Table 1: Agronomic features of some Embrapa cotton varieties in comparison with one standard C-4 variety. Sissoko Research Station-Mali 2009/10 (adapted from Yattara et al., 2011).

Varieties	Agronomic features - 2009/10		
	kg/ha (Seed cotton)	% of the test	% lint
STAM 59A (test)	**2 217**	**100**	**42.7**
BRS 286	3 000	135	42.8
BRS 293	2 967	132	43.1
Araçá	2 933	132	41.2
Aroeira	2 183	98	39.7
Buruti	2 217	100	40.7
Cedro	2 917	132	42.7
Safira	2 433	110	34.7
Seridó	2 333	105	37.2
Average	**2 562**	**-**	**40.7**

enhancement was observed in the progeny of Brazilian and C-4 varieties. Researchers from C-4 countries were provided with access to EMBRAPA's cotton gene bank (Table 1, IER 2010, IER 2011).

- A long-term collaborative research network was established between Brazilian and African scientists.
- The C-4 project created a positive scenario for further cooperation in agricultural research between Brazil and Africa.

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Management of Biotic Stress in Cotton

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Introduction

Cotton is a long duration crop and typically takes about 120-160 days to complete its life cycle. Throughout the growth cycle it is exposed to weeds, insect pests and pathogens, which cause economic damage to yields. Cotton yield losses exceed up to 37% due to weeds (Awan *et al.,* 2015) and its long duration as well as slow growing nature (Nalini *et al.,* 2015). Similarly, 50-60% losses are encountered due to insect attacks (Saini *et al.*, 2009) and 30% by CLCuV infestation (Khan and Ahmed 2005: Khan *et al*., 2015). Management of nutrients, water, weeds, insect pests and diseases at the critical stage of the crop growth is crucial for obtaining good yields.

Weeds

The competition offered by different weeds to cotton plants results in loss of nutrition available for growth of the crop plant and results in considerable reduction of seed cotton

Some of the major weeds of global importance

Scientific name	Common name	Scientific name	Common name
Abutilon spp.	Velvet weed	*Digera spp.*	Large crabgrass
Acanthospermum hispidum (DC.)	Bristly starbur	*Eleusine indica* (L.) Gaertner.	Goose-grass
Ageratum conyzoides (L.)	Goat weed	*Euphorbia spp.*	Asthma herb
Amaranthus spp.	Slender amaranth	*Ipomea spp.*	Ivy morning glory
Bidens pilosa (L.)	Spanish needles	*Parthenium hysterophorus* (L.)	Congress grass
Chenopodium album (L.)	Common lambsquarter	*Portulaca oleracea* (L.)	Common purse-lane
Commelina spp.	Climbing dayflower	*Sida spp.*	Prickly fan-petals
Convolvulus arvensis (L.)	Field bindweed	*Solanum nigrum* (L.)	Black nightshade
Conyza spp.	Canadian horseweed	*Sonchus spp.*	Smooth sow-thistle
Corchorus tridens (L.)	Wild jute	*Sorghum halapense* (L.) Pers.	johnson grass
Cynodon spp.	African stargrass	*Trianthema spp.*	horse pulse-lane
Cyperus spp.	Roadside flatsedge	*Tridax procumbens* (L.)	Coat-buttons
Datura spp.	Devil's snare	*Xanthium strumarium* (L.)	common cocklebur

Source: Cotton Production Practices 2017 (ICAC)

yield. Weeds are reported to be a key limiting factor for cotton yield losses. Weeds not only compete for nutrition but also form leaf canopy over cotton and may hinder the growth of crop plant. Weeds are also considered as an alternative host for CLCuV (Rehman *et al.,* 2017) and several insect pests. Weeds serve as source of reservoirs of viruses inoculum as reported in case of TYLCV (Ghanim 2014). Every crop has a critical period of weed control (CPWC), which refers to the short time duration during which the crop must be weed free. In cotton, the CPWC is the first 15 to 60 days. Maximum yield can be derived when there is at least 95% weed control (Ramachandra *et al.,* 2016).

Effect of weeds on cotton growth

Weeds compete with cotton for nutrients and have the ability to assimilate their biomass much faster than cotton, which results in reduced crop height due to delay in weed removal (Bukun *et al.,* 2004). Number of sympodial branches, fruiting points, boll number and boll weight are badly affected by prolonged delay of weed removal (Velayutham *et al.,* 2002). Significant reduction in boll numbers per plant and boll weight in un-weeded check, was observed by Srinivasan (2003). Un-weeded control produced less no of sympodial branches, boll number plant and boll weight in cotton (Sadangi *et al.,* 2006).

Weeds affect fiber quality

Fiber quality is severely affected by weed density. Fiber length, uniformity, strength and micronaire are characteristics of cotton fiber, which are affected by weeds. The canopy formed by weeds results in restriction of air movement and increase of moisture level which may cause boll rot and quality losses. The addition of foreign material in cotton at harvest may results in reduction of cotton grade and decrease in price. The interference caused by weeds during harvest may also cause loss of cotton quality (Smith *et al.,* 2000).

Weeds act as reservoir of insects

A large of number of cotton insect pests except boll weevil, pink bollworm and cotton leafworm, have a wide range of host plants (Fye 1980). Weeds serve as a reservoir of insect and spider mite pests that may invade and damage the crop. The contribution of weeds to pest problems is highly variable, with greatest dependence on climatic conditions during any particular year. Insect pests such as the lygus bugs, false chinch bugs, beet armyworms, stink bugs and spider mites attack cotton crop after their weed hosts are destroyed (Gross and young, 1977).

Major Pests of Cotton

A large number of insect pests attack the cotton crop. These fauna can be divided in two categories: sucking and chewing insects. Sucking insects mainly include: whiteflies, thrips, aphids, jassids, mealy bugs and mites (Azam *et al*., 2013; Chaudhry *et al*., 2009). Chewing insects are comprised of leafworms, cotton bollworms, pink bollworms and spotted bollworms, which directly damage cotton bolls. It is estimated that about 50-60% losses in cotton yield are caused by sucking and chewing insects (Saini *et al*., 2009). A variety of chemical insecticides have been used to control these insects which have serious threats to environmental and human health (Oerke 2006). The indigenous production of pesticides is on the rise in Asia. In Pakistan pesticides worth US$ 227.88 million were produced in 2013 and annual import value accounted for more than US$ 80.28 million (Economic Survey of Pakistan, 2013-14). The continuous use of these chemical insecticides has resulted in the development of insect resistance to insecticides in almost 500 different species of insect pests (Sanil and Shetty 2011; Schnepf *et al*., 1998).

Insects with chewing type of mouth parts

The cotton bollworms, *Helicoverpa armigera* (Hub.),

Insect pests of global importance

Sap Sucking Insects		Insects with chewing mouth parts	
Scientific name	**Common name**	**Scientific name**	**Common name**
Amrasca spp. & *Empoasca spp.*	Jassids & leaf hoppers	*Pectinophora gossypiella* (Saund.)	Pink bollworm
Aphis gossypii (Glover)	Cotton aphid	*Helicoverpa armigera* (Hubner)	Cotton bollworm
Bemisia spp. & *Trialeurodes spp.*	Whiteflies	*Helicoverpa punctigera* (Wallen.)	Australian bollworm
Thrips spp., Frankliniella spp. & *Caliothrips spp.*	Thrips	*Helicoverpa zea* (Boddie)	Corn earworm
Creontiades spp. &	Mirid bugs	*Heliothis virescens* (F.)	Tobacco budworm
Diparopsis spp.	Red bollworm & Sudan bollworm	*Earias spp.*	Spotted bollworms
Dysdercus spp. & *Oxycarenus spp.*	Cotton Stainers & seed bugs	*Spodoptera spp.*	Cotton army worm
Phenacoccus solenopsis (Tinsley)	Cotton mealy-bug	*Anthonomus grandis* (Boheman)	Boll weevil

Source: Cotton Production Practices 2017 (ICAC)

Heliothis virescens (Fab.) and the other related species *Helicoverpa punctigera* (Wallen.) cause significant damage to cotton across the globe. A single *H. armigera* larva can damage up to 30-40 bolls (Aggarwal et al., 2006). The precise identification of the species is very important because *H. armigera* has evolved resistance to insecticides (Aggarwal *et al*., 2006). *Helicoverpa* adults can produce large number of eggs and because of their mobile nature, populations increase rapidly under favorable environmental conditions (Kurban *et al*., 2005). The pink bollworm *Pectinophora gossypiella* (Saund.) and the spotted bollworm *Earias* spp. are major pests in America, parts of Africa and Asia. The symptoms of the pink bollworm attack include: damaged buds, green bolls with exit holes, rosette flowers, lint discoloration, early boll senescence and burrowed seeds. While the pink bollworm feeds mainly on flowers and young bolls, the cotton bollworms and spotted bollworms can feed on leaves and all other fruiting parts. The spotted bollworms also bore into terminal shoots and stems to cause stunting of plants. Squares, flowers and bolls are damaged completely, thereby resulting in severe economic losses. Early opening is an important symptom when spotted bollworms infest cotton bolls. The presence of predators and other mortality factors influence the survival rate of bollworms. The cotton leafworm, *Spodoptera litura* (Fab.) is an important chewing pest in Pakistan. Eggs are laid in groups. Neonates scrape and feed creating holes in the leaves thereby hindering photosynthesis, which leads to weak bolls and poor productivity. The cotton boll weevil is a major pest in USA and Latin America.

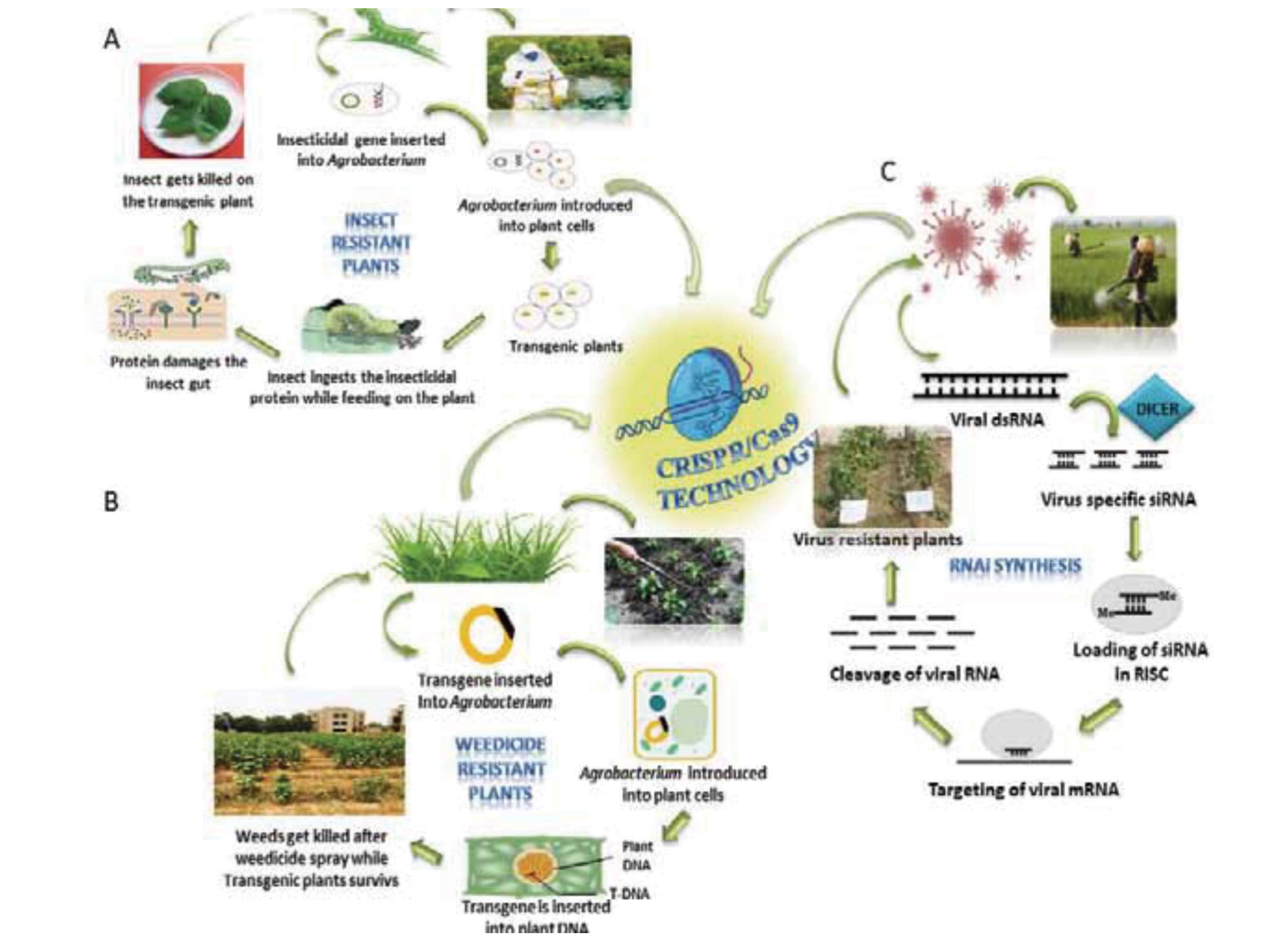
Sap sucking insects

The whitefly- *Bemisia tabaci* (Genn.) is considered to be the most widespread and the most damaging of all the sap-sucking insect pests of cotton crop across the globe. Aphids cause significant damage in Latin America, Europe and parts of Africa. Jassids and leafhoppers are major pests in Africa and Asia. Thrips are major pests in USA and many parts of the world. About 50% yield loss is due to whitefly among all sucking pests flourishing on cotton plant (Khan *et al*., 2015). Whiteflies feed on phloem sap thereby affecting photosynthesis due to leaf damage, sooty mold and stickiness, which results in low yields and poor fibre quality. Whiteflies also transmit the Cotton Leaf Curl Virus (CLCuV), which is a single stranded DNA (ssDNA) virus that belongs to *Gemini viruses* and causes Cotton Leaf Curl Disease (CLCuD). The virus particles that are ingested through stylets reach the salivary glands through the gut and the hemocoel and finally transmit infection during feeding, about 8–12 h after viral acquisition (Brown *et al.,* 1995). The leaf curl disease is a major problem in India and Pakistan. The first severe epidemic of CLCuV appeared in 1992-93 in Pakistan and caused a loss of 7.9 million bales. It was estimated that during 1992-97, CLCuV caused US $5 billion losses to cotton sector in Pakistan (Maharshi *et al*., 2017).

Amongst other sap sucking pests, the Dusky cotton bug, *Oxycarenus spp.* affect seed quality and lint; thrips lacerate leaves and cause serious damage; jassids cause severe leaf damage and symptoms of hopper burn thereby resulting in low yields; aphids cause damage to foliage and transmit viruses; mealy bugs, *Phenacoccus solenopsis* (Tinsley) feed on leaves and stems to stunt the plants and cause yield losses. Mealy bugs have been reported from Nigeria, Benin and Cameroon in West Africa; India, Pakistan, Thailand and Taiwan in Asia and from New Caledonia in the Pacific (Karar 2008). The first outbreak of mealy bug in Pakistan was reported in 2005 and in 2006-07, it affected the whole cotton belt of Pakistan (Abba et al., 2009). It has been observed in India and Pakistan, naturally occurring biological control keeps mealybug populations under control, if the ecosystems are least disturbed by chemical pesticides.

Conventional weed management

In past, the only reliable method of removing weeds was manual hoeing but it was not considered beneficial due



A schematic diagram showing manual and biotechnological approaches to control Insect pests, weeds and viruses in cotton. (A) Representing manual spray method to control insects in cotton and an alternative biotechnological approach (genetic engineering/ CRISPR/CAS9) to produce genetically modified crop**.** (B) Showing manual hoeing for weed removal and an alternative approach (genetic engineering/Crispr/Cas9) to produce weedicide resistant cotton. (C) Showing manual spray method and siRNA and CRISPR/CAS9 approach to produce virus resistant cotton.

to time and labor it required. Non-chemical methods are often found not viable because of input required (Latif *et al.,* 2015). A number of different herbicides have been developed to control weeds. Ikram *et al.,* (2012) tested pre-emergence herbicides such as Pyroxasulfone, Pendimethalin, S-metolachlor and reported that excessive nutrients loss was effectively controlled by the application of herbicides along with significant improvement in the cotton yield and growth. The use of early post-emergence or post-emergence as lay-by application of herbicides is also another option for weeds control in cotton (Jabran, 2016).

Weed Control Using Biotech Herbicide Tolerant (HT) Cotton

Herbicide resistant-cotton became popular in US and other industrialized countries with the development and release of glyphosate-resistant GM cotton by Monsanto in 1995. Considering the tedious methods of manual weeding or tractor based inter-culture, weed management through broad-spectrum potential of certain herbicides on HT-GM-crops gained popularity soon. Glyphosate resistant HT cotton (Round-up Ready Flex) was developed using '*cp4-5-enolpyruvulshikimate-3-phosphate synthase'* (*epsps*) gene. A '*bialaphos resistance (bar) gene*' was used to develop glufosinate resistant HT-cotton. Extensive use of glyphosate resulted in resistance development in weeds notably in *Amaranthus palmeri* in many countries including the US (*THE ICAC RECORDER*, 2006). This prompted the stacking of genetically engineered events to confer multiple-resistance to dicamba, glufosinate and glyphosate in transgenic cotton varieties. The 'three-genes' based herbicide tolerant cotton varieties have been approved for commercial use in the USA. For dicamba resistance, dicamba monooxygenase *DMO* gene from *Pseudomonas maltophilia* (a soil-bacterium) was utilized (Behrens *et al.,* 2007). The application of registered formulation of 2,4-D + glyphosate and dicamba on 2,4-D and dicamba-resistant soybean respectively, sufficiently controlled susceptible and glyphosate resistant pigweeds (Palmer amaranth and tall waterhemp) and common ragweed

(Inman *et al.,* 2016). These formulations did not significantly disturb soil microbial activities related to nutrient cycling in the soybean rhizosphere and confirm their effectiveness to control glyphosate resistant and other herbicide resistant weeds without disturbing beneficial activities of soil microorganisms (Figure 2B). (Nandula *et al.,* 2016). Currently, an estimated 915,000 metric tons of herbicide active ingredients valued at $15.5 billion are used each year to control weeds worldwide and more than 485 biotypes from 252 weed species are resistant to 163 of 281 different herbicides (Fabian Menalled., 2017).

Conventional methods of insect pest management

Integrated pest management (IPM) is the intelligent selection and use of all pest control actions to ensure effective pest control with least negative effects on the ecology and environment (Kranthi, ICAC). Farmers across the globe rely on chemical pesticides that cause problems on several fronts. Thus far 14,644 cases of insect resistance to insecticides have been reported in 597 insect species to 336 insecticide compounds (IRAC., 2016). Out of the twenty insect species that have the greatest propensity for resistance, six of them are cotton pests. Insecticide Resistance Management (IRM) and IPM form the basis for sustainable pest management in cotton. Integrated pest management methods include all strategies that are expected to support ecosystems for a healthier environment. Biological control and cultural methods are preferred to fulfill such an objective. The OECD (Organization for Economic Cooperation and Development) has predicted that the biopesticide share may grow to 20% of the world's pesticide market by 2020 (Whalon and Wingerd, 2003). Genetically engineered cotton must be considered as one of the strategies in IPM to enable long term sustainability and effectiveness of the technology.

Insect pest management using genetically engineered insect resistant (IR) cotton

Plant transformation provides an effective platform for cultivar improvement as well as for studying gene function in plants (Rao *et al.,* 2009, 2011a, 2011b). *Bt*-cotton expresses proteins encoded by crystal (*cry*) and vegetative insecticidal protein (*vip*) genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*). The *Bt* proteins deployed in *Bt*-cotton are considered to be toxic to Lepidopteran insects and safer to human beings and other non-target organisms. After integration into plant genome, these genes are inherited and expressed like other plant genes and have shown good control efficacy on bollworms. Two main approaches have been used to express insecticidal proteins (Jouanin *et al.,* 1998), which targets the digestive system of insect pests (Schuler *et al.,* 1998). Using plant-derived genes, is an important approach, e.g. the genetic information that code for enzymes like proteinase inhibitors, amylase inhibitors, cholesterol oxidase or lectins (e.g. the snowdrop lectin (GNA) of *Galanthus nivalis* L.; Amaryllidaceae). The second approach uses genes from *B. thuringiensis* that express insect-toxic proteins – mostly the *cry* (Schuler *et al.,* 1998) and *vip* genes. Cowpea trypsin inhibitors and Cry toxins were jointly expressed in biotech-cotton varieties commercialized in China. However, majority of the insect resistant GE cotton events are based on *Bt* toxins.

RNAi approaches to combat insect pests and virus attack

Ribonuclei acid interference (RNAi) for gene silencing, is one of the largely used approaches against some insects like whitefly and pathogenic viruses like CLCuV (Yasmeen *et al.,* 2016). RNAi technology has been used to developed GE cotton for resistance against *Geminiviruses* (Zha *et al.,* 2011; Shepherd *et al.,* 2009) and insect pests. Recently, resistance through RNAi has successfully been applied against Bean golden mosaic virus in Brazil (Aragao *et al.,* 2013). Similarly, control of CLCuV through application of RNAi against different genomic regions of the virus has also been reported (Ahmad *et al.,* 2017, Yasmeen *et al.,* 2016). However, in case of RNAi silencing 'knocks-down' occurs in a gene but the cells might still have some gene expression and contain the transcripts. It is also reported in case of CLCuV that the virus takes over the cotton plant in subsequent generations.

CRISPR/Cas9 based insect, virus resistance and weed control in Cotton

Clustered Regularly Interspaced Short Palindromic Repeats CRISPR RNA (crRNA) can help guide the CRISPR associated Cas9 endonuclease to scan the invading DNA and cleave the target sequence. CRISPR/Cas9 is one of the most widely adapted genome engineering systems and has been successfully used in several species ranging from simple microbes to complex plants and animals. The *begomoviruses* (family *Geminiviridae*) associated with cotton leaf curl disease (CLCuD) pose major threats to cotton production in India, Pakistan, and other parts of the Asia. Methods to control these viruses depend not only on controlling production of the circular, ssDNA genome but also two satellite DNAs, alphasatellite and betasatellite. Recently, CRISPR/Cas9 has successfully been used as a tool to engineer virus resistance in plants in general and cotton leaf curl virus (CLCuV) in particular, either by directly targeting and cleaving the viral genome, or by modifying the host plant genome to introduce viral immunity.

Four recent studies confirmed that the CRISPR/Cas9 system could efficiently confer resistance to *Geminiviruses* in plants. *Nicotiana benthamiana* plants expressing the CRISPR/Cas9 machinery exhibited resistance against TYLCV, Beet curly top virus (BCTV), and *Merremia* mosaic virus (MeMV). All of these studies demonstrated that *N. benthamiana* plants expressing the CRISPR/Cas9 system showed reduced viral titers, which ultimately eradicated or significantly reduced disease symptoms (Iqbal *et al.,* 2016). Herbicide resistant rice was developed through discrete point mutations in the rice ALS gene using CRISPR/Cas9-mediated homologous recombination (Sun *et al.,* 2016).

Conclusion

Cotton plants encounter a range of biotic stress factors throughout the season. Insect pests, diseases and weeds are the major constraints in crop production. The recent discovery of RNAi and CRISPR CAS9 offers great opportunities to develop multipronged strategies to mitigate biotic stress factors in an effective manner to ensure sustainable control of insect pests, diseases and weeds in a sustainable and eco-friendly manner.

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Proceedings and Recommendations of the 14th Meeting of the 'Latin American Association for Cotton Research and Development' (ALIDA)

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The 14th ALIDA meeting was held at Maceió, Brazil, on August 28th 2017 on the theme *'New technologies for Boll weevil management'*. The meeting was organized jointly by the International Cotton Advisory Committee (ICAC), the Food and Agriculture Organization of the United Nations (FAO), the Brazilian Agricultural Research Corporation (EMBRAPA), Brazil and the Brazilian Cooperation Agency (ABC / MRE).

Background

The Latin American Association for Cotton Research and Development (ALIDA) was formed in 1986. Thus far 14 network meetings were held in various countries of the region in Argentina (1986, 1997, 2010 and 2011), Bolivia (1999), Brazil (1991, 2003 and 2017), Colombia (1993, 2005 and 2013) Nicaragua (1995), Paraguay (2001) and Peru (1988). The primary objective of ALIDA is to facilitate active interactions amongst researchers of Latin America and also with experts of other countries to discuss challenges, share experiences and knowledge with an aim to strengthen the Latin American cotton sector.

Inaugural Session

In her opening remarks, Mrs. Cecilia Malaguti (ABC/MRE) described IBA and ABC initiatives under the Trilateral South-South Cooperation to strengthen the cotton sector in Africa, Latin America and the Caribbean. Ms. Adriana Gregolin (FAO) welcomed the participants and emphasized the need for combined efforts of ALIDA and FAO projects for the progress of cotton sector in Latin America. In his presidential address, Dr. Sebastião Barbosa (EMRAPA) described ALIDA as a platform for effective exchange of knowledge and research findings for the progress of cotton research in the region. He said that the boll weevil and bollworm are of serious concern to the major cotton growing countries in Latin America and ALIDA can address these problems effectively. Dr. Keshav Kranthi (ICAC) highlighted the rapid strides made by the Brazilian cotton for yield enhancement and the experiences of USA and Mexico in boll weevil eradication which he said could be considered as brilliant case studies to prepare developmental plans for the benefit of Latin America. Ms. Lorena Ruiz (ICAC) reiterated the importance of cotton in the world economy and enlisted the challenges facing Latin America. Engineer Eduardo Román facilitated and compered the meeting.

Participants

Thirty-six participants from nine countries (Argentina, Brazil, Bolivia, Colombia, Chile, Mexico, Paraguay, Perú and USA) attended the meeting.