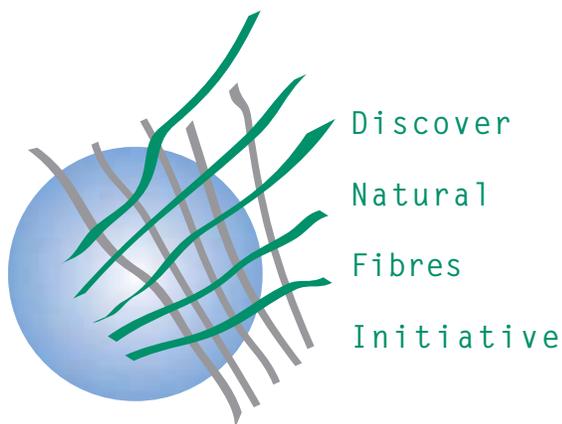




THE ICAC RECORDER

International
Cotton
Advisory
Committee



Technical
Information Section

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Update on Cotton
Production Research



Contents

	Pages
Introduction	2
New Directions in Cotton Research.....	3
Developments and Opportunities in Cotton Breeding.....	15

Introduction

The ICAC started recognizing the “ICAC Cotton Researcher of the Year” in 2009. The award is presented annually and has been given to eight researchers so far. Researchers from universities and public sector research organizations can apply for the award directly or through their heads of institutions. Researchers from all disciplines of cotton production research are eligible for the award. An independent Award Panel, consisting of five experts from at least four countries, representing the major disciplines in cotton, reviews applications and chooses the winner. The composition of the Award Panel is not made public. The ICAC awards the researcher a shield, an honorarium of US\$1,000, a certificate, and the title “ICAC Cotton Researcher of the Year”. Applications are received from February 1 to March 31 and a winner is announced on May 1. More information about the award is available at <<https://www.icac.org/tech/ICAC-Researcher-of-the-Year-Award>>.

The eight winners listed below were asked to give their views on a common subject ‘New Directions in Cotton Research,’ which forms the contents of the first article.

- 2009** Dr. Keshav R. Kranthi, Central Institute for Cotton Research, India
- 2010** Dr. Fred Bourland, University of Arkansas, USA
- 2011** Dr. Sukumar Saha, USDA-ARS, Mississippi State, USA
- 2012** Dr. Andrew H. Paterson, University of Georgia, USA
- 2012** Dr. Yusuf Zafar, National Institute for Biotechnology and Genetic Engineering, Pakistan
- 2013** Dr. Ibrokhim Y. Abdurakhmonov, Center of Genomics and Bioinformatics, Uzbekistan

2014 Dr. Mehboob-ur-Rahman, National Institute for Biotechnology and Genetic Engineering, Pakistan

2015 Dr. Greg A. Constable, CSIRO Agriculture, Australia

The second article on Developments and Opportunities in Cotton Breeding describes how cotton breeding has changed over time and how future breeding looks like with new developments in biotechnology and their applications in cotton breeding. It is imperative that the international scientific community understands that plant breeding is primarily an organismal science and serves as the backbone of research developments. Breeding is comprised of variety development, variety maintenance (including variety approval and certification) and seed production. All of these have continuously changed. The private sector is increasingly involved in some of the important components of the breeding chain, and the role of the public sector has certainly diminished. A mix of different approaches is needed, and there must be agreement as to who is responsible for what. A narrow genetic base and the almost total absence of germplasm exchange among breeders across countries are limiting accelerated achievements in increasing yields. While scientific centers and seed breeding systems will be changing their modalities, they cannot ignore fast approaching molecular-breeding technologies. Marker-assisted breeding and empowerment over directed breeding is the new norm in cotton breeding. Conventional breeding will be replaced by molecular breeding, a joint venture of breeders and molecular biologists. The transitional stage will last a long time and transgenic breeding will become routine.

New Directions in Cotton Research

The ICAC started recognizing the “ICAC Cotton Researcher of the Year” in 2009. The award is presented annually and has been given to eight researchers so far. Researchers from universities and public sector research organizations can apply for the award directly or through their heads of institutions. Researchers from all disciplines of cotton production research are eligible for the award. An independent Award Panel, consisting of five experts from at least four countries, representing the major disciplines in cotton, reviews applications and chooses the winner. The

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The eight previous winners were asked to give their views on a common subject ‘New Directions in Cotton Research,’ which forms contents of this article.

Keshav R. Kranthi

ICAC Researcher of the Year 2009

Director, Central Institute for Cotton Research, Nagpur, India

“What one does is what counts. Not what one had the intention of doing.” Pablo Picasso

There may be myriad noble intentions. But, they will make sense only when these intentions become the soul of whatever one does. New directions in cotton research must essentially incorporate a vision for sustainable future that respects and nurtures nature as a noble yet pragmatic intention.

Progress in cotton science and technologies over the past twenty years has been commendable. In particular, genetically modified biotech cotton became a game changer in some countries. With the sheer brilliance of science, bollworms were relegated to the rear and weeds were laid to rest. But will this last forever? Bollworms are returning and herbicide use is being questioned. As the current technologies fade, new technologies are needed to keep up the momentum. But they come at an additional cost. Will the benefits remain sustainable? Where do we go from here? Sustainability is the watchword. Cotton research needs new directions. For sustainability to be achieved, the following objectives must underpin the new research roadmap:

- Utilize genomics knowledge to create designer plants;
- Exploit the power of indigenous native genetic resources;
- Forecast market dynamics and impending threat of insect pests, nematodes and diseases;
- Develop technologies with environmental and social responsibility; and
- Drive back to nature with robust science for a sustainable future.

It is imperative that research be underlined with clear intentions, eventually moving away from chemical-intensive

productive systems to work towards technologies that work in harmony with ecology and nature. This is possible only with a strong commitment for a robust road map based on solid basic science. Research results from basic and fundamental aspects must serve as inputs in strategic research projects to be efficiently translated into applied research so as to enable the development of useful eco-friendly technologies that assist in yield improvement, resilience to biotic and abiotic stress factors, provide superior quality fiber, reduce human drudgery and work in consonance with the environment.

Genomics for Designer Plants

The science of genetics, plant breeding, genomics, proteomics, metabolomics and genetic engineering opens opportunities to create designer plants that produce high yields of premium quality cotton irrespective of salinity, water stress, prolonged drought, heat, insect pests, pathogens, nematodes etc. Discovery of new genes that enhance cotton fiber quality, enable plants to withstand and overcome drought, excess water, heat, salinity, diseases, nematodes and insects have opened up new avenues for host plant resistance to biotic and abiotic stress factors.

Huge data on ‘expression profiling’ are now available with information on 18,500 genes and about 0.5 million expressed sequence tags (ESTs). Annotated genome sequences of *Gossypium raimondii* (D genome) and *Gossypium arboreum* (A2 genome) are now available in public database. Molecular genetic information unraveled more than a thousand quantitative trait loci (QTLs) on at least 30 to 40 economically important traits. The NCBI database contains about 0.7 million sequences. Research papers describe about 49 genetic maps and at least 25,000 markers. This huge scientific knowledge must be exploited to create ‘designer varieties’.

Exploit the Power of Native Genetic Resources

Indigenous genetic resources in the context of cotton production systems mainly comprise native cotton species, insects and microorganisms. Indigenously evolved cotton species, varieties and native land races are innately endowed with abilities to withstand severe biotic and abiotic stresses. Such native races must be used to lay the foundation for genetic robustness in production systems. Research approaches must be oriented towards genetic enhancement of native races through QTL (Quantitative Trait Loci) pyramiding to develop multi-adversity resistant cultivars with adaptability to climate change and possessing superior fiber traits and other desirable qualities. Plant breeding objectives must essentially be oriented towards creating germplasm and cultivars that have high resource-use-efficiency for profitable farming in marginal ecologies with minimum chemical inputs.

Indigenously available biological resources, such as nitrogen fixing plants, indigenous parasitoids and predators of insect pests, insect-pathogens and biological control organisms, must be identified and nurtured to establish sustainable cotton production ecosystems. Native leguminous nitrogen fixing plants can act as cover crops without competing with cotton. Such species must be identified for natural weed management and soil health enhancement.

Forecasting Market Economics, Insect Pests, Nematodes & Diseases and Diagnostic Tools

Research on stochastic modeling of market intelligence helps in decision support and forecasting probability of volatility in economics and uncertainties of market dynamics. Similarly, research on temporal and geo-spatial dynamics of insect pests, natural enemies, and diseases with reference to climate change and impact of technology interventions can help in developing models to forecast probable changes in biotic stresses, such as insect pests and diseases. Development of simple immunochemical, biochemical and molecular diagnostic tools to detect diseases, races, biotypes, biotech cotton, nutrient deficiencies, pesticide purity etc., optimizes input use and enhances the value of inputs in precision farming.

Research on the chemical ecology of plant volatiles, allomones and kairomones in trophic relationships will help in the development of eco-friendly pest and disease management strategies. Studies on ecological tri-trophic interactions between insects and plants will strengthen integrated pest management (IPM). Research on ecology and eco-toxicology influencing pollinators will help in yield enhancement.

Technologies with Environmental and Social Responsibility

Technological interventions are generally designed with an intention to solve specific problems or to improve the existing production systems. Cotton research projects broadly

aim to improve varieties, production practices, crop health management; technology transfer, economic and social impact.

Cotton production systems comprising of land preparation, tillage, seed viability, nutrient and soil moisture management, weed management, insect pest and disease management, harvesting, storage, transport and marketing need technological interventions. Technologies can be processes, strategies, products, chemicals, implements or machinery. Each of these interventions exerts an influence on other components of the production system. For example, the introduction of biotech Bt-cotton hybrid seeds in India resulted in reduction of insecticide use for bollworm control, but increased insecticide usage for the control of sap-sucking insect pests. Consequently, sap-sucking pests, such as leafhoppers and whiteflies, developed resistance to almost all the recommended insecticides and caused whitefly outbreaks in the Punjab state (North Region of India) in 2016. The pink bollworm developed resistance to Bt-cotton (Cry1Ac) in 2009 and to Bollgard-II® (Cry1Ac+Cry2Ab) in 2014 in India. Introduction of biotech cotton as Bt-cotton hybrids resulted in doubling of nitrogenous fertilizers and expenditure on insecticides in India. Farmers and technologists were unsure of the biotech cotton varieties suitable for specific agro-ecological conditions to be selected from the huge list of more than 1,000 Bt-cotton hybrids. Thus the technology has now reached a stage of fatigue and uncertainty of performance, which have economic and social implications. Research on insect resistance management (IRM) holds importance for sustainability of such powerful technologies. It is imperative for research to address the issue of assessing the short-term and long-term implications of technological interventions from economic, ecological, environmental and sociological perspectives before the technology is approved for use. For example, short duration varieties are known to be less vulnerable to the damaging peaks of insect pests and diseases. They gain resilience to overcome and escape moisture stress and peak occurrence of insect pests and diseases, especially under rain dependent farming systems. Such technologies may not need intensive chemical interventions as are required for long duration varieties.

Back to Nature for Sustainable Future

With an uncertain climate looming large every year, like many crops, cotton also becomes vulnerable. Different cotton ecosystems respond differently to the changing climate. There is a need to identify key elements of conservation agriculture that have the highest potential to enhance soil health, input-use efficiency of land, water and nutrients within specific ecological niches.

Research must be intensified to develop cotton-based cropping systems, comprised of nitrogen fixing legume crops (fodder and pulses) and microbial bio-fertilizers (*Azolla*, *Anabaena*, *Azotobacter*, phosphorus solubilizing microorganisms (PSM), Arbuscular Mycorrhiza (AM) etc.). Apart from nitrogen

fixing, many legume crops are known to assist in establishing micro-ecology that strengthens naturally occurring biological control of insect pests and pathogens to support self-sustaining IPM ecology and integrated farming systems with animal husbandry. In addition, research must be oriented to develop strategies to improve soil carbon content by improving locally available processes for crop residue recycling, vermi-composting, green manure, biochar preparation and application. Identify robust strains of microbial resources and bio-fertilizers to enhance availability of phosphorus, potassium and other secondary and micronutrients.

It is extremely important to ensure that the biological diversity of natural ecosystems is properly documented in order to understand the impact and inter-relationships of different

organisms through ecological life table studies for different organisms. Research must be intensified on temporal and geographical dynamics of changes in the diversity of the flora and fauna, especially insect pests, pathogens and their natural biological control organisms present in specific ecologies with reference to climate change.

In sum, recent advances in biological information on cotton, gene discovery, biotechnology, molecular biology, nano-technology, solar energy, information technology etc., must be consolidated to form the foundation of future research that can exploit these advances to develop a road map that not only ensures high yields, all round profitability and improvement in fiber quality but also nurtures the ecology to establish sustainable farming and a healthier environment.

Fred Bourland

ICAC Researcher of the Year 2010

Center Director, Northeast Research and Extension Center, University of Arkansas, USA

Focus On - Negative Association Between Fiber Quality and Cotton Yield May be Neutralized

In the current market situation, high fiber quality is essential to ensure and enhance the marketability of cotton, but research reports over the past sixty years have documented that fiber quality and yield are negatively related. If this negative relationship did not exist, fiber quality would have been concurrently improved as breeders have selected and released higher yielding varieties. By growing varieties that possess high fiber quality, cotton producers may have a competitive edge over other production areas and the entire cotton industry will be enhanced.

Poor relationships that are not genetically controlled by the same or tightly linked genes can usually be broken, but considerable effort and focus may be required. In recent years, developing lines that yield well and produce excellent fiber quality has become a high priority in the University of Arkansas Cotton Breeding Program. A fiber quality index (Q-score) was developed and used to evaluate fiber quality. Q-score is an index that includes measurements for up to six HVI fiber properties (length, micronaire, length uniformity, strength, short fiber index, and elongation), and may range from 0 to 100. Using high selection pressure for fiber quality in early generations ensures that only high fiber quality lines will be advanced in a breeding program. Q-score does not include a measure of trash because samples are often hand-harvested and ginned without the aid of lint cleaners. Consequently, visual leaf pubescence ratings are used as an indication of the hairiness of lines and the likely trashiness of their ginned lint. Q-score and leaf pubescence ratings are used to characterize lines developed by the breeding program and entries in the annual Arkansas Cotton Variety Test.

Since 2010, three conventional cotton varieties and 12 germplasm lines were developed and released by the University of Arkansas (UA) Division of Agriculture. Most of these lines possess improvement for most lint yield components, host plant resistance, morphological, and fiber quality traits. The three varieties possess both high yielding ability and high fiber quality.

Data from the 2005 through 2015 Arkansas Cotton Variety Tests were examined to determine the status of the relationship between lint yield and fiber quality, and to examine relationships involving leaf pubescence. In these years, 40 to 78 cotton varieties and advanced breeding lines were annually evaluated in irrigated, replicated tests at four sites spanning 200 miles north to south in Arkansas. Data extrapolated from these tests included lint yield and Q-score of each entry averaged over the four locations, and leaf pubescence ratings made at one site.

Average Q-scores ranged from 56 in 2008 to 65 in 2010 with no obvious trend over years. During that time, Q-score tended to be negatively correlated with lint yield (significant in five of the 11 years), but the relationship of Q-score and lint yield was non-significant in 2013 through 2015. In 2015, 13 of 42 entries produced Q-scores of 70 or more. Six of these high fiber quality varieties were in the upper quartile for yield across the Arkansas locations. These six varieties included entries developed by Phytogen, Monsanto and the University of Arkansas Cotton Breeding Program. These data suggest that some new cotton varieties adapted to the Mississippi River Delta of Arkansas may have neutralized the negative association between lint yield and fiber quality (as measured by Q-score).

Leaf pubescence ratings were positively correlated with lint yields in each year from 2005 through 2015 (significant in eight of the 11 years). Thus, higher yields tended to be related to increased hairiness of varieties. As evidenced by three of the four highest average leaf pubescence ratings being found in past three years, leaf hairiness of varieties appears to be increasing. Leaf pubescence was significantly related to Q-score in only two of the 11 years. In those two years, a relatively low, negative relationship was found, which indicated that more hirsute varieties had lower fiber quality. However, the low magnitude and occurrence of these correlations suggests that Q-score and leaf pubescence are nearly independent.

Thus, lint yields of varieties in the Mississippi River Delta, USA, tend to still be negatively related to fiber quality in terms of both Q-score and leaf pubescence. Although overall relationships have not changed, some varieties may have broken this negative relationship, i.e. possess both high yielding ability and superior fiber quality. To address this possibility, varieties that produced a Q-score ≥ 70 were designated as having superior fiber quality. From 2005 through 2013, the proportion of varieties that had Q-score ≥ 70 exceeded 20% in only one year. In 2014, nine of the 34 entries (26%) produced

Q-score of ≥ 70 . Five of these varieties (15% of the entries) produced above average lint yield and had a Q-score ≥ 70 . None of these five varieties were rated as smooth leaf, but all had intermediate ratings (between smooth and very hairy). In 2015, 15 of the 42 varieties (31%) produced Q-scores of ≥ 70 . Ten of these varieties (24% of the entries) produced above average lint yield. Upper quadrant lint yields were produced by six of these 15 varieties. These six varieties included varieties developed by Dow, Monsanto, and the University of Arkansas. Two of these six varieties were rated as smooth leaf, one had an intermediate rating, and two were rated as being very hairy.

Since the negative relationship between lint yield and fiber quality has become less negative or perhaps neutral, researchers should be able to continue identifying lines that provide both high yield and high fiber quality. Although lint yield is positively related to the density of trichomes on leaves, some smooth leaf lines now possess both high yielding ability and high fiber quality.

The development of varieties that produce high yields of high quality cotton will certainly assist cotton producers in our current tight market, but will also have long-term benefits to the entire cotton industry.

Sukumar Saha

ICAC Researcher of the Year 2011

Genetics and Sustainable Agriculture Research Unit, USDA-ARS, Mississippi State, MS, USA

The world population is projected to reach over nine billion people by 2050. The rapid increase in population demands that current production for global food and fiber security be almost doubled within the next three decades. Farmers will have to fulfill this demand under conditions of rapidly declining agricultural resources, including land and water under the pressure of weather volatility. The cotton producers are facing some serious challenges, including yield stagnation, loss of genetic diversity, and demands for superior fiber qualities due to changes in textile technologies and competition from synthetic fibers. We have to find innovative solutions by integrating advanced technologies with conventional methods of agricultural practices to control pests, diseases and weeds and produce more stress-tolerant plants for sustainable cotton production. This paper highlights some important research areas focused on integrated approaches of novel biotechnologies, genomics and conventional breeding methods for future sustainable cotton production.

Pest and Weed Control Research

The advanced technologies of genetic engineering to develop plants resistant to both insects and herbicides provided the maximum economic impact to cotton farmers since 1996 among all of the major crops. The future success of this

technology will be based on the following specific strategies:

- Selecting the effective gene cassette composed of the most efficient promoter and genes of interest;
- Identifying the best event providing the best level of transgenic expression with desirable plant phenotype;
- Breeding the transgenic trait into elite cotton lines and developing a suitable management practice to achieve the desired effect in the elite lines; and
- Documenting the safety of the technology through regulatory research program. Recent studies showed that the new emerging tool of RNAi technology to block the expression of specific genes would have great impact in transgenic research, such as improving seed or fiber qualities.

Genetically engineered herbicide-tolerant and insect-resistant cotton lines will provide significant economic benefits to growers and the environment. However, it is important to have a strong ongoing regulatory research program and a safety network against the development of resistant weeds or insects and to study the ecological impact of any new transgenic technologies to control weeds or pests. For example, Bt toxin will not have adequate insecticidal effects

on sap-sucking insects, such as aphids, whiteflies, plant hoppers, and other plant bugs. This may create a condition where many minor pests will evolve as the major pests in cotton fields. In addition, the overuse of a single product or herbicide to manage weeds may contribute in the selection for herbicide-resistant weeds in cotton fields. The future research on stacking multiple resistant genes against a pest or weed through transgenic technologies in conjunction with an integrated insect and weed management strategy, such as herbicide or crop rotation or combination of both methods, and effective crop management system will be the critical factors in sustainable cotton production.

Next Generation Sequencing Technologies

The rapid development of the low cost next-generation sequencing technologies with the recent publication on decoding the cotton genome, such as A, D genome diploid species as well as AD tetraploid species of *G. hirsutum* and *G. barbadense*, have opened up a new paradigm in nearly all areas of basic and applied cotton research. These reference sequences provided a platform in conjunction with the rapid increase of functional genomic resources to collect valuable information on the association of phenotypes with functional genes for economically important fiber and biotic/abiotic resistance traits. Due to low cost and advancement in next generation sequencing technologies, soon RNA seq approaches will have ripple effects to open many new areas, such as large-scale study of the gene regulation, transcriptomes, proteomes and understanding the metabolic pathways to control economically important traits. The sequencing technology will help to understand the association of gene expression with differential epigenetic markings in the sub genome of the allopolyploid AD genome. Given the rapid innovations of cost effective sequencing technologies, an international collaborative effort to study and analyze genome-wide sequence variation and its association with diverse phenotypes in a large number of cotton accessions will help, like Arabidopsis, to understand the molecular control of important traits in cotton. Such valuable information will be very useful to identify the precise target gene sequences for improvement of specific traits using new genome editing technologies, such as ZFNs, TALENs, and CRISPR/Cas. The genome editing will not only help in characterizing the potential functions of the genes but also will help create new varieties without introducing any foreign genes by manipulating the genetic code of the target genes. Perhaps such lines will be more acceptable to mitigate the unfavorable opinion against GMO in public forums.

Marker Technologies

With the continuous declining sequencing costs, it is expected that in the near future the next generation of molecular marker development effort will be based primarily on genotyping by sequencing. The development of sequence-based SNP marker strategy will play the major role in future molecular mapping of QTLs and marker-assisted selection programs.

New technologies such as SNP chip consisting of high density SNP arrays, each containing ~63,000 Infinium assays (<http://www.illumina.com/applications/agriculture/consortia.html>) will be important tools in future molecular mapping research.

Conventional Breeding Methods

Future success in the genetic improvement of cotton will be dependent on an integrated approach of conventional breeding methods with new emerging technologies. Discounted prices for short-staple fibers with poor fiber qualities and yield will be the major concerns for cotton producers, so fiber quality and resistant traits against biotic and abiotic stresses will be key target points in cotton breeding programs. However, to maximize the economic benefits, cotton producers will be interested in improving some other novel traits such as seed qualities (e.g. gossypol-free cotton seed as an important source of protein or seeds with modified fatty acid composition). With rapid changes in climate as a result of global warming, high temperature stress will be a major factor in future cotton production. For example, heat stress could limit male and female gametophyte development or cotton fruit sets. Improving cotton cultivars with enhanced tolerance to limited moisture and heat stresses would be critical factors to mitigate yield losses with changing climate. The genetic diversity in the breeding gene pool of Upland cotton is narrow and considered to cause recent yield stagnation, decline of fiber qualities and increasing vulnerability to climate change. Wild tetraploid species are sanctuaries of many useful novel genes that remain unknown and underutilized in various cotton breeding programs. Interspecific germplasm introgression is normally constrained by genetic incompatibilities between the species. We have developed a series of chromosome substitution lines (CS) from *G. barbadense*, *G. tomentosum* and *G. mustelinum* in collaboration with Dr. David Stelly at Texas A&M University. Our results unveiled that CS lines will complement conventional breeding methods to unlock the gateway for discovery and targeted introgression of beneficial genes in future Upland cotton breeding programs.

Due to the globalization of agriculture, it is expected that commodity prices are likely to decline, and efficient agricultural management with superior traits in elite lines will be the key factors to combat future challenges. It is also important to note that cotton is a major cash crop in both developed and developing countries, where both environmental and economical impacts and the role of private industries and new technologies are very different in such complex agricultural systems. A coordinated global effort will have significant impact in future cotton research to provide maximum economic benefits against the primary challenge from synthetic fiber. The past records on the success story of cotton biotechnology demonstrated that a coordinated effort from private and public institute partnership at the global level would maintain the flow of scientific marvels providing maximum economic benefits to cotton stakeholders for sustainable production in the 21st century.

Andrew H. Paterson

ICAC Researcher of the Year 2012

Regents Professor and Head, Plant Genome Mapping Laboratory, University of Georgia, Athens, GA, USA

With a high quality reference sequence of the cotton D genome in hand that ranks among the best assembled plant genomes, together with draft sequences of the A, D, F and AD tetraploid genomes of *G. hirsutum* and *G. barbadense* now published, what are the next steps toward translating the promise of genomics into impact on both fundamental science and on-farm cotton productivity and quality?

In my view, two questions are central, each with several dimensions as follows:

What is the Spectrum of Diversity of the *Gossypium* genus, i.e. What is the Raw Material Available for Cotton Improvement by Conventional Means?

A genome sequence provides only one version of the ‘spelling’ (sequence) of each gene in a genome – variations in the spelling of a gene reflect botanical diversity at an elemental level. Some variations have no functional consequences, encoding the same amino acid or occurring in noncoding DNA. Other variations may have striking consequences, for example causing a truncated protein or one that is chemically incapable of performing its intended function. Indeed, the pattern of variations across a diverse sampling of genotypes is informative – ‘essential’ genes or nucleotides being intolerant of variations, while genes contributing to diversity may have a superabundance of variations.

With the basic gene set of cotton now known, economical methods for massively-parallel ‘re-sequencing’ can be applied to capture the spectrum of variations in a gene, among genotypes within a species, or species within a genus. Numerous cotton scientists are now engaged in the sequencing and analysis of their favorite cultivar/species, and one can anticipate that most of the relatively common variations on most cotton genes will be identified in the next 5 to 10 years. An essential element for this process is gene banks; the richness of diversity in gene banks will largely determine the richness of our knowledge of cotton diversity.

An intriguing fringe benefit of the rapid characterization of cotton gene sequence diversity is likely to be much greater insight into the consequences of cotton polyploidy. We now know that the predominant mechanism responsible for allelic differences between tetraploid cottons and their diploid progenitors is not random mutations but unidirectional DNA exchanges between homeologous chromosomes (i.e., homeologous gene conversion events or HeGCE) that have doubled the copy number of specific alleles. Striking spatial (across the genome) and temporal (across cotton evolution) patterns implicate this mechanism both in the generation of diversity that may have facilitated the evolution of the superior fibers of tetraploid cottons, and in silencing of abundant retro

transposons derived from the progenitor A genome.

How Do Scientists Relate *Gossypium* Diversity to its Phenotypic Consequences?

Rich plant genomic resources now available have motivated formulation of a host of ‘hypotheses’ – about roles of particular genes in the quality and productivity of agricultural crops, the ability to reduce demands on environmental resources, and the opportunity to address challenges posed by global climate change. For example, thousands of genes have been found to show ‘footprints of selection’, or ‘mutations inferred to have striking functional consequences’, or ‘expression patterns correlated with a phenotype’. How do we bridge the gap between the richness of hypotheses about genes implicated in physiological functions and phenotypes, and the limited time and funding available to thoroughly investigate individual candidate genes for such roles?

Much has been, and will continue to be, learned about the functions of plant genes by analogy – that is, by discovering the function of a similar gene in a botanical model and deducing its function in cotton. Major investments have been made in several botanical models, most notably *Arabidopsis thaliana*, the first plant to have its genome fully sequenced, which may have shared common ancestry with cotton 83 to 86 million years ago, making them rather close relatives in the context of evolution. Most cotton genes have recognizable ‘homologs’ (relatives) in *Arabidopsis*, and the facility of *Arabidopsis* genomics (and that of other botanical models) will continue to elucidate gene functions that translate with varying degrees of accuracy to the corresponding cotton genes.

While analogy to *Arabidopsis* or other genes will teach us much about cotton, it is not likely to teach us how cotton does things that *Arabidopsis* does not, for example how cotton produces seed-borne single-celled epidermal fibers that are long and strong enough to be spun into yarn and woven into fabric. Nearly 30 years ago, molecular genetics provided the means to identify the locations in the genome of genes conferring variations in agriculturally important traits, and these techniques have long been applied to cotton fiber yield and quality components, as well as many other traits, by using linked DNA markers. Methodological improvements such as ‘nested association mapping’ are being advanced in cotton, but will require major coordinated efforts to develop the phenotypic information necessary to take full advantage of these techniques.

Likewise, new technologies for disrupting the function of specific genes and observing what task fails to be performed are quickly being adapted from their origins in other organisms for application in cotton. Such techniques as virus-induced gene silencing (VIGS) and CRISPR-Cas system for plant genome editing are likely to find growing application in relating cotton genes to their functions.

Yusuf Zafar

ICAC Researcher of the Year 2012

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Focus On - Declining Rate of Cotton Production in Pakistan

Pakistan is Rapidly Losing its Supremacy in Cotton Production, as Total Production Has Been Either Stagnant or Even Declined in the Last Two Decades. The latest arrival data for 2015/16 show that Pakistan will produce 1.6 million tons in 2015/16 compared to 2.3 million in 2014/15. This decline in production of almost 30% and the cumulative loss of nearly US\$4 billion to the national economy is in addition to negative socio-economic impact in the cotton producing areas. Moreover, the local textile sector has to import about 400,000 tons of raw cotton this year (2015/16) to meet the shortfall. Serious decline in production occurred because of heavy rains, pests attack and harsh weather conditions on cotton crop. Pakistan has a relatively modest infrastructure for supporting cotton research that resulted in generating useful knowledge on cotton genetics and genomics. All these efforts were recognized by different national and international agencies, including two Pakistani researchers were awarded "Researcher of the Year" by ICAC in 2012 and 2014, respectively. So, what could be the possible reasons for such a dismal performance?

The tetraploid cotton *G. hirsutum* was introduced in the Indian sub-continent during British rule in 1929. The East India Company also laid a strong foundation of commodity-based research centers (cotton, rice, wheat etc.). After independence, Pakistan continued the system with minor changes at various levels. The cotton breeders of Pakistan released over 80 new improved cotton varieties and developed a highly effective integrated plant protection management (IPM) and later integrated crop management (ICM) system. All aspects of cotton production, including fiber quality, made substantial progress. The production of raw cotton began with less than a million bales in 1950 and reached 14 million bales in 1992. Such a spectacular performance was made despite limited funds and various natural and man-made calamities. The onset of a cotton leaf curl virus (CLCuV) epidemic in 1992 jolted the cotton researcher community in Pakistan very badly. However, with the help of international community (CFC/ICAC, USDA, ADB, etc.) and the government of Pakistan's focused attention (though 2-3 years late), this lethal disease was partly overcome. New varieties were developed with moderate resistance to CLCuV, and thus recovered the damage done by the disease. At the same time insect-resistant biotech cotton was introduced in neighboring countries (China and India) and illicit trade and uncontrolled

entry of highly susceptible varieties (not well adapted to the local environment) completely collapsed the varietal approval system. Weak implementation of existing Seed Act of 1976 and Cotton Act of 1965 was ignored and cotton production in the last ten years has hovered around two million tons, even exceeding 2.4 million tons in 2004/05, and a long-term average yield just shy of 800 of kg/ha of lint.

The reasonable adapted and applied research, presence of cotton research centers with good infrastructure, availability of field research stations in almost all eco-climate zones, a well-placed variety evaluation and approval system, all the required ingredients were present. Still cotton production continues to decline (what about last year??)! The single major reason is "Bad governance." The GoP is unable to cope and adjust to the demands of the recent era (WTO, UN-Cartagena Protocol, IPR etc.). The outdated Seed Act of 1976, which presently suited the bogus seed companies was not modified. The amended draft bill has been with government agencies for the last 16 years and still needs to be enacted. Similarly, the Plant Breeder Rights bill (obligatory under WTO) has been hanging round for the last 10 years. Pakistan made fantastic advances in cotton biotechnology and genetic engineering. Two national institutes gathered critical mass and sophisticated infrastructure, which paved the way for producing GM cotton indigenously. Pakistan is a signatory of Cartagena protocol and ratified this multilateral agreement in 2010. The federal EPA established a National Biosafety Centre and attended all meetings of the CPB, but was extremely slow in developing rules and placing system for evaluation and granting approval for GM crops in Pakistan. Only temporary approval for 5 years was granted to Bt-cotton developed by a few centers, which covers nearly 98% of the area of nearly 3 million hectares. At present more than 50 cases of GM crop testing, evaluation and final approval are with the NBC, which is reactivated during this month after a dormancy of nearly 3 years.

My analysis of the current situation is that, in the present era of collective governance, the government should be a good facilitator and a strong regulator. These functions must work hand in hand in order to reap the benefits of research, especially of modern biotechnology research wherein Pakistan has invested heavily and technically is very strong. The research, development and release of biotech varieties are heavily regulated and researchers are limited by cumbersome regulations. It is unfortunate that, despite making tremendous success in developing modern tools of cotton R&D in the country, the benefits of such researches are unable to reach the end users (farmers).

The present system inherited some problems from the past while others are related to adherence to international rules and regulations that prohibit fast application and utilization of research results. The best way forward for the present system is to implement recommendations made by the cotton sector in Cotton Vision 2020, enact the Amended Seed Act of 1976, the Plant Breeders Right bill and finally re-activate the National Biosafety Centre with a permanent infrastructure and trained human resources. This action requires little or, in some cases, no extra funding. Once all components are in place, increased investment by the private sector and a revival of public sector

research will accelerate. Short-term fixes are temporary solutions and cannot guarantee consistent progressive growth. Long-term vision and solution of issues for a country that has a monocrop (cotton) economy with a textile sector accounting for more than 65% of exports and main contributors to foreign exchange earnings and job providers are very critical for the cotton sector in Pakistan.

Disclaimer: The opinions expressed here are solely of the author and has no relation in any form to any organization to which he has already served or is presently serving.

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Focus On - Some New Directions and Priority Tasks for Worldwide Cotton Genetics, Breeding, Genomics and Biotechnology Research

Cotton (*Gossypium* spp.) is the most important natural fiber as well as an important byproduct (including feed and food) producing crop worldwide, grown in more than 80 countries to fulfill the needs of humanity. According to the ICAC, world cotton area is forecast to be 31.1 million hectares, with a total production of 22.5 million metric tons in 2015/16. World cotton consumption has been fluctuating; it reached its peak in 2007 (27 million tons) and has been declining since 2011 (~23 million tons), and now measures 24.3 million tons in 2014/15. Cotton production forecasts call for a continued decrease, with an almost 9% decline (to 23.8 million tons) in 2015/16 compared to 26.3 million tons in 2014/15.

This prompts the world cotton research and production community to substantially increase the average world yield, with subsequent stabilization and sustained cotton production. There is a huge need for solving issues associated with improvements of fiber quality without affecting yields and other key agronomic traits, tolerance to existing and emerging abiotic and biotic stresses, feed and food product qualities and staying competitive with manmade fibers while being non-hazardous to the environment.

To address these challenges and limitations, the cotton research community has made extensive research efforts and formed large-scale collaborative projects on the development of cotton genomics and genetic resources, characterization of germplasm resources, genetic mapping of key agronomic traits, development and application of modern “omics” technologies for cotton improvement. The global cotton research groups have completed the sequencing of whole genomes of two ancestor-like diploid *D*₅ cottons as well as two cultivated allotetraploid *G. hirsutum* and *G. barbadense* cotton genotypes. These efforts have expanded

our understanding of cotton genomes and genetic signatures behind the key cotton traits, so that subsequent usage may result in great achievements in cotton improvement programs. These seminal achievements, however, generated many needs, new directions, priority tasks, and grand challenges to be faced by cotton research community, some of which I will try to highlight below.

The detailed inventory of World Cotton Germplasm Resources showed that there are between 53,000 and 63,946 cotton germplasm accessions preserved in all cotton-growing countries. To better utilize cotton germplasm resources worldwide, there is an urgent need for: use of molecular markers with shifting from SSR-based characterization toward SNP-based analyses and genotyping by sequencing methods; wider application of association mapping, including nested-association mapping (NAM) and genome-wide association mapping (GWAS) methods, to facilitate reliable marker-assisted and genomic selection approaches; and evaluation of core sets of worldwide cotton germplasm from each country using DNA technologies for molecular diversity assessment. Such detailed analyses of existing international germplasm resources is the key, and exchanges of “only” useful and “needed” accessions may be effectively utilized in solving the key issues of a recipient country.

Consequently, the above-mentioned tasks require the development of high-throughput phenotyping platforms (cotton “phenomics”) to make effective and detail phenotypic evaluations in a large number and over a short period, which is an immense challenge in all plant sciences, including cotton. There is a need for ‘reference’ regional germplasm evaluation nurseries worldwide where cotton research community could phenotypically evaluate their sets of germplasm accessions and priority breeding/mapping populations across globally different environmental conditions. This would help to map biologically meaningful marker-trait associations taking into account very diverse environmental impacts accelerating

genetic improvement programs. Furthermore, these efforts would help to better utilization of existing genetic diversity to breed superior quality cotton cultivars. Most importantly, application of modern genomics tools and a large number of high-throughput SNP based DNA markers and new generation, high-throughput genotyping/phenotyping platforms would make the “breeding by design” and “virtual breeding” approaches possible for efficient cotton improvement.

With the availability of genome sequences and a large number of SNP marker collections, there is a need for the analysis of copy number variations (CNVs) in cotton genomes/genotypic accessions, and to link them to the key traits. Cotton mapping studies/groups should also think about using molecular phenotyping (i.e., using molecular process such as protein–RNA interactions, translation rates, etc.) in QTL mapping that would help to precisely link the sequence variation(s) to its phenotype(s). Genetical genomics approaches (i.e., use of expression QTLs) must be extensively applied in cotton research.

Although, as mentioned above, tremendous achievements have been made in the past 2 to 3 years toward sequencing key cotton genomes, more priority tasks lie ahead of us in this direction. A need exists to: 1) improve the sequence length that would solve the many incorrect sequence sites and genome assembly challenges which cotton genomics currently faces; 2) physically match all reference molecular genetic maps of cotton with sequenced reference genomes; 3) sequence the remaining cotton genomes utilizing better methods and the third generation sequencing platforms; and 4) initiate largely coordinated multiple species (e.g., the “IKP” project) and intragenomic accession (e.g., “1001 Arabidopsis accession sequencing”) sequencing efforts (including exome, transcriptome and whole genome) in the next phase of sequencing cotton genomes. In particular, for example, sequencing of “1002 Upland cotton accessions” would be most exciting and useful project for the cotton research community, which would generate more genomics resources and tools for cotton improvement programs. The extension of such intraspecies accession re-sequencing projects for ELS and other allotetraploid and their progenitor genomes should be a long-term target of future cotton research.

The completion of above highlighted tasks would also result in effective use of all variations existing among cotton germplasm resources and its ecotypic populations, design efficient GWAS analysis and consequent genomic selections as well as tools/software programs for better analyzing cotton genomes and improving genome assembly issues. Exploring a polyploidy crop, future cotton research benefits more from the sequencing of many polyploids, and their subgenomes that would increase our understanding the complexity of polyploidy, gene silencing, epigenetics, and biased retention and expression of genes after polyploidization. It also would help to discover all natural variations and lost genes during crop domestication that should be useful for restoring

key agriculturally important traits, as well as for cotton evolutionary, speciation and taxonomy studies in the future.

Addressing and completion of above-mentioned large cotton genome/species/germplasm accession sequencing tasks demand the development of better bioinformatics tools for handling, organizing, systematizing and visualizing of “Big Data” generated from cotton genomes. A future post-genomics task of the cotton research community would be to link the sequence variation(s) with phenotype(s), trait expression, and epigenetic and adaptive features of cottons to their growing environment and extreme conditions. This would make sequenced cotton genomes “functional” and biologically meaningful. To address these tasks, the cotton research community must institute combined approaches to develop bioinformatics, proteomics, metabolomics, phenomics, genomic selections, genetical genomics, reverse genomics, system biology, etc. This requires a special attention and funding of cotton bioinformatics direction.

There is a task for development and translation of the yet unexplored concept of “personalized agriculture” to cotton breeding and farming, requiring extended efforts on development of inexpensive high-throughput multi-accession/species genome sequencing and plant phenotyping platforms and efforts (as mentioned above), plant proteome and metabolome profiling tools and instrumentation by utilizing small amount single-cell derived samples. Development of chemical genomics for cotton would be an important new direction for the future, helping to provide a way for “personalized” agriculture for sustaining cotton production worldwide.

On the cotton biotechnology side, there is a huge need to have concentrated efforts on timely application of novel transgenomics (e.g., RNAi) and genome editing tools (GEENs such as ZFNs, ODMs, TALENs, CRISPR, etc.) for cotton, and to utilize complex effects of cotton developmental genes to simultaneously improve key traits and overcome negative trait correlations. There is a need to accelerate novel “biotech cotton varieties” using these novel technologies, extending the field trials and on-time commercialization of RNAi or genome-edited organisms (GEOs)/cultivars. These are currently present in a very limited state in cotton. The greatest attention, however, must be paid to proper regulatory policies, by providing understanding and removing confusion of regulatory agencies and stakeholders; otherwise, cotton production may not readily benefit from these efforts.

All of the highlighted new directions and tasks ahead of the cotton research community require the preparation of well-qualified next generation genomics, bioinformatics, and molecular breeding scientists with the capability to utilize modern computing and instrumentation platforms and genomics/bioinformatics knowledge, areas in which cotton research suffers from significant limitations currently. This requires urgent awareness, attention, and investment.

Although they may sound ambitious at the present time, these tasks and new directions in cotton ‘omics’ sciences will help to address many key issues and challenges regarding to simultaneous improvements on fiber, yield, and maturity, resistance to biotic and abiotic stresses of cotton, providing competitiveness of cotton fiber over synthetics. However, to accomplish the goal of needed tasks highlighted above,

there is a need for support from the International Cotton Advisory Committee (ICAC) and its member governments, the International Cotton Genome Initiative (ICGI), the International Cotton Researchers Association (ICRA), and strong international collaboration among the world cotton research community, which requires extended efforts and significant investments.

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Focus On - Cotton Improvement for Environmentally Stressed Economies

Cotton Provides Natural Fiber to the Textile Industry Worldwide, and is the Backbone of Economy of Many Developing Countries. Sustaining cotton yields under stressed environments is and will continue to be a major issue worldwide. Also, the changing climate further worsens the climate crises—that has been witnessed recently in Pakistan where an approximately 35% reduction in yield is expected in current season (2015/16) compared with the previous year.

Efforts towards achieving sustainability in cotton production are handicapped by lack of genetic diversity in the adapted genetic material, limited knowledge about the genetics of most complex traits and non-availability of robust DNA markers. Consequently, current breeding practices remain unchanged. Bringing new genes under plough from untapped genetic resources (unutilized germplasm and wild cotton species) are not very successful because of the linkage drag of many unwanted genes, which negatively impact the resultant phenotype. Before introducing alien alleles from an untapped genetic resource, it is important to know about genetic variants through deploying next generation sequencing (NGS) tools—previously not possible. In this regard, “re-sequencing” of germplasm accessions, obsolete cultivars and land races can be undertaken to characterize genome-wide variations. Consequently, new SNPs (including insertions/deletions and substitutions), copy number variations, etc. would be identified. These variations can be translated into functional diversity, and thus DNA markers can be designed that would set a firm foundation for initiating intelligent breeding programs. If the resources are limited or ploidy level (ancient or recent) is high (especially in tetraploid cotton)—thus making computational analysis difficult, a reduced representation approach (for example exome capturing) or sequencing the transcriptomes may help in detecting variations in functionally active genes. The identified genetic variations can also be used to mine diversity within cultivated cotton varieties. For example, the cotton species evolved in a particular region containing important genes helped in adaptation in a corresponding region would help in developing cotton varieties with high adaptability. *G. arboreum* has been evolved in drought-prone

areas, and presently its cultivation is restricted to marginal land (less than 2% area). It was replaced by the *G. hirsutum* in Indo-Pak region. Presently, the *G. hirsutum* cotton varieties (especially in the post GM-cotton era) express their yield potential under high input environments—making the crop vulnerable to insect pests and diseases, and also to harsh environments. Similarly, the natural genetic variations present in obsolete cultivars can be re-introduced in modern cultivars using the generated genomic information. Thus the genetic information would help in selecting cotton genotypes/strains with the traits involved in adaptation to the corresponding environments without losing their yield potential—enabling cotton production to be sustained.

Though a significant number of genes present in the cultivated species and their progenitors have been identified using NGS tools, these don’t necessarily reflect the whole diversity present in the species. The germplasm present in seed banks could be characterized phenotypically in a range of environments using high-throughput phenotyping platforms—recording data both under controlled as well as field conditions in a non-destructive way. Also, the transcript sequencing and biochemical analysis of diverse accessions grown under different environments would help to gather knowledge about phenotypic variation that is not possible solely with genome sequencing. In this way, one can identify QTLs accurately and efficiently, which can be linked to DNA markers.

TILLING (Targeting Induced Local Lesions IN Genomes) is another approach for inducing mutations randomly by exposing cotton seed to chemical mutagens. The resultant stable mutant lines can be re-sequenced to identify the mutations across the whole genome (genome wide) and/or exome regions can be sequenced (exome capturing approach) for identifying mutations that can be linked to functional diversity. This is a very straightforward strategy for enhancing genetic diversity—a potential buffer to the spread of diseases. The mutagenized populations of either tetraploids or diploids have shown improvement in lint quality and also the ginning out turn (GOT) percentage. Breeding for high GOT (more than 45%) should be the ultimate aim of cotton breeders. Adapted cultivars with high GOT potential can be the best target to improve GOT percentage. Newly identified tools,

such as ZFNs and CRISPR-Cas9, can induce mutations in target genes without disturbing the whole genome. However, their potential is yet to be realized commercially.

Resistance to insect pests in cotton has been engineered using genes (*cry* genes) excised from a soil bacterium—success has been demonstrated. Now the resistance conferred by these genes has been broken down in different parts of the world. For example, single gene protection enjoyed for couple of years in Pakistan has been overcome due to the infestation of *Helicoverpa armigera* in June 2014 on early sown cotton crop and *Pectinophora gossypiella* in 2015/16. During 2015/16, unexpected drought, heavy rainfall, storms and floods, which not only enhance the boll rotting and lodging but also led to weeds outcompeting the cotton crop—resulted in reduced boll number and size. Thus, stressed climatic conditions together with the infestation of pink bollworm badly hammered cotton production in Pakistan. Also, the potential of converting minor pests into major pests is another threat to cotton sustainability. For example, before the cultivation of Bt-cotton in Pakistan, mealy bug and dusky bug remained unnoticed on cotton—indirectly controlled by the application of insecticides used to kill lepidopteron pests. Now, however, chemical control measures are taken to control these two newly emerged pests.

Such scenarios can emerge in other cotton-growing countries. Other than educating the cotton farmers on taking control measures, it is important to increase the range of novel genes derived from other alien sources under the tissue specific promoters which would help in combating the insect pests much more effectively. Also the genes and/or their transcription factors (DREBs, ERFs, ZIP, WRKY etc.) conferring tolerance to biotic and abiotic stresses from plant sources including wild species can be characterized followed by their introduction in cotton. It is worth mentioning that the protein expressed by the *cry* genes did not interact with the complex biochemical pathways involved in shaping phenotypes as these were novel. However, genes of plant origin may interact with host protein—may change the expression of the transgene. New horizons of controlling insect populations such as production of intact RNA molecules of essential proteins in plastids should be undertaken. Novel herbicide tolerance genes (other than EPSPS) are required to suppress the emergence of herbicide-tolerant weeds in cotton. However, the plant source genes would have high chances of acceptance by the end user. Identifying new marker genes (other than antibiotic-resistant genes) would be another step towards increasing the acceptability of GM-crops worldwide.

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Focus On - Integration of All Research Disciplines for Future Production Systems

There are many disciplines in cotton research – and each discipline has a wide range of levels from basic to applied, so discussing new directions is a daunting challenge. My areas of specialization have been applied plant breeding and crop physiology, so I will be more comfortable in concentrating on those aspects. However, I have interacted with many other disciplines, so where appropriate, I will highlight the opportunities across research boundaries. In fact, no single research discipline and level will solve all future cotton problems and it is the coordination and collaboration of different research groups that will have the greatest impact in developing solutions and making new discoveries.

Half the world's cotton has lint yields less than 800 kg/ha. Many of those crops are rainfed and the low yield potential reduces grower confidence to invest in pest control or fertilizer inputs. It is a challenging question as to whether research investment into such cropping systems has return on investment. Such questions are becoming more common.

Breeding

Good traditional breeding practices will continue to be important. The fundamentals are appropriate population sizes,

using the best parents with good genetic diversity and accurate testing of candidate genotypes in the cropping systems of interest.

Breeding with multiple GM traits has become complex and that situation will continue, particularly to introgress multiple traits into new (improved) conventional germplasm. Although breeding with multiple GM traits is slower, the same breeding procedures are required with GM traits as for conventional; a simple backcross and bulk will not necessarily recover elite yield. Our experience is that there is diversity in yield performance within backcross-generated GM populations, so careful evaluation of elite lines is required.

Diseases are significant constraints for cotton in most production systems, so cultivars with (multiple) disease resistance are a priority. The focus in Australia has been on Bacterial Blight, Verticillium Wilt, Fusarium Wilt and Cotton Bunchy Top (a virus similar to Blue Disease), with the objective of having resistance to all diseases in all cultivars. There is potential for other diseases, particularly viral, to appear and pre-emptive research is necessary to prepare contingency plans for such an occurrence.

Cotton is grown primarily for its fiber, but competition with man-made fibers (MMF) has resulted in continued reduction of cotton's use in textiles. Clearly an important direction

for cotton is to improve demand from spinners and fiber quality improvements are required. We have coined the term Integrated Fiber Management (IFM) to highlight the need for considering fiber quality across the whole production chain and research disciplines. Plant breeding might improve fiber quality potential, but good agronomy and ginning are required to achieve that potential. Economic viability (price) will continue to be a challenge for producers, as cotton fiber competes with MMF. Research on improving fiber quality therefore may also need to assess reducing loss of market to MMF rather than increasing cotton unit value.

Collaboration Between Breeding and Other Disciplines

One opportunity for improved outcomes from research is improving the organizational structure in research teams. Better integration of research disciplines is required in some cases. Compare the structure of large commercial biotechnology companies with the situation 15 years ago: Breeding and molecular teams are now more integrated and other resources, such as weather data, are being incorporated into customer services. Other research agencies could replace fragmentation of research groups with coordination. The establishment of an International Cotton Researchers Association (ICRA) has one aim to facilitate more communication and collaboration between researchers.

Molecular Biology

The development and use of GM cotton resistant to Lepidopteran pests has substantially reduced insecticide inputs (by 80% in Australia) and improved control of those pests. Likewise GM cotton resistant to glyphosate or glufosinate has reduced residual herbicide inputs while improving control of some weeds. Resistance management of these GM traits has not been perfect – hence the need for stacking insect- or herbicide-resistant genes. However cotton is much better off environmentally and the regulatory lessons have been learnt.

It appears that the number of new GM traits being developed is slowing down, but modern molecular marker tools will facilitate better early generation screening in breeding. This area of research has been mentioned and covered in all contributions to this document and it is clear that such marker technology will become more prevalent, for example to screen breeding material, which contains new sources of disease resistance. Breeders and plant pathologists will still need to search for new sources of disease resistance. SNP-based markers of elite germplasm will assist with more rapid backcrossing of GM traits. Thus it is vital that cotton breeders and biotechnologists work closely together.

Agronomy

We have found that selection for increased yield has resulted in cotton cultivars with increased waterlogging tolerance, nutrient use efficiency, water use efficiency and even net leaf photosynthesis rate. These improvements, as well as reduced emissions, are expected from all industries and cotton research is being directed towards a better understanding of interactions between genotype, environment and management to deliver more sustainable cropping systems. Collaboration between researchers can develop integrated cropping systems for pests (IPM), weeds (IWM), diseases (IDM) and fiber quality (IFM). Development of cotton cropping systems, which are water and nutrient use efficient, are also vital and close collaboration between breeders and crop physiologists is necessary.

The Future/Conclusions

There are many challenges facing cotton, not the least being competition with MMF. In addition, potential climate impact on rainfed and irrigated cotton production systems may reduce production or at least cause variability of production. Changes to water availability may also occur with competition between urban and agricultural demands. These effects may change where cotton is produced. Nevertheless, there is remarkable cotton research capacity across the world and, with proper coordination, solutions can be found to enable cotton to flourish as a commodity.

Developments and Opportunities in Cotton Breeding

Breeding is the development of superior varieties/cultivars/genotypes/germplasm lines, and even hybrids, for commercial production or utilization in breeding programs. Cotton breeding has been going on for centuries and is certainly much utilized/more widely explored than any other scientific approach to agriculture. Contributions from breeding are so immense that other disciplines have only endeavored either to recover the true value of breeding efforts or tried to protect achievements acquired from breeding. These are the breeders who domesticated cotton, though mainly through selection, to be grown as an annual crop and produce lint that has enriched consumption value. An examination of many cotton research programs shows that the initial efforts in breeding were upgraded into research stations and institutes, which emerged as multidisciplinary focal research centers on cotton. Instances where the inverse happened and the breeding of varieties was added to an existing entomological or agronomic research program may exist, but are rare. In cotton, breeding is the leader and at times was regarded as the central axis of any cotton research program.

Target plant breeding started about 10,000-12,000 years ago when man observed that if a seed falls on the ground it germinates and produces a new plant (Roupakias, 2014). Just one hundred years ago researchers were still struggling to accept the Mendelian Law of Inheritance and the Law of Independent Assortment. Mendelian genetics was ignored for almost 25 years due to hesitation in admitting the existence of genes or accepting that heritable characters are genetically controlled and cannot just be transferred as if acquired. The genes assort independently without any outside influence.

The extensive research done on cotton became more formal and was easier to understand after it was discovered that there are genes that carry a blueprint of the characters to be expressed under a given set of growing conditions. Such discoveries, unimaginable in the early years of cotton research, were severely questioned and remained shelved for about half a century. The theory of evolution did not satisfactorily address many concerns, and it was practically impossible to give up the long-held belief in the inheritance of acquired characters. Fortunately, however, the laws of inheritance of characters and the independent assortment of genes were rediscovered and applied. Thus began the science of formal breeding we know today.

The Cotton Breeding of Yesterday

Only three methods of breeding have been employed throughout the world, i.e. introduction, selection and hybridization. Varieties have been imported from other countries and directly adopted for commercial cultivation.

This is probably the most obvious and easiest way to improve production based on improving the genetic background of cultivation material. Cotton production itself was initiated by introduction in the Indian subcontinent, it initially failed and then succeeded. The reliance on introductions has diminished since it has been understood that there is a science behind the carryover of characters and they cannot merely be manipulated based on production conditions. Introductions do not have an impressive history of success, although varieties developed in one part of the world do have a chance to excel in performance when grown under more suitable conditions within a country or across countries.

Selection from within a population, having not been produced directly through hybridization, has also proved successful in the past. The material from where selections were made often comprised adopted/commercially grown varieties. The major limitation to the selection method of developing varieties has been a lack of sufficient variability, as selection had to rely either an existing variability in the population resulting from natural out-crossing or natural mutations. Drastic deviations from existing populations were not expected and usually there was no fear of adaptation issues.

Because of the limitations described above, efforts were made to induce variability in the existing homozygous populations via mutagenesis. Chemical mutagens were employed without much success. However, radiation was extensively used in a hit and miss fashion in many countries in the 1960s and 70s. Gamma rays were more effective than other sources of radiation on cotton for desirable mutations. A number of different doses were tried, and it was found that 30 and 35 krad induced chromosomal changes with a minimum number of deleterious mutations. Pakistan probably benefitted the most from gamma radiation in the form of developing heat-tolerant varieties. The variety NIAB-78, developed through radiation was once planted on over two million hectares in Pakistan. Although the variety showed continuous segregation at low level, apart from commercial use, NIAB-78 provided a base for developing heat-tolerant varieties, which was the most important factor limiting yield until early 1980s. There are many different ways of using radiation to induce beneficial mutations, including radiating wet/soaked seed, pollen grains, using varying doses, and using different sources of radiation; all have been tried. Scientists have concluded that the high number of deleterious effects and undesirable linkages induced by radiation rendered this technique too costly and inefficient to continue.

The hybridization procedure has existed parallel to the other two approaches, but hybridization has not been employed on a large scale for several reasons:

- Hybridization is a long process involving the crossing of two parents, selection from segregating populations starting from F₂ generation onward, and attaining genetically pure breeding lines before they are carried forward for commercialization.
- Populations that segregate starting from the F₂ generation have to be grown in sufficient numbers so that the maximum number of combinations can express themselves. A small F₂ population means that all the possible combinations cannot occur in the field, and subsequent generations will also be limited in the number of expressions they can exhibit, either through the bulk selection method or with progeny rows. Managing large segregating populations and various segregating generations of each population requires huge resources.
- Selection from segregating populations in the field requires vigilant breeders' judgment on what should be carried forward and what not. Fiber quality was used as a criterion for rejecting single-plant selections or bulk populations, but only when the rest of the material had been discarded. It was common that breeders always had difficulty in deciding which plants and progenies to reject, and it was easier for them to select more rather than fewer plants and lines. The cost of carrying huge populations from one generation to the next restricted attempts to try more combinations and crosses.
- Nevertheless, hybridization continues as a modern method of developing varieties, but the practice relies mostly on the experience and judgment of breeders, rather than science. In other words, breeding is practiced more as an art of selecting better plants that will continue performing better in subsequent generations and will become commercial varieties.
- Unfortunately, breeders lacked a precise test that would ensure success. However, a big team of breeders attempting a large number of crosses has a higher chance for success because they are screening more combinations. Nevertheless, there is no way to guarantee that large teams will develop a superior variety than a small group of breeders attempting a few crosses every year.
- Mutagenic control of various characters, negative correlations among desired characters, and other factors complicate and restrict the flow of varieties. There were gene combinations that every breeder desired to introduce into varieties, but they were not successful because they could not pick and choose genes carrying the desired characters.

Various efforts were made to overcome some of the constraints to successful breeding of superior lines. These included using backcrossing to retain or transfer a limited number of characters, the same technique now used to transfer biotech genes. Single crosses, double crosses, varieties crossing

with segregation populations and many more options have been tried, but only back crossing proved its worth. The development of varieties with single, double and even triple biotech gene transfers is possible because of the experience learned from conventional breeding.

Variety Development and Seed Production

In spite of all the limitations it was unequivocally recognized throughout the world that comparative advantage in cotton production was in large part based on its superior seeds. This meant that farmers could purchase improved seed and imitate the innovation by planting and increasing it locally. The genetic superior hidden behind the seed was least acknowledged.

Development of a variety and seed production are two separate tasks. The role of seed production was recognized much after the impact of breeding was acknowledged. Then followed the importance of variety maintenance. A breeder could develop a good variety and it might be lost due to lack of a good seed production system. Once a variety is developed, it is necessary that varietal purity is maintained and high germination seed is provided to farmers. Grading, proper packing, instructions on the package, timely delivery, proper seed rate to be used, among others are linked to the success of a variety.

Many countries have realized the fact that variety maintenance and seed production should be separated, with the latter being transferred to private seed companies. Variety maintenance, that has a role from breeders, is the next task that private companies took over from the public sector. This change has just been completed in Australia, China, India, Pakistan and the USA. Turkey has joined the same system in the last few years. Among these countries, there is not any country where all the three stages i.e. variety development, maintenance and seed production, are in a single hand without any competition. In the USA, USDA breeders develop germplasm lines that could be used by the private sector in the development of varieties but university breeders are free to develop varieties for commercial adoption. They were doing so until the biotech genes became part and parcel of any commercial variety. In other countries the public sector competes with the private sector to develop and commercialize varieties.

What is Breeding Currently Going Through?

Breeding is increasingly conducted within the private sector, although it will take many more years until this shift is complete. The public sector in many countries see private sector breeding as a challenge to their authority and have sometimes been reluctant to cooperate.

Public sector breeding programs have often been judged in terms of the number of varieties released for commercial production and the area planted to such varieties. Some

breeders fear that financial support from governments will dissipate if they surrender the right to commercialize varieties. However, the shift to private sector breeding is a reality, and this shift allows public sector resources to be better utilized in other areas of research that require higher attention than received in the past. Variety approval and seed certification are two aspects of the planting seed development chain that could stay with the public sector.

Breeding programs, whether in the private or public sector, are faced with issues that will have long-term consequences if proper measures are not taken. Constraints are often openly discussed, but solutions are not given, or if solutions are mentioned, pathways are not shown to resolve them. Hence the elimination of constraints is not expected soon, and the consequences are potentially severe and long lasting.

The major issues confronting cotton breeding programs are:

- The genetic base of current varieties is narrow. Only a limited circle of varieties are hybridized to produce newer varieties, hence the breeders end up with only a slight chance of achieving improvements over existing varieties. Individual companies or public sector breeding teams are using their own varieties for the sake of maintaining their known and accepted stylized series. They are doing so to have a higher success rate in commercializing new varieties than they would have if they attempted to cross two diverse genotypes. So, breeders themselves have fused this problem into their breeding programs, and they are the ones who must change their approaches. A breeding process can produce change in the composition of a population only if there is variation from which to select.
- Germplasm exchange has almost disappeared. Germplasm availability is the extent of freedom that a breeder has to obtain and use any genetic material that exists in or outside a country. The result of stringent plant variety protection in individual countries is restricting breeders' capacity to freely use any genetic material. These restrictions inhibit further development of innovations. It is true, to some extent, that while some countries have been collecting significant amounts of new germplasm, they are not reporting significant rates of providing germplasm beyond their own borders. No statistics are available to report in cotton, although CGIAR data show this trend in other crops.
- The advent of biotechnology and intellectual property laws has increased the market for improved seed. These changes made it easier for commercial breeders/companies to be rewarded and to recover the cost of their investments. But, at the same time, intellectual property protections limit the ability of farmers or rival breeders to reproduce seed. Private seed companies have benefitted the most from the expansion of the seed industry, to an

amount exceeding US\$2 billion. Public sector breeders and programs have not been compensated for their work to the same degree as private programs, resulting in shrinkage of public sector breeding programs.

- The focus in cotton breeding has shifted to short-term objectives to achieve quick returns. Budgets for long-term fundamental scientific research are shrinking, which is not in the ultimate best interest of achieving breakthrough achievements.
- The rise of molecular genetics, particularly with the commercialization of biotech crops, has accelerated the shift toward private sector plant breeding. Many scientists believe that as conventional breeding is replaced by biotechnological approaches, public sector programs and institutions will retreat from classical plant breeding. This, in turn, has a negative effect on breeding education at universities and research work at public institutions (Roupakias, 2014). Biotechnology is relatively new and can be done in large centralized laboratories; there has been a rapid expansion of biotechnology research. This research is essential, but a reduction has also occurred in public sector plant breeding efforts, which could result in a lack of progress in the development of elite germplasm and effective commercial cotton cultivars (Constable, 2015). Conventional breeding and molecular genetics are complementary and both are needed to fundamentally improve cotton varieties.

The most relevant Plant Variety Protection related laws are Plant Variety Protection (PVP), the International Convention for the Protection of New Varieties of Plants ("UPOV Convention"), Intellectual Property Rights (IPR), Convention on Biological Diversity (CBD) and patents. Up until the 1960s, plant genetic resources were traditionally more openly shared, moved rather rapidly around the globe and rapidly utilized. Most sources of origination were in the public sector and, therefore, the plant genetic resources were conceived of, and treated like, public goods. The organizations and individual scientists were free to collect and use genetic materials from any part of the world in their breeding programs. Would that was not the case at the time of developing short duration fertilizer tolerant wheat and rice varieties, the occurrence of green revolution would have been delayed significantly. In the last fifty years, however, an increasing proportion of plant genetic resources have been subject to various forms of capture, as a result of advances in applied bioscience and the promotion of exclusive legal protections. As such, they have been converted into appropriated, private goods. International intellectual property rights are practically non-existent. The current intellectual property rights are mostly territorial in nature, and they are acquired and enforced on a country-by-country basis. The feeling among the stakeholders shows that no one took advantage over the others because of plant variety protection and other restrictions.

High Potential – A Challenge for Breeders

Since the acknowledgment of genetics as carrier of characters and furtherance of physiological understandings on how the cotton plant reacts to the biotic and abiotic factors, breeders have admitted that they have a high challenge of reaching near to the genetic potential. The genetic potential (sometimes referred to as theoretical yield) could not be assessed in quantitative terms. The plant morphology dictates that total number of bolls on the plant could be as many as the number of leaves minus the number of branches, but the question then arises of how big a boll (boll weight) will develop. The proportion of lint to the seed is another critical factor inhibiting the calculation of quantitatively accurate theoretical yield. It is undoubtedly admitted that only a portion of the real potential is realized. Realization of genetic potential is considered to be impossible or unreachable. The factors hindering the research to reach genetic potential are many, interdependent, complex and misunderstood or even insurmountable.

Breeders talk of recoverable potential, by which they mean how much potential can be extracted/realized under a given set of production conditions. While the genetic potential may be closer among cotton-producing countries, recoverable potential, which is influenced by the production conditions, varies hugely among countries and even among farmers within countries. The author does not agree with Constable (2015) who stated that the theoretical yield in Australia under best irrigated condition is about 5,034 kg lint/ha. According to him, Australia reached 3,500 kg lint/ha under best conditions in 2015. This means that breeding and all other allied disciplines together have a target of increasing yield by 44%. The author is of the opinion that the indeterminate nature of the plant does not permit us to quantify the upper limit.

Cotton Breeding of Tomorrow

Cotton breeding is in a high-transition stage in which the way that genetic principles are applied is changing. It is quite possible that 'conventional breeding', in conjunction with transgenic breeding, complemented with even newer developments in biotech approaches, will find a common name. The name for this new breeding approach is not known, but it might be something like 'directed breeding,' wherein the breeder will have a specific, predetermined target, and the breeder will hybridize with certainty. Future breeders will not be working with hit-and-miss trial methods and with an unlimited wish list of targets. Rather, breeders will have a source for the anticipated outcome.

The long process of selection, currently followed for the sake of producing a homozygous population, has to be shortened. The production of haploid plants and the doubling of chromosomes in the cotton genome have long been targeted, but additional options may become available with the progress we are making. The other time-consuming aspect of breeding

is multi-location performance testing of varieties under varied sets of agronomic practices. This also needs to be changed. It is not cost-efficient to test candidate varieties at many locations, under various sets of agronomic situations, and then make selections based just on normal conditions.

Cotton, especially *G. barbadense*, is highly sensitive to photoperiodic complexities and thermal conditions. Researchers have talked about 'global varieties' that will perform equally well anywhere in cotton-producing areas. Imagine if a good team of breeders is allowed to focus on innovative developments rather than breeding varieties based on luck and having to wait 12 to 14 years to know the fate of their breeding lines.

As a breeder myself years ago, I recall that many exceptional germplasm lines were deficient in only one or two traits, but those traits were so important that the germplasm lines were discarded. Discarding such genotypes, which is routine, is a waste of genetic resources. Useful gene(s), for example genes for fiber length or strength, that are deficient in an otherwise exceptional germplasm line can be transferred through genetic engineering, for which molecular markers are available or could become available. The deficient lines can be used as recurrent parents for marker-assisted accelerated back-cross breeding methods. Transgenic breeding has already allowed researchers to find suitable genes in related and non-related species and induct them into their desired lines.

The biggest change is, of course, going to come from biotechnology applications. It is obvious that many more biotech cotton varieties with single-gene and pyramid-gene traits will be available soon. These varieties will not be limited to the herbicide-tolerant or insect-resistant traits currently available, but will exhibit unique features. Over the next 10, 20 or 30 years, breeding will be employed to transfer specific new traits into current varieties. Quoting many researchers, Abdurakhmonov (2013), stated that the 21st century's "omics" science and innovative genomics tools are the most promising approaches, in combination with contemporary cotton breeding knowledge and strategies. The strategies include (1) accelerated development and success of transgenic, cisgenic and intragenic biotech crop technologies with complex effects targeted to improve the intrinsic yield in cotton, and (2) decoding of cotton genomes and the mapping and characterization of the genetic basis of complex traits (referred to as quantitative trait loci-QTLs) that provide better exploitation of existing genetic diversity of cotton germplasm and gene pools, and a widening of the genetic diversity of commercialized cotton cultivars using modern marker-assisted selection (MAS), marker-assisted backcross selection (MABS) and genomic selection (GS) programs.

Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers (Jiang, 2013)). Classical markers include morphological markers, cytological markers and biochemical/protein markers. DNA markers have developed into many

systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) (Collard et al., 2005), such as RFLP (Restricted Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), SNP (Single Nucleotide Polymorphism), etc.

The biotech cottons, commercialized over the past two decades, undoubtedly increased farmers' income wherever they were adopted. It may be remembered that farmers have benefitted from biotech cotton because of decreased insecticide use, lower cost of production and overall increased operational yields. The breeding programs will emerge like molecular cotton breeding labs. Molecular breeding implies molecular marker-assisted breeding (MAB) and is defined as the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant or animal traits on the basis of genotypic assays. Molecular markers are the firm landmarks in the genome of an organism rather than the normal genes because mostly they do not have the biological impacts and may or may not relate with phenotypic expression of a trait. Research on genetic improvement for developing new varieties will be based on utilization of classical breeding techniques as well as new DNA markers and gene transformation technology. Molecular markers will become an important tool in plant breeding and some complexities linked to DNA-based assays currently hindering its use in practical plant breeding will ultimately be overcome. Cotton breeding (i.e. crossing selected parents and planting large segregating populations from F₂ on in the field for selecting those few genotypes with superior or novel attributes) requires a DNA marker system that is reliable and capable of screening large populations for trustable results. DNA markers linked to a particular characteristic, agronomic or quality-related, will be identified and utilized. Work is already going to find closely associated markers.

The use of marker-assisted technology will be most easily employed when a particular feature is controlled by a small number of genes, and their impacts are little influenced by ambient conditions. Unfortunately, many genes control lint yield and fiber quality properties, each inducing only a small effect. For example, Shen *et al.* (2011) stated that advanced-backcross quantitative trait locus (QTL) analysis of an interspecific *G. hirsutum* × *G. barbadense* population showed that 28 fiber length QTLs were identified, including qFL-*chr1* on chromosome 1 of the A-sub genome. The *G. barbadense* allele at this QTL contributed to longer fibers and explained up to 24% of the phenotypic variance. Managing these quantitative traits is even more difficult because of the number of genes involved. DNA markers associated with QTLs for improved fiber quality, such as length, strength and uniformity, will be explored for Pima cotton. It might take many years to overcome challenges, such as the simultaneous improvement of yield and fiber quality, but molecular

technologies will certainly accelerate the process of improving the cotton genome. The Cotton Marker Database at <http://www.cottonmarker.org/cgi-bin/cmd_search_marker_result.cgi>, has hit 9,027 records, and most of them were reported after 2000 and many in the F₂ or BC₁ populations.

Malik *et al.* (2014) have presented a good review of the role of molecular markers in cotton genetic improvement, including future prospects for the practical utilization of new molecular technologies. An overview (of literature) of genetic diversity studies in cotton using different kinds of markers, i.e. RAPDs, SSRs, AFLPs, ISSRs (Inter Simple Sequence Repeats) and SNP, showed that thousands of upland and barbadense populations, and a number of diploid cultivated and uncultivated species genotypes, have been studied in the USA, China, India, Pakistan and many other smaller cotton-producing countries. Enormous work is going on to develop more efficient DNA markers for plant breeders and geneticists in order to develop cultivars of cotton in more efficient ways. It is hoped that SNP markers will have a large influence on molecular-assisted selection and mapping studies in the future due to an abundance of sophisticated detection systems that will be developed.

Summary

It is imperative that the international scientific community understands that plant breeding is primarily an organismal science and serves as the backbone of research developments. Breeding is comprised of variety development, variety maintenance (including variety approval and certification) and seed production; all of which have continuously changed. The private sector is increasingly involved in some of the important components of the breeding chain, and the role of the public sector has certainly diminished. A mix of different approaches is needed, and there must be agreement as to who is responsible for what. While scientific centers and seed breeding systems will be changing their modalities, they cannot ignore the fast approaching molecular-breeding technologies. Marker-assisted breeding and empowerment over directed breeding is the new norm of cotton breeding. Conventional breeding will be replaced by molecular breeding, a joint venture of breeders and molecular biologists.

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New Publications

Dictionary of Cotton

This is a joint publication of the ICAC with the International Cotton Researchers Association (ICRA). Thirty-three researchers from around the world have defined over 2,000 terms used in cotton production, processing and use. The publication is available at US\$50 (including shipping). Send your orders to publications@icac.org or visit the web page at www.icac.org.

Climate Change and Cotton Production in Modern Farming Systems

The Technical Information Section of the ICAC has published extensive review articles from time to time. The topic was selected based on current needs of the cotton research community. The sixth review article of the ICAC on cotton production research is on the topic of Climate Change and Cotton Production in Modern Farming Systems. Dr. Mike P. Bange of the Commonwealth Scientific and Industrial Research Organization, Australia, along with 11 other researchers from Australia and USA authored the publication. The article outlines research approaches to address future climate change and provide details for the formation of robust frameworks to evaluate the impact of projected climatic changes. Worldwide, cotton is broadly adapted to growing in temperate, subtropical, and tropical environments, but growth may be challenged by future climate change. Production may be directly affected by changes in crop photosynthesis and water use due to rising carbon dioxide and changes in regional temperature patterns. Indirect effects will likely result from a range of government regulations aimed at climate change mitigation. While there is certainty that future climate change will impact cotton production systems; however, there will be opportunities to adapt. This review begins to provide details for the formation of robust frameworks to evaluate the impact of projected climatic changes, highlight the risks and opportunities with adaptation, and details the approaches for investment in research. Ultimately, it is a multi-faceted systems-based approach that combines all elements of the cropping system that will provide the best insurance to harness the change that is occurring, and best allow cotton industries worldwide to adapt. Given that there will be no single solution for all of the challenges raised by climate change and variability, the best adaptation strategy for industry will be to develop more resilient systems. Early implementation of adaptation strategies, particularly in regard to enhancing resilience, has the potential to significantly reduce the negative impacts of climate change now and into the future.

The publication is a joint venture of CABI and the ICAC. ICAC and CABI jointly published this article. The 61 page publication is available from <http://www.cabi.org/bookshop/book/9781780648903>.