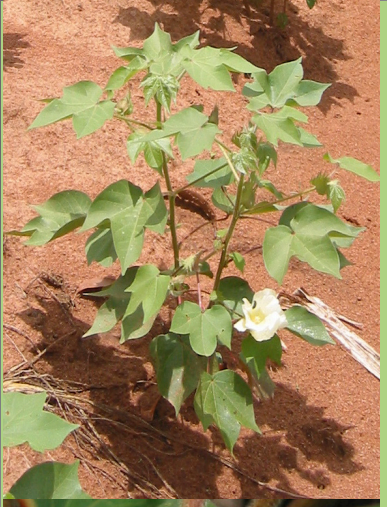

THE ICAC RECORDER

International Cotton Advisory Committee

Technical Information Section

VOL. XXVII No. 3
SEPTEMBER 2009

Update on Cotton Production Research

Discover natural fibres 2009

Contents

Introduction

This issue of the *ICAC RECORDER* is comprised of three articles. The first article 'Challenges and Opportunities in Cotton Production Research' is from the ICAC Cotton Researcher of the Year 2009. Dr. Keshav R. Kranthi of the Central Institute for Cotton Research, Nagpur, India was awarded a shield at the 68th Plenary Meeting of the ICAC. Dr. Kranthi made a presentation at the Technical Seminar during the Plenary Meeting on his vision for cotton production research. Dr. Kranthi is an entomologist, so his paper is dominated by entomological research, but he also presented his futuristic views on many other aspects of cotton research. He said that new technologies are being invented at a greater speed than ever before. Insect resistant transgenic crops, RNA interference, mutated genes to overcome insect resistance, molecular signaling, gossypol free seed, pheromone and pesticide precision application technologies, gene mining and the availability of markers for economically important traits, are opening many new avenues in cotton research. While significant progress has already been made in many fields, there are strong indications that new technologies to develop biotech cotton that could scare insects, make biotech resistant bollworms susceptible to the toxin, develop photoperiodic insensitive cotton and develop 'global cotton' varieties that could be grown successfully under varied agro-climatic conditions are being developed. Dr. Kranthi also suggested the establishment of an International Cotton Research Institute in a developing country.

Five other papers were presented in the Technical Seminar held on September 10, 2009 during the 68th Plenary Meeting in Cape Town, South Africa. The topic of the Technical Seminar was 'Biosafety Regulations, Implementation and Consumer Acceptance.' All papers will be published separately and made available free of charge on the ICAC web page but only in English. Because non-English speaking researchers cannot make full use of the Technical Seminar papers, I have decided to include summaries of the all papers in the second article.

Dr. Ali Jafari Mofidabadi, Director, Cotton Research Institute, Iran has contributed the third article in this issue of THE RECORDER, entitled "Producing Triploid Hybrid Plants Through Induced Mutation to Broaden Genetic Base in Cotton". Dr. Mofidabadi used gibberellic acid as a growth regulator for obtaining interspecific hybrids between tetraploid and dioploid species of cotton. He used two *hirsutum* varieties as female parents and two *arboreum* varieties as male parents. Fertilized flowers were treated with gibberellic acid in three different concentrations. Normally, *G. hirsutum* would not produce fertile seeds but Dr. Mofidabadi was able to get fertile seeds. However, there were significant differences among cross combinations. More details on the methodology, treatments and successful combinations are given in the third article.

Technical Seminar 2010

The 2010 Technical Seminar will be on the topic "How to lower the cost of cotton production". The location of the 69th Plenary has not been decided yet.

Applications Invited for Research Associate Program

The Technical Information Section of the ICAC will conduct a Research Associate Program in April 2010. The Research Associate Program of the ICAC provides an opportunity to receive additional training in cotton research, marketing, statistics and economics. The 2009/10 Research Associate Program will be conducted for ten days starting on April 20 and ending on April 30, 2010. The program will be conducted on the topic of: Improving Productivity of Cotton. Candidates will be responsible for their cost of international travel to and from Washington, DC while the ICAC will provide lodging and a per diem of US$50/day (not exceeding a total of US$500 for the duration of the Program) for food and miscellaneous expenses. Additional information is available at <http://www.icac.org/cotton_school/research_associate_prog/english.html>.

The ICAC RECORDER (ISSN 1022-6303) is pulbished four times a year by the Secretariat of the International Cotton Advisory Committee, 1629 K Street, NW, Suite 702, Washington, DC 20006-1636, USA. Editor: M. Rafiq Chaudhry <rafiq@icac.org>. Subscription rate: $200.00 hard copy, $165.00 electronic version.

Candidates from ICAC member countries must apply through either the ICAC Coordinating Agencies in their countries or delegate to the Standing Committee of the ICAC. Their contact details are available at http://www.icac.org/. Candidates from non-member countries can participate in the program by paying a tuition fee of US$4,000 per candidate.

The last date to apply is October 31, 2009. Applications should be addressed to Dr. Terry P. Townsend, Executive Director of the ICAC at <terry@icac.org>.

Challenges and Opportunities in Cotton Production Research

Keshav R. Kranthi, Central Institute for Cotton Research, Nagpur, Maharashtra, India

Global cotton production has increased over the years, with significant increases in the last 5 years. During 2007/08 a total of 26.3 million metric tons were produced. But, the share of synthetic fibers, consumed at the end use level, overtook cotton in 1995 and continues to increase. The share of cotton in global textile consumption is decreasing and has reached 39% in 2008. This is concerning. The issue relates mainly to rising production costs and stagnating prices. If cotton production costs can be reduced significantly using sustainable low cost production techniques, cotton can claim its due share in textiles again.

Some recent interesting research developments

The distance between output of fundamental science and its application for technology development has narrowed significantly in recent times. Some very exciting developments have been happening in science over the recent immediate past especially in agricultural sciences. Molecular sciences have pervaded almost all fields of agricultural science leading to exciting breakthroughs, especially in cotton improvement for resistance to biotic and abiotic stresses, herbicide resistance and fiber quality enhancement. Several new concepts in pest management have emerged that have great potential to change the way insects pests, nematodes and pathogens can be managed in a highly precise manner with least effects on non-target flora and fauna. In recent times, new technologies are being invented at a greater frequency than ever before. Insect resistant transgenic crops, RNA interference (RNAi), mutated genes to overcome insect resistance, molecular signaling, *Wolbachia* based control, pheromone and pesticide precision application technologies, nanotechnology, molecular analysis of genetic diversity in crops, allele mining, gene mining, availability of markers for economically important traits, pests, pathogens and organisms of biological control etc., have been signaling a new era in crop improvement.

Insect resistant biotech cotton that silences gossypol

Bollworms survive on cotton because they have an enzyme called P450 monooxygenase *cyp6AE14*, which digests gossypol. The new biotech cotton expresses dsRNA of the enzyme. When bollworm eats the dsRNA the enzyme is silenced and undigested gossypols remain in the stomach that kills the insect. The cytochrome p450 *cyp6AE14* genes of the cotton bollworm were silenced to disable the bollworm from feeding on gossypol in cotton plants (Mao *et al.*, 2007). The technology has immense potential in pest management that can be sophisticated to the extent of being extremely specific for the control of target pests alone. The RNAi technology is in the forefront of all the 'state of art' technologies for pest management. Ever since the publication in Nature, 1998 and the noble prize awarded to Drs. Andrew Fire and Craig Mellow in 2006, for their discovery of dsRNA based silencing of specific genes through RNAi (RNA interference), the technology has fired the imagination of researchers all over the world.

Prospects of developing low gossypol seed varieties through biotech cotton

Low gossypol seed can be possible through biotech cotton expressing *cyp6AE14* genes from pink bollworm and *Helicoverpa* to be expressed in seeds. The gene sequences are known and seed specific promoters are available. These can be used to develop low gossypol seed varieties.

RNAi-mediated elimination of toxic gossypol from cottonseed

Recently, Keerti Rathore and his team at the Texas A&M University, USA utilized RNA interference to inhibit the expression of the δ-cadinene synthase gene in a seed-specific manner, thereby disrupting a key step in the biosynthesis of gossypol in cotton. Compared to an average gossypol value of 10 µg/mg in wild-type seeds, seeds from RNAi lines showed values as low as 0.2 µg/mg. Importantly, the levels of gossypol and related terpenoids that are derived from the same pathway were not diminished in the foliage and floral parts of mature plants and thus remain available for plant defense against insects and diseases. Further, they reported that the germinating RNAi seedlings are capable of launching

terpenoid-based defense pathway when challenged with a pathogen. Thus, the silenced state of the δ-cadinene synthase gene that existed in the seed does not leave a residual effect that can interfere with the normal functioning of the cotton seedling during germination.

Designing genes to kill Bt resistant bollworms

Mutated cadherin alleles in Cry1Ac resistant *H. armigera* insects from field population were found to confer resistance. In an extremely useful study, Soberon *et al*. (2007) showed that susceptibility to the Bt toxin Cry1Ab was reduced by cadherin gene silencing with RNA interference in *Manduca sexta*, confirming cadherin's role in Bt toxicity. Native Cry1A toxins required cadherin to form oligomers, but modified Cry1A toxins lacking one alpha-helix did not. The modified toxins killed cadherin-silenced *M. sexta* and Bt-resistant *Pectinophora gossypiella* that had cadherin deletion mutations. The author suggested that cadherin promotes Bt toxicity by facilitating toxin oligomerization and demonstrate that the modified Bt toxins may be useful against pests resistant to standard Bt toxins.

Insecticide resistant bollworms can be made susceptible

Bollworms have been found to have developed resistance to insecticides by over-expressing a few enzymes selectively that degrade insecticides. Some of the examples are, cytochrome p450 (cyp6b7) over expresses in pyrethroid resistant *H. armigera*; a protease over-expresses in Cry1Ac resistant *H. armigera*; esterase E9 over expresses in Methomyl resistant *H. armigera*; and esterase E5 over expresses in quinalphos resistant strains. The genes responsible for insecticide resistance can be effectively silenced through RNAi so that the insects show susceptibility to the toxins.

Biotech crops that can scare pests

It is now proven that new biotech crops that scare insects can be developed. Insects release chemicals called alarm pheromones when they are scared by their enemies. This warns their colonies to escape. New biotech crops express alarm pheromones that scare specific insects. The alarm pheromone for many species of aphids, which causes dispersion in response to attack by predators or parasitoids, consists of the sesquiterpene (E)-farnesene (*Ef*). High levels of expression in *Arabidopsis thaliana* plants of an *Efsynthase* gene cloned from *Mentha piperita* were used to cause emission of pure *Ef*. These plants elicited potent effects on behavior of the aphid *Myzus persicae* (alarm and repellent responses) and its parasitoid *Diaeretiella rapae* (an arrestant response). Also new lectin genes have been found to be effective against sucking pests and are being used to develop cotton crops that can resist sucking pests.

Gadgets for pest scouting

Simple gadgets can be designed to scout insect pests, without having to count any insects. Some plants have been found to help cotton fight pests. Insects make ultrasonic sounds or release pheromones or cause plants to emit ethylene that can be detected by simple gadgets for farmers to precisely detect insect infestations, even from home.

Border plants that help cotton crop fight pests

Insect injury causes signal transduction. The signal transduction pathways leading to the release of plant volatiles have been found to alert other plants in the neighborhood. The scent of jasmine reduces populations of jassids, aphids, and the *H. armigera*, and enhances populations of predators and parasitoids in cotton fields.

Development of 'Global Cotton'

Cotton is sensitive to photoperiod and thermal conditions and does not adjust easily to new environments. Genetic engineering can help to develop cotton varieties that can grow anywhere in the world. Researchers should be able to exchange germplasm without any restrictions for the betterment of cotton.

Developing photoperiod insensitive, biotic and abiotic stress resistant biotech cotton varieties

Cotton is sensitive to photoperiod and thermal conditions and does not adjust easily to new environments. For example, it took about 60-70 years for *G. hirsutum* and 150 years for *G. barbadense* to adapt to Indian climatic conditions. This clearly indicates that each of the individual cotton genotype has a specific photoperiod and thermal requirement for optimal performance. Therefore it would be appropriate to identify the highest yielding genotypes for extremely specific geographical zones that have a common photo and thermal profile across the season. Genetic engineering can help to develop cotton varieties that can grow anywhere in the world. Manipulation of Rubisco activase can alter photoperiod and thermal sensitivity to enhance the adaptability of cotton to a wide range of environments. Drought responsive element binding proteins (DREB) *rd29A* genes for drought, high-salt & cold stress have been identified and used in several crops including cotton. Superoxide dismutases (SOD) confer chilling stress and is being explored for its utility in cotton**.** Biotech cotton varieties for other traits such as drought and disease (leaf curl virus) management have not yet been released commercially and have immense potential in many countries. Herbicide resistant biotech cotton in small-scale production systems should find a useful place. Careful planning and design of alternative placement of intercrops are needed to avoid the direct effect of herbicide on them and to ensure that cotton does not become the sole crop in the production systems

because of the new weed management biotechnology use.

Combating the recent resurgences of minor pests

Insecticide use on cotton declined significantly after the introduction of insect resistant Bt cotton. As a consequence, several minor pests have been resurfacing in the cotton ecosystems mainly in India and China. Recent reports show that new pests such as the mirid bugs and mealy bugs have been causing significant economic damage, thereby necessitating the continuance of insecticides for pest management. RNAi should be used to develop insect and disease resistant varieties. The insect resistant products developed through RNAi will give India a competitive edge over other countries that have been developing biotech-crops[o]. Efforts should be made to identify 'insect-species-specific' genes present in the insect gut that are functionally important for feeding, digestion and other biological activities. There is a need to identify effective siRNAs and/or miRNAs and their targets. Gene sequences and the novel structures must be explored for their utility for crop protection through conventional or transgenic approaches for the management of cotton insect pests such as the bollworms, mealy bugs and new pests.

Biotech cotton with new genes and gene pyramids

Insect resistant biotech cotton has contributed immensely to pest management mainly by causing a significant reduction in the insecticides used to control bollworms. More importantly, farmers in developing countries are no longer stressed with impending bollworm infestations that would otherwise cause severe damage to the crop and thereby reduce production. It is important to develop biotech cotton management strategies so that the full benefits from the technology can be harnessed and the technology can be sustainable for the longest possible time. Bollworm resistance management strategies have not been followed as prescribed in many developing countries, and the problem needs to be addressed on priority basis. Alternative genes (new Cry genes, lectins, protease inhibitors, genes from nematodes etc.) and RNAi based crop protection against insect pests, should be introduced as soon as possible through biotech cotton for more effective pest management. Insect resistant biotech crops (pigeon pea, chickpea, tomato and other vegetables, etc.) that serve as alternate host plants for bollworms should be developed with genes that are not used in biotech cotton. Use of the same Cry genes in all crops will enhance the chances of resistance development in insects.

Impact of climate change on cotton production

Cotton crop productivity is sensitive to climate-induced effects like temperature, rainfall, radiation, CO_2 concentration, and changes in soil, pests and diseases. Work carried out at the Central Institute for Cotton Research (CICR), India, indicates that select conventional cotton varieties/hybrids are well adapted to elevated CO_2 levels due to better morphophysiological and biochemical attributes**.** Elevated levels of CO_2 significantly increase plant height, node number, sympodia number, leaf number, leaf area, dry matter production, reduced shedding of bud and bolls an delayed senescence of leaves. Productivity of cotton in terms of total number of bolls and weight increased significantly with an increase of 73%. Fiber quality improved significantly under elevated CO_2 atmosphere. The photosynthetic rate in cotton varieties increased by 34-45% while stomatal resistance decreased significantly. Microbial population increased in soil under elevated CO_2 atmosphere. Elevated CO_2 atmosphere of 650 ppm and temperature of 40 degrees centigrade was found to be optimum for growth of cotton plants. Although it appears that cotton crop will do better in the changed atmospheric scenario during the later part of the 21st century, studies indicate that the pest problem will be aggravated further leading to an increased use of pesticides. By and large, research in India indicates that the impact of climate change on cotton production and productivity will be favorable.

Mechanization of cotton production

Cotton production is labor intensive in almost all developing countries. Cotton production demands labor all through, starting from sowing to harvesting which include several operations including inter-culturing and hand weeding. Cotton in several countries is cultivated in small-scale production systems, which demand smaller machines that are affordable for small scale farmers. Several attempts are underway to develop machines for picking and other important operations in cotton cultivation in small-scale production systems. Recently, a 3-row, self-propelled check row planter with a pneumatic metering mechanism was developed and evaluated in India. The field capacity was 0.5 hectare per hour with 88% field efficiency. The cost of operation was US$4.0 per hectare, which is remarkably less than any other traditional method. In addition, a self-propelled inter-row cultivator was developed and tested on cotton in India. The field capacity was found to be about 0.3 hectare per hour with 48-98% field efficiency. Small-scale, two-spindle machine pickers are being developed and tested for Indian conditions. Research needs to be done to ensure that new machines are developed such that crop production operations are not stalled in rainy days, which is normally the case with labor-intensive operations.

Organic cotton

Biotech cotton is not eligible for certification as organic. The rapid adoption of insect resistant biotech cotton cultivars into many cotton growing agro-climatic zones reduces the benefit of organic cotton and limits the spread of organic cotton cultivation to new areas. At this juncture, it would be possible to promote organic cotton only into the desi cotton (*G. arboreum* and *G. herbaceum*) belt in India. Presently,

about 0.5 million hectares are under desi cotton cultivation in India. Initially, organized organic cotton cultivation may be promoted in these areas where the input use in cotton is less and is traditionally non-chemical. Currently, Bt cotton is not permitted in the non-traditional cotton growing states – Orissa, West Bengal and the NE states that grow cotton on about 100,000 hectares. There is further scope for expanding organic cotton into these areas.

Yield and quality enhancement through molecular breeding

Plant breeders all over the world have so far subjected germplasm resources to intensive breeding, so as to enhance yield, fiber quality traits, high oil content or resistance to biotic or abiotic stresses. Such programs also inadvertently result in the narrowing of the genetic base. There is a need to take a re-look at the entire germplasm collection once again in light of the use of molecular markers and the genes that are currently available. The markers and genes identified recently for economically important traits can provide an elegant tool to convert some high yielding germplasm lines into elite cultivars. Out of the 50 cotton species, 5 are included in the primary germplasm pool, 21 as secondary, and 24 as tertiary germplasm pool, based on the relative genetic accessibility. There are several high yielding germplasm lines that are deficient in just one or two economically important traits such as fiber strength or length or susceptibility to biotic or abiotic stresses. Useful genes can be transferred into cultivars through genetic engineering or desired traits, for which molecular markers are available that can be back-crossed into the lines through accelerated marker assisted breeding. In addition to its lint, the oil and protein portion of cottonseed also represents significant economic value. As far as possible, plant-breeding programs should also ensure that the newly developed cultivars have reasonably high levels of oil and proplant-breeding

High yielding elite germplasm lines, which are inferior in only one or two of the desirable traits such as fiber quality or resistance to biotic or abiotic stresses, should be chosen as recurrent parents for marker assisted accelerated back-cross breeding methods. Another set of high yielding germplasm lines should be identified, which possess the trait of interest, and can be used as donor parents. Recently, 2,937 SSR primer pairs have been identified as highly informative which target unique genomic sequences and amplify about 4,000 unique marker loci in a tetraploid cotton genome. Chromosome-marker bins, each 20 cM in size, were constructed on the genetic linkage map containing the markers. Thus 207 marker bins were assigned for a total of about 4,140 cM, which is approximately the size of the tetraploid cotton genetic map. The markers can be used effectively to tag quantitative traits of interest in the already characterized germplasm pools and thereafter be utilized in marker assisted breeding programs for genetic enhancement of elite lines and genotypes to develop high yielding cultivars. Genes conferring strength and fineness can be identified from Ramie and utilized to enhance fiber traits in cotton through genetic transformation. Sucrose phosphate synthase and extensin genes have been shown to enhance fiber length and strength and can be further explored

Issues in fiber quality testing

Cotton fiber quality assessment through instrumentation is still a challenge. There are no rapid internationally acceptable uniform methods of testing cotton for neps, stickiness and maturity. The testing procedures are still time-consuming in many countries. There is an imminent need to invent simple and rapid testing equipment and procedures for fiber quality evaluation that can give a preliminary assessment before the fiber can be subjected to the high volume instrument and other tests to ensure better returns for the producers.

High yields with narrow spacing and nutrient management

How can yields be increased in developing countries? Yields in developing countries mostly in Africa have been stagnating. Ultra narrow row spacing is highly popular in China, Uzbekistan and several other countries where plant populations of 100,000 to 200,000 per hectare give yields of 7,000-8,000 kilogram of seedcotton per hectare. The same approach should help other countries to identify and develop varieties through 'ideotype breeding' of compact genotypes suited for ultra narrow tow spacing, with specific fiber traits for specific locations. Additionally, the compact genotypes with specific fiber traits can be converted to insect resistant biotech cotton. Such location, specific high yielding varieties will ensure sustainable production in major cotton growing countries of Asia and Africa in the future.

The need for a global institute on cotton

There are research institutes on many crops, but not on cotton. Together the governments can move forward for the betterment of cotton, perhaps only through a global institute 'International Cotton Research Institute-ICRI' that may be set up in any of the developing countries, and which addresses all our problems together without having to restrict ourselves to technologies with intellectual property rights issues, especially in Asia and Africa.

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Biosafety Regulations, Implementation and Consumer Acceptance

Every year during the Plenary Meeting of the ICAC, the Technical Information Section organizes a Technical Seminar on a selected topic. The Seminar, also called the Meeting of the Committee on Cotton Production Research of the ICAC, is usually held on Thursdays, and 6-8 speakers are invited to make presentations on important aspects of each topic. The 2009 Technical Seminar was held on the topic 'Biosafety Regulations, Implementation and Consumer Acceptance.' Six papers were presented from five countries, including a special paper from the 'ICAC Cotton Researcher of the Year 2009,' Dr. Keshav R. Kranthi, Central Institute for Cotton Research, Nagpur, India. The paper by the first 'ICAC Cotton Researcher of the Year 2009' has been published in full and the other five papers appear in the present article in the form of summaries.

Regulatory Requirements and Technology Diffusion: The Case of Biotech Cotton

Idah Sithole-Niang, Head, Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe

The currently available commercial transgenic cottons were obtained by using recombinant DNA and transformation technology to introduce a foreign gene into the target genome.

A promoter drives expression in the plant, and the gene is introduced into the cells of a desirable cotton variety using one of following techniques.

- *Agrobacterium*-mediation
- Particle bombardment using the gene gun
- Pollen tube pathway

Table 1 shows the biotech cotton events that had been commercialized by 2009. The table indicates the year when biotech cotton was first approved for commercial release by a country.

The most significant events were: Bollgard cotton, MON 531/757/1076, carrying a Cry1Ac gene driven by the 35S Cauliflower mosaic virus (CaMV) promoter with the neomycin phosphotransferase (*npt*II) and the aminoglycoside adenyltransferase (*aad*) genes as selectable markers.

Table 1. Approval of Biotech Cotton for Environmental Release by Country

Country	Year & Event
Argentina	1998 (MON 531/757/1076)
Australia	1996 (MON 531/757/1076)
Brazil	2005 (MON 531/757/1076)
Burkina Faso	2008 (15985)
China	1997 (various)
Colombia	2003 (MON 531/757/1076)
India	2002 (MON 531/757/1076)
Japan	1997 (MON 1445/1698; MON 531/757/1076)
Mexico	1997 (MON 531/757/1076)
South Africa	1997 (MON 531/757/1076)
USA	1994 (BXN)

Biosafety Considerations

Once produced, all biotech cottons undergo risk assessment studies based on three components before they can be released into the environment:

- Environmental risk assessment (including effects on non-target organisms, potential for weediness and concerns over gene flow and consequences thereof);
- Food and feed safety (in terms of toxicity, nutritional equivalence, allergencity and digestibility);
- Socio-economic considerations.

In developing countries, if the biotech cotton has not been developed locally, the initial entry point into the system will be to apply for confined field trials. Once the results of the confined field trials are deemed satisfactory, the country might then opt for commercial release. In that case, a field trial is set up for seed multiplication so that the material can be bulked and used for the subsequent food and feed safety tests that the country wishes to conduct; otherwise, the risk assessment at this stage may also comprise an evaluation of the documents submitted by the applicant. The final decision however, may be based on socio-economic considerations and that may have nothing to do with the performance or safety of the technology.

National Biosafety Framework System

Regulation of biotechnology is a requirement under the Cartagena Protocol on Biosafety. The protocol is a legally binding instrument under the Convention on Biological Diversity. The primary objective of the Convention on Biological Diversity is to develop a global framework for the conservation and sustainable use of biological diversity. Most African countries are signatories to the Cartagena Protocol on Biosafety. To-date, 9 African countries have fully developed national biosafety frameworks, 13 have

Table 2. Status of National Biosafety Frameworks (NBFs) in Africa

Fully developed NBFs	Interim NBFs	Work in Progress	No NBFs
Algeria, Burkina Faso, Egypt, Kenya, Mauritius, Malawi, South Africa, Tunisia and Zimbabwe	Ethiopia, Ghana, Madagascar, Mali, Mozambique, Namibia, Nigeria, Rwanda, Senegal, Sudan, Tanzania, Uganda & Zambia	Benin, Botswana, Cameroon, Congo, Democratic Republic of Congo, Djibouti, Eritrea, the Gambia, Lesotho, Liberia, Libya, Niger, Seychelles, Swaziland & Togo	Angola, Burundi, Cape Verde, Chad, Comoros, Cote d'Ivoire, Equatorial Guinea, Gabon, Guinea, Guinea Bissau, Mauritania, Sao Tome & Principe, Sierra Leone & Somalia

interim biosafety frameworks, 15 are in the process and 16 have none. (See table 2).

There is a need to develop national biosafety frameworks that are better focused and more streamlined in order to harmonize the different national frameworks and facilitate trade and the trans-boundary movement of biotech crops.

The South African Regulatory System

Gillian Christians, Registrar Genetically Modified Organisms Act, Department of Agriculture, Pretoria, South Africa

South Africa implemented the Genetically Modified Organisms (GMO) Act of 1997 (Act 15 of 1997), also called the GMO Act, in December 1999, and since then all activities involving biotech crops are conducted in compliance with permits issued under this Act. Due to the growing importance of biosafety and related issues, the South African Government elevated the GMO unit, which until then had been operating under the Directorate Genetic Resources, to a full-fledged directorate. The Bio-safety Directorate has two regulatory bodies i.e. the Advisory Committee and the Executive Council, in addition to a Registrar and inspectors. The Registrar, who is appointed by the Minister of Agriculture, Forestry and Fisheries is responsible for the administration of all activities within the scope of the GMO Act.

Biotech applications are subjected to a multidisciplinary process of scientific evaluation by the expert panel of scientists that make up the Advisory Committee that acts as a national advisory body on all matters relating to biotechnology issues. The Advisory Committee consists of ten scientists appointed by the Minister of Agriculture, Forestry and Fisheries. Extended pools of experts from various disciplines support the Advisory Committee. The Advisory Committee, together with subcommittee members, is responsible for the evaluation of risk assessments of all applications as related to food, feed and environmental impact. Its findings are then submitted to the Executive Council in the form of a recommendation.

The Executive Council is the ultimate decision-making body and currently consists of officials from six government departments/ministries (Agriculture, Forestry & Fisheries, Health, Environmental Affairs, Labor, Trade and Industry and Science and Technology) and the chairperson of the Advisory Committee. With the implementation of the GMO Amendment Act 2006, the Council will additionally include in the near future, members from the Department of Water Affairs and the Department of Arts and Culture. The Council is tasked with advising the Minister of Agriculture, Forestry and Fisheries on all aspects concerning the development, production, use, application and release of biotech products, and to ensure that all activities with regard to biotech products (importation, exportation, transit, development, production, release, distribution, contained use, storage and application) are performed in accordance with the provisions of the Act. Approved biotech activities are regulated by way of permits issued by the Registrar and the accompanying permit conditions are monitored for compliance by inspectors of the Department of Agriculture, Forestry and Fisheries.

The existence and enforcement of the GMO Act in South Africa provides the country with a decision-making tool that enables its authorities to conduct a science-based, case-by-case assessment of the potential risks that may arise from any activity involving a particular genetically modified organism. Despite the ten years elapsed since their adoption in South Africa, biotech crops have almost exclusively incorporated traits for insect resistance and or herbicide tolerance. As biotechnology advances beyond the realm of agronomic traits, the regulatory system will be challenged to respond to the emerging biotechnology applications. The directorate must therefore continue to pursue efforts to strengthen its regulatory framework, exploit capacity building initiatives and participate in regional and international biosafety engagements.

Biotech Cotton in International Trade

Richard Haire, Queensland Cotton Corporation Ltd., Brisbane, Queensland, Australia

There have been numerous assessments of the economic, social and environmental merits of biotech crops, and they all share common conclusions as below.

- Biotech crops have led to a material reduction in the use of insecticides.
- Biotechnology had a positive impact on community perceptions about our industry's efforts to promote sustainability in crop protection practices.
- Biotech crops have reduced the occupational health and

safety risks associated with the storage, handling and application of pesticides.

- Biotech cotton gives enhanced yields and improves the production reliability of cotton.

The net economic, social and environmental benefit has been unambiguously positive. However, access to biotechnology applications is governed by strict licensing conditions that essentially seek to protect the technology developer's intellectual property rights, eliminate the potential for a secondary market in the product through the retention of seed for future planting and defend the technology from systematic failure. The primary products of currently approved biotech cotton traits are seed, fiber, cottonseed oil and cottonseed meal.

Seed - The pricing strategy for planting seed seems to be based on the principal of "charge as much as the market will bear."

The studies done on its impact on gross margins reveal that, while the value of the technology is relatively consistent, there is wide disparity in pricing. Australia, for example, pays six times the license fees paid by India and the United States but enjoys 84 percent of the benefit that India does and receives double the economic benefit of the USA.

Cotton lint - There has been no observed difference between the fiber characteristics of biotech cotton and those of conventional varieties, and its spinning-ability does not appear to have been affected. On the contrary, there is evidence to suggest that the introduction of herbicide resistance has had a direct and positive impact on the leaf and vegetable matter content of cotton. Other than the restriction related to certification for organic production, there is neither a regulatory nor market differentiation between biotech and conventional cotton and there is no material demand preference for one version over the other.

Cottonseed oil - Global production of cottonseed oil for the 2007 season was estimated at 5.2 million metric tons with approximately 3.6 million tons coming from biotech cotton varieties. The oil is sold in either its raw form or in end use product form without restrictions across the world. Cottonseed oil finds its way into the food chain through its use in table spreads (margarines), salad dressings and as cooking oil. Scientists describe cottonseed oil as being "naturally hydrogenated" because the saturated fatty acids it contains are the natural oleic, palmitic and stearic acids. These fatty acids make it a stable frying oil that needs no additional processing and does not form trans fatty acids There is currently no market segmentation for cottonseed oil derived from biotech seed.

Cottonseed meal - Cottonseed meal accounts for approximately 40 percent by weight of fuzzy cottonseed, depending on the particular extraction process used. It is a high protein stock feed. For the 2007 year, over 10 million tons of cottonseed meal was produced globally with almost ¾ of it from biotech seed. When biotech cotton was introduced, in both the United States and Australia, there was some market interest in segregating biotech cottonseed meal from conventional cottonseed meal. However, within 2 years, market demand became generic.

Market Response to Biotech Cotton Seed

Amadeo Nicora, Instituto Nacional de Tecnología Agropecuaria -INTA, Argentina

Argentina established the regulatory framework for commercialization of biotech crops in 1991. The National Advisory Committee on Agricultural Biotechnology (CONABIO) was established under the Secretary of Agriculture, Livestock, Fisheries and Food (SAGPyA) with representatives from various institutions from the public and private sectors for regulatory activities. In Argentina, it takes 5-6 years for a new material to move from the first evaluation phase up to commercial release. Currently, there are seven biotech cotton varieties registered in the records of the National Seed Institute. A stacked gene variety comprising the Bollgard and Roundup Ready genes was recently approved, but its entry in the records of the National Seed Institute is still pending.

According to the National Seed Institute in 2007/08, the total area planted to biotech cotton was 27%, but that figure increased to over 70% in 2008/09. The use of the cottonseed obtained is as follows: 63% goes to crushing for oil extraction, 28% is fed to livestock as raw seed, 5% is exported and about 5% is used to plant cotton. Regarding seed exports, most is exported to Chile, followed by Spain (21%) and Uruguay (9%). Recent data shows that exports of cottonseed oil (semi-refined) are destined for Algeria (43%), Korea (31%), China (18%) and Chile (8%). Cotton seed cake is primarily exported to Chile, the Netherlands and Brazil. In Argentina, cottonseed is usually blended with cereals as a source of proteins.

The National Institute for Agricultural Technology (INTA) has undertaken research on milk and meat from livestock fed on raw cottonseed.

Specific studies designed to compare the performance of cows fed on biotech and non-biotech materials to detect differences in milk production and the chemical composition of the milk revealed no significant differences in the variables analyzed. These results indicate that when the diets of dairy cows are supplemented with seeds from biotech cotton varieties containing Bt and RR genes, their performance, in terms of consumption, production and chemical composition of milk, is similar to that of cows fed seed supplements from non-biotech varieties.

Cottonseed marketing practices in the domestic market do not differentiate between biotech and non-biotech origins and refer exclusively to the differential contributions by destination: industry or fodder.

Improving Confidence in Biotech Cotton

Jocelyn Webster, AfricaBio, South Africa

After its commercial release in South Africa in 1996, biotech cotton quickly spread among small and large cotton growers. Although cotton area in South Africa has been decreasing, yields have gone up, and over 90% of the area planted to cotton is still under biotech varieties. Studies have shown that large cotton farmers adopt biotech cotton mainly because of savings in spray labor (63%) and higher yields (32%). All other factors form only 5% of the reasons for adopting biotech cotton by large growers in South Africa. Small growers in South Africa benefit from higher yields and income, savings on insecticide costs and safety in terms of reduced handling of chemicals. The two implications of biotech cotton faced by small growers in South Africa are the same as in other countries, i.e., higher seed cost and emergence of secondary pests.

Some of the reasons for slow adoption of biotech cotton in the world are continued concerns about possible food and environmental safety, weak regulatory capacity in potential countries, complexity of trade in biotech crops, high regulatory barriers leading to restriction or slow access to beneficial technologies and high barriers that may restrict competition in seed market and reduce options for farmers. Confidence in biotech cotton can be improved through following means.

- Ensure effective, stringent and transparent enforcement of biosafety regulation
- Showcase the benefits of biotech cotton
- Address arising concerns
- Highlight socio-economic benefits, and
- Regular consultations with farmers are critical for harnessing their support and addressing their needs.

Biotech crops can contribute to improved food security and poverty alleviation in Africa. Commercialization of biotech cotton in South Africa and Burkina Faso, and confined field testing in Kenya, Malawi, Uganda and other developing countries shows that farmers in Africa are able to access the benefits of biotech crops. However, they need good governance, financial support, skills training, market access, the support of competent extension service and an adequate rural infrastructure.

Producing Triploid Hybrid Plants Through Induced Mutation to Broaden Genetic Base in Cotton

Ali Jafari Mofidabadi, Cotton Research Institute, Gorgan, Iran

Abstract

Gibberellic acid was used as a growth regulator to obtain interspecific hybrids between tetraploid and dioploid species of cotton. Two commercial *G. hirsutum* varieties (Sahel and Siokra) were used as female parents; pollen grains from Hashem Abad and Kashmer (*G. arboreum*) were used to fertilize emasculated flowers. Pollinated flowers were treated with different concentrations of gibberellic acid to overcome the flower abscission barrier. The Chi-square tests showed that different gibberellic acid concentrations produced significant differences (α=0.05) in cross combinations for boll development. Highly significant differences in hybrid boll setting were observed between control plants and hormone growth regulator plants. The maximum boll development (92%) was observed in Siokra x Hashem Abad when the pollinated flower was treated with 100 ppm gibberellic acid at 70-80 days after pollination; in contrast, only 2-3% of pollinated flowers led to boll formation when gibberellic acid was not applied. The number of seeds set per boll varied from non-mature seeds to an average of 2.8 seeds per boll. Additionally, the seeds were not as well developed as those of the self-pollinated female parents. The hybrid plants were found to have either more vigorous growth than both parents, or to be at an intermediate level between the two parents for some traits.

Introduction

Cotton belongs to the genus *Gossypium* and has genetic resources both in domesticated and wild forms (Bhale, 1999). The species cultivated in Asia, *G. arboreum L*. and *G. herbaceum* L., are diploids with 2n=26 chromosomes, while *G. hirsutum* and *G. barbadense,* species cultivated in the New Word, are allotetraploids with 2n=52 chromosomes (Ravikesavan *et al*., 2002). The world's commercial cotton production is dominated by tetraploids, thus, there is a constant need to broaden its available genetic base (Stewart and Hsu, 1978). Resistance to certain pathogens and insects, male sterility and certain morphological plant traits possessed by Old World cotton (diploids) are potentially useful for incorporation into tetraploid cottons for higher production (Stewart and Hsu, 1977).

Cotton breeders have been trying to obtain hybrids between diploid and tetraploid species for a long time, (Gill and Bajaj, 1987), but it has been difficult, and some times impossible, to obtain many hybrids under in situ conditions because of several incompatibility factors. Meanwhile the diploid species

that cross directly with upland cotton produce sterile triploid F_1 hybrids. Such sterile hybrids have to be treated with colchicine to produce fertile hexaploids (Joshi and Johri, 1972).

While Mirza *et al*., (1993) and Moradi (1997) have recommended exploring ovule-embryo culture. Amalraj (1989), and Joshi and Johri (1972) suggested application of gibberellic acid (GA_3) and naphthaleneacetic acid (NAA) during hybridization between distant parents (between species). Many attempts have been made at in vitro nutrition of immature embryos, but because of cross pollinated flower abscission and the long time that cotton embryos take to develop, embryo rescue has not proved to be a reliable method for obtaining hybrid plants. Therefore, in this study, different concentrations of gibberellic acid were used to overcome the flower abscission problem of pollinated tetraploid flowers so that the embryos might develop into fully-grown seeds.

Materials and Methods

Attempts were made to achieve field crosses to produce interspecific hybrids between selected parent species based on different genetic distances. In all cross combinations, the female parents were tetraploids and the male parents were diploids. Seeds of selected parents were planted in rows spaced at 90 cm, with the same distance between plants. Ten rows of seed parents and two rows of pollen parents were planted in each experimental unit. All the recommended cultural practices, such as fertilizing and irrigation were carried out at the required times. To prevent flower abscission and foster boll retention, pollinated flowers were sprayed with three different concentrations of gibberellic acid during one week. Hybridization followed manual emasculation and pollination under field conditions. Emasculation was carried out at the appropriate time, in the evening, and the emasculated flowers were pollinated the following morning. In order to reduce the incidence of boll shedding, gibberellic acid was sprayed at the pedicel base in concentrations of 50, 100 and 250 ppm during 7 days after pollination. The fourth entry in the experiment (control) was non-application of gibberellic acid, or no treatment. The number of bolls set was counted at the time of harvest and analyzed using a Chi-square test.

Table 1: Cross Combinations and Gibberellic Acid Treatments

Tetraploid Parent	Diploid Parent	Gebberellic Treatment
Sahel	Hasham-Abad	50 ppm
Sahel	Kashmer	50 ppm
Siokra	Hasham-Abad	50 ppm
Siokra	Kashmer	50 ppm
Sahel	Hasham-Abad	100 ppm
Sahel	Kashmer	100 ppm
Siokra	Hasham-Abad	100 ppm
Siokra	Kashmer	100 ppm
Sahel	Hasham-Abad	250 ppm
Sahel	Kashmer	250 ppm
Siokra	Hasham-Abad	250 ppm
Siokra	Kashmer	250 ppm
Sahel	Hasham-Abad	Control/no treatment
Sahel	Kashmer	Control/no treatment
Siokra	Hasham-Abad	Control/no treatment
Siokra	Kashmer	Control/no treatment

Results and Discussion

There was a total of 16 cross combinations. Eighty flowers were emasculated and pollinated; one tetraploid and one diploid parent were used for each cross combination (Table 1). The results showed that out of 1,280 cross-pollinated flowers, 326 bolls (26%) were set 70-80 days after pollination. These findings contrast with the results reported by Stewart and Hsu (1978) and Moradi (1997), who were able to obtain triploid hybrids from crosses between tetraploid and diploid genotypes using a growth regulator hormone without in vitro embryo culture. The data on number of bolls set, counted by each cross at 70-80 days after pollination, showed that there were significant differences (using Chi-square test at α=0.05 level) among parents for cross ability and hormone treatment for boll retention (Table 2). Moradi (1997) has reported similar findings. Successful results have been obtained using embryo rescue along with growth regulator hormone for diploid and tetraploid cotton species by Stewart and Hsu (1978).

As given in table 2, the maximum boll retention of 92% was observed in a cross Siokra x Hasham Abad when treated with 100 ppm gibberellic acid. The boll retention data of only 3% in the control for the same combination showed that higher boll setting was all due to 100-ppm gibberellic acid. On the average of all cross combinations, only 2.5% of pollinated flowers led to the development of harvestable bolls without gibberellic acid. The number of seeds per boll varied ranging from no mature seeds at all to an average of 2.8 seeds per boll. The bolls and seeds were not as well developed as those from the self-pollinated female parents. In general, the hybrid plants were more vigorous than either parent and in some cases the morphology of hybrids was intermediate between the two parents for several traits.

The average number of retained bolls was 55% when a tetraploid variety was kept as maternal parent and cross-pollinated flowers were treated with gibberellic acid. Inclusion of four controls lowered the boll setting percentage to 42%. The mean effect of hormone treatment concentration showed that there are highly significant differences among gibberellic acid concentrations. The data (Table 3) showed that 100-ppm concentration of gibberellic acid gave maximum boll setting in all cross combinations. Differences among cross combinations treated with 100 ppm were insignificant. Boll setting for 100 ppm in four cross-combinations ranged from 47% to 92%

Table 2: Effect of Different Crosses and Gibberellic Acid on Cross Ability

Crosses	GA_3 ppm	% Boll Retention
Siokra x Hashem-Abad	50	50
	100	92*
	250	57
	0	3
Siokra x Kashmer	50	70
	100	86*
	250	74
	0	2
Sahel x Kashme	50	38
	100	57*
	250	39
	0	2
Sahel x Hashem-abad	50	20
	100	47*
	250	30
	0	2

*= Significant differences at 5 % level

Table 3: Mean Effect of Gibberellic Acid on Hybridization

Gibberellic acid (ppm)	Cross ability %
100	70.5*
50	40.5
250	50.0
0	2.25

*= Significant differences at 5 % level

Table 4: Mean Effect of Parent on Hybridization

Parent	% Cross ability
Siokra x Hashem-Abad	50.5*
Siokra x Kashmer	58.0
Sahel x Hashem-abad	24.8
Sahel x Kashmer	38.0

*= Significant differences at 5 % level

while boll setting in the control did not exceed 3% in any combination.

The mean effect of parents for successful cross ability indicated that Siokra had more potential for successful crossing with two different diploid parents compared to the variety Sahel (Table 4). Bhale (1999) came up with similar findings in his experiments. He stated that okra leaf type cotton has a higher potential for cross ability than normal-leaf varieties. Based on this observation, it is recommended that at least 50 meters isolation distance should be kept in seed production of okra leaf type varieties to avoid any cross-pollination effects. Among the crosses between Siokra and diploid parents, the highest cross ability was observed between Siokra x Hashem-Abad (Table 4).

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