases in productivity that were achieved along the years, and breeding programs have contributed significantly to the increased productivity and production of cotton in Brazil. Genetic gains are achieved continuously with germplasm improvement associated with biotech traits. Throughout the breeding process, knowledge of the environmental characteristics and of the production system, and from these to define which characters/traits are necessary, holds a strategic role. Whereas lint yield and fiber quality remain the primary goals regardless of the production system, breeding for the tropical Brazilian cerrados necessitates taking into account a set of highly relevant characters to be pursued through conventional improvement. Because of the combination of high relative humidity and high temperatures, fungal (Ramularia leaf spot and Ramulosis), bacterial (bacterial blight) and viral (cotton blue disease) diseases are favored. Nematode species, such as the root-knot, reniform, and root-lesion nematodes, are also widely distributed in the cotton-growing areas. Varieties with short flowering and maturation intervals are desirable in some parts of the Brazilian cerrados where planting occurs in mid-February.

The adoption of GM cotton varieties brought significant contributions to the management of weeds and insects (worms). The resistance to worms provided by the biotech trait is an important tool to be used in integrated pest management programs. Temperature and soil humidity during the cotton-growing season are extremely favorable for

weeds, and herbicide tolerance is a very important tool for integrated weed management. The process that leads from the identification of a potential useful gene to a new GM variety is a very long, and some important decisions can affect the efficiency and final results. The goal in the GM conversion process is to obtain a converted line harboring the transgenes in a genetic background with equal or superior agronomic performance, as compared to that of the recurrent elite germplasm. The proper choice of recurrent and donor parents, and the number of backcross generations to be performed have a large influence on time and resources. After the backcross generations are completed, performing selfing is required to fix different alleles in a homozygous state. In the case of the introgression of events with stacked genes, in which many loci are involved, the identification of individuals with all or most loci in homozygous state after one round of selfing (F_2 plants) is harder and expensive. A strategy to reduce the necessity of overly large populations is the " F_2 enrichment" scheme, in which two successive generations of selfing are performed.

Modern cotton production systems demand biotech traits that provide solutions and higher efficiency of pest control. However, biotech traits need to be deployed in high quality germplasm with characteristics suitable for the environment and production systems.

4. Addressing the Challenges of Sustainable Cotton Production under Competition in China

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This paper provides firstly a quick overview of agriculture in China, and then a brief analysis of cotton production under restructuring in a context where the strengthening of agriculture has gained momentum. The very recent measures targeted at strengthening agriculture are assessed through the prism of sustainability, namely the three commonly acknowledged pillars of social, environmental and economic aspects. The contemplated actions to enhance agriculture, with implications for cotton production, look like a set of challenges whose chances of being successfully overcome are appraised through a retrospective analysis of a few achievements related to former challenges.

In China, agriculture has lacked attractiveness for several decades since the economy has been liberalized. Rural families on tiny farms lag behind in terms of income; they suffer from a continuously growing income gap in spite of an increasing share of wages through off-farm activities. Families have been abandoning farming, making land available to increase the size of remaining farms, while strong labor constraints imply that the mechanization of more cultivation practices has become more crucial than ever.

Cotton production, especially in the two traditional production regions of Yellow River Valley and Yangtze River Valley,

is particularly touched by the above-mentioned evolution of agriculture. Cotton has become less and less attractive compared to the competing crops, notably cereals, due to lack of governmental support, in addition to the increased cost of labor and fertilizers, as well as those of insecticides, in spite of, or because of, almost twenty years of biotech cotton adoption.

Agricultural policy measures elaborated in March 2016 can be related to each of the three sustainability pillars. More precisely, about half of the measures correspond to one of the three social, environmental and economic dimensions, and the other half falls in between two dimensions.

Retrospective analysis of a few innovations like China-specific technique of transplanting, the widespread use of commercial cotton hybrids, the evolution of insecticide spraying devices, the development of cultivation machines adapted to moderate scale farming (although a breakthrough achievement in mechanized harvesting has yet to come) show a successful process of recurrent technology development based on a huge scientific and technical network motivated by an important potential market. In the last few decades, China has demonstrated its capabilities to overcome technical challenges, but many challenges ahead, related to measures recently announced, fall out of the technical sphere (like decentralization of land contracts, insurance, credit guarantees, etc.). Organizational and institutional innovations are required; they call upon successful interaction between producers and other stakeholders, be they public or private, and should differ from top-down and administratively oriented procedures.

5. Understanding Cotton Fiber Development

Lili Tu, Wenxin Tang, Yang Li, Kai Guo, Nian Liu and Xianlong Zhang, Huazhong Agricultural University, China, (Presented by Xianlong Zhang)

Higher quality fiber equates to a more comfortable textile and better productivity in the spinning mill. So manipulating fiber developmental processes to improve quality is a common target for breeding and biotechnology. Researchers at the Huazhong Agricultural University isolated a gene encoding a calcium sensor, GhCaM7, based on its high expression level relative to other GhCaMs in fiber cells at the fast elongation stage. Overexpressing GhCaM7 promotes early fiber elongation, whereas GhCaM7 suppression by RNAi delays fiber initiation and inhibits fiber elongation. GhCaM7 overexpression fiber cells show increased ROS levels compared to wild type, while GhCaM7 RNAi fiber cells have reduced levels. H₂O₂ enhances Ca²⁺ influx into the fiber and feedback-regulates the expression of GhCaM7. GhCaM7 can modulate ROS production and can be regarded as a molecular link between Ca²⁺ and ROS signal pathways in fiber early development.

A novel truncated α -expansin, GbEXPATR, was found to be specifically expressed at the fiber elongation stage in Gb. To compare the functions of GbEXPATR and the normal full-length form of this gene, GbEXPA2, in fiber elongation,

transgenic cotton lines with RNAi and over-expression of these two genes were produced. The cell wall composition and the fiber quality of the transgenic lines were altered with the GbEXPA2 and GbEXPATR expression level changes demonstrating a role for α -expansin in cell wall remodeling. In particular, GbEXPATR, that lacks the carbohydrate binding domain 2, had a strong effect on cell elongation through delaying secondary cell wall synthesis and, as a result, enhanced fiber length, fineness and strength.

MicroRNAs (miRNAs) play important roles in plant development. We constructed seven fiber RNA libraries representing the initiation, elongation and secondary cell wall synthesis stages. A total of 47 conserved miRNA families and seven novel miRNAs were profiled using small RNA sequencing. In addition, 140 targets of 30 conserved miRNAs and 38 targets of five novel miRNAs were identified through degradome sequencing. Histochemical analyses detected the biological activity of miRNA156/157 in ovule and fiber development. Suppressing miRNA156/157 function resulted in the reduction of mature fiber length, illustrating that miRNA156/157 plays an essential role in fiber elongation.

Ascorbate peroxidase (APX) is an important ROS scavenging enzyme and we found GhAPX1AT/DT encoded one member of the previously unrealized group of cytosolic APXs (cAPXs) which were preferentially expressed during the fiber elongating stage. Suppression of all cAPX (IAO) resulted in a 3.5-fold increase in H₂O₂ levels in fiber and caused oxidative stress, which significantly suppressed fiber elongation. The fiber length of transgenic lines with over-expression or specific down-regulation of GhAPX1AT/DT showed no obvious changes. However, fibers of over-expression lines showed higher tolerance to oxidative stress. Differentially expressed genes (DEGs) in 10 DPA fiber of IAO lines identified by RNA-seq were related to redox homeostasis, signaling pathways, stress responses and cell wall synthesis, and the DEGs up-regulated in IAO lines also up-regulated in the 10 DPA and 20 DPA fiber of wild cotton compared to domesticated cotton.

6. Smallholder Cotton Farming: Sustainability Matters

Joe C. B. Kabissa, Tanzania

Up to 80% of the annual global cotton output is produced by smallholder farmers living in the developing countries of Asia, Africa and Latin America. In sub-Saharan Africa most of the cotton-producing countries depend heavily on cotton for economic development and poverty alleviation. In developing countries cotton farming is the occupation of smallholders who depend on family labor to produce relatively low input cotton under rainfed conditions. In cotton farming, smallholders respond to price and other incentives, both negative and positive, and this has a bearing on their potential to increase productivity by greater use of purchased inputs. In sub-Saharan Africa in general, and Francophone Africa in particular, the cost of producing cotton is one of the lowest

in the world. Nevertheless, rather than increasing, yields have stagnated or are in the decline and so the sustainability of cotton farming is under threat. Some of the existing and emerging challenges were presented, including what needs to be done in the context of the global cotton market.

7. The First 60 Million Years of Cotton Improvement and What May Lay Ahead

Andrew Patterson, Professor, University of Georgia, Athens, USA

A reference genome for cotton based on the compact genome of *Gossypium raimondii*, together with draft genomes for *G. hirsutum*, *G. herbaceum*, and *G. longicalyx* and their subsequent analyses, have provided new insights into key events in cotton's evolutionary history, starting with a 5x multiplication of the entire genome about 60 million years ago, followed by the merger of the A and D sub genomes about 1-2 million years ago, and including a rich history of intergenomic exchange that has doubled the dosage of many cotton alleles in striking patterns across time and across the genome. The reference genome provides new insights into fiber evolution including many candidate genes that warrant further investigation, as well as a valuable resource for mitigating many long-standing challenges in cotton improvement.

8. Integrated Pest Management to Complement GM Traits

Keshav R. Kranthi, Director, Central Institute for Cotton Research, India

The history of cotton pest management presents an interesting journey replete with examples of powerful technologies losing out to the power of insect resistance. Many a times pest management is complicated by human interventions that disrupt natural ecology, thereby resulting in the shuffling of insect pests from minor to major importance and vice versa. Subsequently, pest control ammunition is consolidated with focus on the newly emergent pest. These interventions may lead to other problems for such a transient process to continue in a cyclic manner over time. This transience in pest management places farmers in precarious situations, especially when the most recent powerful technologies start crumbling. The cases of synthetic pyrethroids, neonicotinoids and biotech cotton present clear examples of how it barely took a decade for the technologies to enter the 'desperation phase' from the 'exploitation phase'.

India presents an excellent case study of how new 'pest control technologies' influence changes in pest dynamics, thereby warranting the development of new technologies. For example, synthetic pyrethroids were introduced into India in 1981 to control the two major insect pests, pink bollworm, *Pectinophora gossypiella* and *Spodoptera litura*. The two insect species were effectively controlled by the pyrethroids, but by 1986 two different insect species American

bollworm *Helicoverpa armigera* and whitefly *Bemisia tabaci* emerged as major problems. The problem was more severe on American cotton *Gossypium hirsutum* hybrids where pyrethroid usage was high. Insecticide application intensified to an extent of 15-30 applications per season, resulting in high levels of insecticide resistance in these pests. New insecticides with novel modes of action were introduced. Both pests exhibited high propensity for resistance to almost all groups of insecticides that were used for their control. In 2002, Cry1Ac based biotech cotton was introduced in India to control the 'insecticide-resistant' *Helicoverpa armigera*. Pink bollworms developed resistance to Cry1Ac Bt-cotton in 2008. Bollgard II (Cry1Ac+Cry2Ab) was introduced in 2006 as an effective control measure against the pink bollworm, but the worms developed resistance to Bollgard II by 2014. By 2009 the pink bollworm was reported to have developed resistance to Cry1Ac Bt-cotton. It is presumed that ecological disruption by pyrethroids may have been the cause. Indiscriminate use of pyrethroids also resulted in whitefly and American bollworm resistance by 1990 to almost all insecticides recommended for their control.

The case of pink bollworm resistance to Bollgard II (Cry1Ac + Cry2Ab) exclusively in India within six years, in contrast to sustained susceptibility of the pink bollworm to Cry toxins in Australia, China and USA despite 19 years of selection pressure, exemplifies the problem of incorrect 'technology stewardship' in India. The acceleration of pink bollworm resistance development is probably related to two main factors:

- i. The deployment of insect resistant biotech cotton technology only in 'commercial cotton hybrids' in India in contrast to 'straight varieties' in all other countries.
- ii. Extending the crop duration of insect resistant biotech hybrids in India for 2-4 months beyond the normal season of six months, thus providing continuous food source and thereby facilitating multiple cycles and additional generations of pink bollworms.

These two factors intensified selection pressure. There was a third factor that may have also accelerated resistance development. More than 1,600 different biotech hybrids of variable maturity and duration were approved for cultivation in India, thereby providing continuous attractant cues and a steady source of food for the pink bollworm for almost 3-4 months in the year. The first factor is the most unique to India. Bolls on the hemizygous (for Bt toxins) hybrid plants contain seeds, which segregate for the Cry toxins. For example, at least 25% of the seeds in each boll of Bollgard hybrid plants do not contain the Cry1Ac toxin. Similarly at least 6% of the seeds in each boll of Bollgard II hybrid plants do not contain the Cry1Ac or Cry2Ab toxins. Pink bollworm larvae that contain alleles conferring resistance to Cry toxins in homozygous (Cry-RR) condition survive on all seeds, whereas generally older instars of larvae that have the resistant allele in heterozygous condition (Cry-Rr) survive on the developing

Bt containing seeds in the green bolls. Larvae initially survive on non-Bt seeds and the older instars of heterozygous (Cry-Rr) allele survive on seeds carrying Bt, while larvae without resistant alleles die after feeding on raw Bt-seeds. Thus, the Cry-R allele gets conserved in the pink bollworm populations because of the unique condition of Cry toxin segregation in biotech cotton hybrids only in India and not in other countries, where all developing seeds in green bolls of biotech cotton varieties contain Cry toxins that kill the heterozygous (Cry-Rr) larvae.

Neonicotinoids were introduced in the mid-1990s. The efficacy of imidacloprid as seed treatment was most crucial for biotech cotton hybrids because more than 90% of the hybrids were susceptible to leaf hoppers, whiteflies and thrips. Imidacloprid seed treatment protected the crop for the first two critical months against sap-sucking insects. In combination with the Bt-toxins, neonicotinoids as seed treatment and foliar sprays and new insecticides, such as spinosad, emamecting benzoate and indoxacarb, which were introduced during 2000-2004 for bollworm control on non-Bt cotton, provided comprehensive pest control at least for the first five years after Bt-cotton hybrids were introduced in 2002/03. These technologies contributed to the increasing trend of yields during 2001-2007. Insect-resistant biotech cotton spread like a wildfire to saturate the cotton area in the country by 2009/10. By 2007/08, whiteflies and leaf hoppers developed high levels of resistance to imidacloprid and yields started showing a declining trend thereafter. Pink bollworm resistance to Bollgard-II is further influencing the yield loss. In all these instances, the common feature was indiscriminate use of the technologies with scant regard to the principles of IPM (Integrated pest management) and IRM (insecticide resistance management) principles.

While new insecticide molecules and new biotech products using biotechnology can be constantly developed, it is important to note that these provide only short-term gains and need continuous replacement, which comes at a price. Apart from adding to the cost of cultivation, the powerful impact of the matrix of technologies also disrupts ecosystems. Many a times, these pest control technologies enter into a stage of uncertain efficacy because of insect resistance to insecticides, thereby leading to intensive and extensive over-use. Pest management strategies eventually turn fragile.

Implementation of IPM and IRM principles in the deployment of 'insect resistant biotech features' is crucial for the long-term sustainability of pest management. For example, insect-resistant biotech genes could have been durable if deployed in pure line varieties that were homozygous for Cry toxins, of short duration (<150 days), early maturing and resistant to leaf hoppers. This would have obviated the need for insecticides either as seed treatment or foliar application for the control of sucking pests or bollworms. Early maturing, short duration varieties in India escape bollworms due to the mismatch between the peak occurrence of bollworms and the peaks of flowering and boll formation. In addition, insecticide

recommendations for the rotation of chemical groups with different modes of action when necessary on biotech cotton crop would have resulted in the minimal use of insecticides for durable pest management and a delay in the development of resistance by bollworms to Bt toxins and the resistance of sap-sucking insects to neonicotinoids.

Report on ICGI Papers

David M. Stelly, Chair of the International Cotton Genome Initiative

International Cotton Genome Initiative (ICGI) speakers described advances that are increasing the power, precision and practical relevance of research on the genomics of cotton and related species. In many cases, new technologies revealed additional complexities of *Gossypium* germplasm and genomes – at evolutionary, taxonomic, structural, compositional, hereditary, epigenetic and functional levels.

The rapidly advancing state of cotton genomics was evident at the meeting and contrasted sharply with the 2006 meeting in Brasilia – just one decade ago -- when ICGI first discussed the desirability of focusing early common genome-sequencing efforts on *Gossypium raimondii*. As explained in the resulting white paper (2007), this non-cultivated tree-like Peruvian species would seem from an agricultural standpoint to be unlikely target for sequencing, but it has the smallest and thus the least complex genome of all known *Gossypium* species, and was thus considered to be the best choice. In 2012, the first high-quality genome assembly was published for *G. raimondii*. Since 2006, large numbers of SSRs and other types of DNA markers were developed, mapped and used for germplasm characterization, trait dissection and very limited marker-assisted selection. Rapid development of high-density intraspecific and interspecific SNP maps, and the global use of 10,000s of SNP became feasible in 2014, when the CottonSNP63K Array was released. Last year, 2015, the report of two draft genome assemblies for cultivated cotton signaled a major leap forward for cotton. These important technical and scientific advances in cotton genomics prefaced the 2016 ICGI biennial meeting held in conjunction with WCRC-6.

- Joshua Udall from the USA discussed structure of the *Gossypium* genomes, including the use of “homeo-SNPs” (one-base differences in sequence between A versus D subgenomes) to facilitate informatic analysis of regular SNPs (one-base differences in sequence between different individuals), and to facilitate comparative informatic analysis between genomes of diploid species and the A and D subgenomes of tetraploid species. Those comparisons will also likely improve sequence assemblies for genomes of the A-genome diploids.
- David Fang from the USA reported progress in using genotyping by sequencing (GBS) of isogenic lines and in bulked-segregant analysis, in some cases fine-mapping mutant genes and QTLs of interest. Prospective

applications to MAJIC populations were noted.

- Brian Scheffler from the USA reported a marker-anchored physical framework for the AD genome of upland cotton and high congruence with scaffolding the widely used D5 genome assembly and one of the two newly reported (2015) AD genome assemblies. The integrated physical framework should facilitate development of a reference-grade genome assembly for Upland cotton.
- Vamadevaiah Hiremath from India demonstrated by qPCR that 6 transcription factors were differentially up-regulated in some drought tolerant genotypes in water-stress treatments.
- Zhongxu Lin from China analyzed *G. hirsutum*/*G. barbadense* Sea Island SSRs in hybrid BC₁, reciprocal BC and F₂ populations, and found instances of differential recombination rates in male and female parents, segregation distortion and hybrid breakdown.

Breeding and Applied Genomics

- Lucia Vieira Hoffmann of Brazil indicated that EMBRAPA is preserving and characterizing Brazil's native and naturalized cottons. Their collection includes about 1,350 lines, and HVI analysis of about 500 accessions revealed some could be useful resources for fiber improvement.
- Maite Vaslin de Freitas Silva of Brazil reported on genetic resistance to cotton blue disease (CBD), which is caused by cotton leaf roll dwarf virus (CLRDV) and aphid-transmitted. New genetic and functional evidence from cotton and arabidopsis indicated that the cotton resistance involves Cbd2, an arginyl tRNA transferase (ATE) implicated in leading targeted proteins to protein degradation.
- Johnie Jenkins of the USA described development of a random-mated complex population developed from 18 *G. barbadense* chromosome substitution lines and 3 *G. hirsutum* lines. The success of introgression and complexity were validated by marker-based analysis.
- Muhammad Tehseen Azhar from Pakistan reported wide differences among diploid *Gossypium* species in terms of their susceptibility or tolerance cotton leaf curl virus (CLCuV), based on both natural transmission or from CLCuV-infected *G. hirsutum* grafts.
- Farshid Talat from Iran reported chloroplast genomes of three D-genome species were completed and compared to each other as well as to 14 other *Gossypium* species.

Functional Genomics

- Shuangxia Jin from China discussed multiple experiments on cotton's defense system to whitefly infestation and identified several candidate genes for control of phloem-feeding pests. Transcriptome analysis of cottons resistant and susceptible to whitefly (*Bemisia tabaci*) at multiple

time periods after exposure indicated that WRKY40 and a copper transport protein as "hub" genes that may regulate cotton defenses to whitefly infestation. Virus-induced silencing of GhMPK3 increased susceptibility to whitefly. miRNAs were also key to the defense system.

- Vasu Kuraparthi from the USA reported positional cloning of a HD-Zip transcription factor gene capable of causing okra leaf. An 8-bp deletion in the promoter leads to what regarded today as normal leaf shape, whereas the ancestral type is subokra.
- Ayyanagouda Mahantgouda Patil conducted an *in silico* analysis in India to identify genes that are significantly up- or down-regulated, putative transcriptional factor binding sites and transcriptional factors related to fiber development.
- Uzma Qaisar from Pakistan conducted a meta-analysis of microarray data from short- and long-fibered *G. hirsutum* and extra-long-fibered *G. barbadense* to identify over 1,400 genes differentially expressed according to fiber lengths. Two seemed especially significant, ethylene responsive transcription factor wrinkled-1 (*wri1*) and a vacuolar processing enzyme (*vpe*) gene, which completely correspond to fiber lengths in cotton.

Comparative Genomics and Bioinformatics

- Jing Yu from the USA reported significant improvements in holdings and functionality of CottonGen, the main global resource for cotton genomics data. SNP lists, maps, a dedicated CottonSNP63K page, RNASeq data and GBS data were added, and are viewable through an implementation of the JBrowse genome viewer. Synteny can be viewed in GBrowse-Syn, and new metabolic pathways are available through Pathway Tools. Other new genome, trait, map and marker data, and new or improved search tools were also added.
- Daniel Peterson from the USA posited his generalized observations and insights on contemporary bioinformatics, particularly where these lead to constraints and underperformance. Some can be addressed by collective actions of individual researchers, while amelioration of others requires major adjustments by the field, including new methods that better utilize high-performance super-computing systems.
- John Yu from the USA discussed recent advances in sequencing of genomes from D- and A-genome diploid species (D5 and A2), as well as the AD-genome of tetraploid species. A benefit of having each of these is that much of the overall AD-genome is amenable to separation into the A- and D-subgenomes of *G. hirsutum*, which makes it more feasible to track down effects on important traits, e.g., fiber traits.
- Qian-Hao Zhu from Australia presented research on homeodomain-leucine zipper (HD-Zip) transcription

factors in cotton. HD-Zips are unique to the plant kingdom and often help regulate genes involved in plant development and response to abiotic/biotic stresses. Over 70 HD-Zips occur in Upland cotton. Expression tends to be tissue-specific. Three of them exhibit differential response to infection by *Verticillium*.

- Ishwarappa Katageri of India reported that whole-genome sequencing of *G. arboreum* and *G. herbaceum* was used for large-scale *in silico* SNP identification. He also reported an initial CottonSNP63K-based analysis of 178 *G. hirsutum* x *G. barbadense* RILs to map out major QTLs affecting key traits.

Breeding and Applied Genomics

- Todd Campbell from the USA reported efforts to identify unique sources of fiber quality. He identified germplasm line MD 15 as a unique source of high fiber quality, and that MD 15 fiber quality likely resulted from transgressive segregation selected for during its development.
- Lili Tu from China reported advanced phenotypic validation of multiple genes putatively affecting fiber development and quality. These included a calcium sensor, GhCaM7, which affects ROS signal pathways in fiber early development, GbEXPATR, which lacks a carbohydrate binding domain and delays secondary cell wall synthesis and thereby enhances fiber length, fineness and strength.
- Amanda Hulse-Kemp from the USA reported on the development of 3 independent CottonSNP63k-based linkage maps from 1 F₂ and two sets of reciprocal RIL

populations from common parents ‘Phytogen 72’ (PHY72) and ‘Stoneville 474’ (STV474). These collectively provide the best intraspecific maps developed to date, and two corresponding immortalized RIL mapping populations that constitute a portable platform for future cotton research.

- Khezir Hayat, a Ph.D. student from Turkey, described the development and plans for a GBS-based interspecific *G. hirsutum* x *G. barbadense* mapping project.

Sustainability At The WCRC-6

Sustainable production and its impact on cotton production practices was one of the highly emphasized subjects at the Conference. There were a number of sessions wherein papers on cotton agronomy in the context of sustainable production technology were presented. One session was devoted to ‘Measuring sustainability in cotton farming systems’ wherein work on the lines of guidelines and parameters set by the ICAC’s Expert Panel on Social, Environmental and Economic Performance of cotton (SEEP) were presented. Two plenary papers (abstracts given above) addressed the sustainability issue from the perspective of smallholders and how to address challenges of sustainable production. In the concluding session, the Organizing Committee presented a report on the overall sustainability of the Conference other than the technical presentations. The event organizers will plant 3,600 trees in order to neutralize the greenhouse gas emissions generated by the event. Organization of the Conference left significant positive social, environmental and economic impacts in many ways. The Organizing Committee presented the following chart indicating sustainable gains in quantitative terms.



Participation

471 researchers from 40 countries and five international organizations attended the Conference.

WCRC-6 List of Participants by Country

Country	No. of Participants	Country	No. of Participants
Argentina	8	Mozambique	5
Australia	24	Myanmar	2
Bangladesh	2	Netherlands	1
Benin	1	Nigeria	3
Brazil	212	Pakistan	13
Burkina Faso	1	Peru	4
Chad	1	Poland	2
China	23	Portugal	5
Colombia	4	Spain	1
Ecuador	1	South Africa	3
Egypt	4	Sudan	6
Ethiopia	1	Syria	1
France	1	Tanzania	2
Germany	1	Togo	1
India	37	Turkey	11
Indonesia	2	Uganda	3
Iran	6	USA	58
Israel	1	Uzbekistan	2
Kenya	3	Vietnam	1
Mali	3	Zambia	2

International organizations:

CABI	= 1
CIRAD	= 2
Food and Agriculture Organization	= 2
International Atomic Energy Agency	= 2
International Cotton Advisory Committee	= 2
Total	= 471